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Complete this list EACH TIME a beam is obtained with a new electrospray/instrument combination, or after any maintenance operations have been carried out.

<table>
<thead>
<tr>
<th>Parameter:</th>
<th>Used:</th>
<th>Recommended:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Instrument/Electrospray Identification; Date:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sample:</td>
<td>Positive ion:10ng/µl Gramicidin S or Leucine Enkephalin in 1:1 H2O:Methanol +1% acetic acid. Negative ion:30pmol/µl Lauryl sulphate in Methanol or100ng/µl Raffinose in 1:1 acetonitrile:water</td>
<td></td>
</tr>
<tr>
<td>Flow Rate:</td>
<td>10-50 µl/minute</td>
<td></td>
</tr>
<tr>
<td>Signal Obtained:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bath Gas:</td>
<td>250l/hour @ 7 bar, N2</td>
<td></td>
</tr>
<tr>
<td>Nebuliser Gas:</td>
<td>Full on (8-12l/hour), 7 bar, N2 or air</td>
<td></td>
</tr>
<tr>
<td>Source Slits:</td>
<td>Wide open</td>
<td></td>
</tr>
<tr>
<td>Collector slits:</td>
<td>Wide open</td>
<td></td>
</tr>
<tr>
<td>Other Slits:</td>
<td>Wide open</td>
<td></td>
</tr>
<tr>
<td>Resolution:</td>
<td>Wide open</td>
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</tr>
<tr>
<td>Magnet Position:</td>
<td>571-572 (2⁺ ion) or 265 (M⁻ cation) or 503</td>
<td></td>
</tr>
<tr>
<td>Magnet Limits:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Span:</td>
<td>1 or 3 x 10⁴ ppm</td>
<td></td>
</tr>
<tr>
<td>Detector:</td>
<td>300</td>
<td></td>
</tr>
<tr>
<td>Bath Heater:</td>
<td>60-80</td>
<td></td>
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**Vacuum Pressures:**

| Source Ionisation: | ~ 3-5 x 10⁻⁶ mbar |
| Source Backing: |       |              |
| Analyser Ionisation: | ~ 1 x 10⁻⁶ mbar |
| Analyser Backing: |       |              |
| Inlets 1 (Turbo Backing): | ~ 8-18 x 10⁻² mbar |

**Voltages**

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<thead>
<tr>
<th>Parameter</th>
<th>Slider Position</th>
<th>Readback</th>
<th>Measured</th>
<th>Recommended</th>
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<tbody>
<tr>
<td>Needle Voltage:</td>
<td>~ 8kV (variable)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Counter Electrode:</td>
<td>(———)</td>
<td>~ 1050V above * Vacc.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sampling Cone:</td>
<td>~ 50V above * Vacc.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skimmer Lens:</td>
<td>~ 60V above * Vacc</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skimmer (Accel. Volts):</td>
<td>(———)</td>
<td>4kV</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hexapole:</td>
<td>~10V below * Vacc</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ring Electrode:</td>
<td>~ 20V below * Vacc</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RF Neon First illuminates at:</td>
<td>500 600 700 800 900</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(⁺ for positive ionisation)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tbody>
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(Use sheets 2 and 3 for additional data.)
Sheet 2, Electrospray Checklist, Manual Tuning Controls

(Complete if relevant)

**Pot Positions, 4kV Positive Electrospray Beam:**

<table>
<thead>
<tr>
<th>Foc 1</th>
<th>Foc 2</th>
<th>IE</th>
<th>Y Foc</th>
<th>Z Foc 2</th>
<th>Z Foc 3</th>
<th>R1</th>
<th>C2</th>
<th>R3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ion Rep BC</td>
<td>Z Def</td>
<td>Y Def</td>
<td>Z Def 2</td>
<td>Z Def 3</td>
<td>R2</td>
<td>C3</td>
<td>R4</td>
<td></td>
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</table>

**Pot Positions, 4kV Negative Electrospray Beam:**

<table>
<thead>
<tr>
<th>Foc 1</th>
<th>Foc 2</th>
<th>IE</th>
<th>Y Foc</th>
<th>Z Foc 2</th>
<th>Z Foc 3</th>
<th>R1</th>
<th>C2</th>
<th>R3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ion Rep BC</td>
<td>Z Def</td>
<td>Y Def</td>
<td>Z Def 2</td>
<td>Z Def 3</td>
<td>R2</td>
<td>C3</td>
<td>R4</td>
<td></td>
</tr>
</tbody>
</table>

**Note:** Record the pot positions as for a 24 hour clock, eg. “16:30”.

**Other Comments:**
# Sheet 3, Electrospray Checklist, Data System Tuning

(Complete if relevant)

<table>
<thead>
<tr>
<th>ES POS/NEG* SETUP</th>
<th>Parameters Name</th>
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</thead>
<tbody>
<tr>
<td>Focus #1</td>
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</tr>
<tr>
<td>Beam Centre</td>
<td></td>
</tr>
<tr>
<td>Focus #2</td>
<td></td>
</tr>
<tr>
<td>Needle Voltage</td>
<td>%</td>
</tr>
<tr>
<td>Counter Electrode</td>
<td>r/b</td>
</tr>
<tr>
<td>Sampling Cone</td>
<td>%</td>
</tr>
<tr>
<td>Skimmer Lens</td>
<td>%</td>
</tr>
<tr>
<td>Skimmer</td>
<td>r/b</td>
</tr>
<tr>
<td>Hexapole</td>
<td>r/b</td>
</tr>
<tr>
<td>Ring Electrode</td>
<td>%</td>
</tr>
<tr>
<td>Bath Heater</td>
<td>%</td>
</tr>
</tbody>
</table>

Fill in these values as appropriate, or use the Print Screen menus to obtain hardcopies. Also hardcopy the Peak Display if required.

<table>
<thead>
<tr>
<th>SLIT ADJUST</th>
<th>Parameters Name</th>
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<tbody>
<tr>
<td>Source</td>
<td>%</td>
</tr>
<tr>
<td>Collector</td>
<td>%</td>
</tr>
<tr>
<td>Alpha</td>
<td>%</td>
</tr>
<tr>
<td>Z2</td>
<td></td>
</tr>
<tr>
<td>Z3</td>
<td></td>
</tr>
<tr>
<td>Z4</td>
<td></td>
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<table>
<thead>
<tr>
<th>LENS ADJUST</th>
<th>Parameters Name</th>
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<tbody>
<tr>
<td>Ion Energy</td>
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<tr>
<td>Y-Def #1</td>
<td></td>
</tr>
<tr>
<td>Z-Def #1</td>
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</tr>
<tr>
<td>Rotate #1</td>
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<tr>
<td>Y-Focus #1</td>
<td></td>
</tr>
<tr>
<td>Z-Def #2</td>
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<tr>
<td>Z-Focus #2</td>
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<td>Rotate #2</td>
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<td>Curve #2</td>
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<tr>
<td>Curve #3</td>
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<tr>
<td>Z-Focus #3</td>
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<td>Z-Def #3</td>
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<tr>
<td>Rotate #4</td>
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<table>
<thead>
<tr>
<th>INSTRUMENT</th>
<th>Parameters Name</th>
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</table>

Other Comments:
Electrospray Installation Checklist

1. Select ES source in OPUS and check voltage limit is set to 4 kV.
2. Vent source housing, while monitoring vacuums.
3. Isolate inlet 1 rotary pump by closing speedivalve on E2M18 pump at rear of instrument.
4. Disconnect and blank off pumping lines to source ball valve and ball valve actuator.
5. Open speedivalve on inlet 1 pump. The inlet 1 vacuum should rapidly recover to its normal value.
6. Remove ball valve actuator assembly and blank off port.
7. Remove complete front flange assembly.
8. Mount electrospray interface on source housing, using two dowels from original front flange.
9. Connect turbo pump backing line. Plug in turbo pump water cooling lines.
10. Install electrospray cradle, connecting the two wires (and gas line if Mk.4 cradle), and sliding the steel collar over the electrospray transfer lens.
11. Connect the cables from the electrospray trolley to the instrument side panel. These include ground, mains power, D-type and fibre optic connections.
12. Establish OPUS communication with electrospray trolley. Open DECterm window and type SIOSLOAD. At the ETH prompt type WB 681700 1. To return to $ prompt, press Ctrl Z twice. The message "ES unit present on optic loop" will appear in OPUS.
14. Complete all remaining connections to electrospray interface. Connect high voltage and ground cable to electrospray probe.
15. When inlet 1 pressure is less than 0.2 mbar, open butterfly valve. Close pumping line to side probe lock.
16. Switch on turbo pump, using switch on trolley front panel. Select full speed via ES Vacuum menu in OPUS.
17. Connect bath gas and waste gas lines to interface. Connect 7 bar nitrogen supply to trolley.
18. When turbo is running at full speed, and vacuum pressures are satisfactory, find an ion beam using a standard test compound (gramicidin S or leucine enkephalin for +ve ions; lauryl sulphate or raffinose for -ve ions).
19. Assess interface performance, and record all relevant interface parameters, for instance using the Electrospray Checklists provided, for future reference.
APCI Installation Checklist

1. Select API source in OPUS and check voltage limit is set to 4 kV.
2. Vent source housing, while monitoring vacuums.
3. Isolate inlet 1 rotary pump by closing speedivalve on E2M18 pump at rear of instrument.
4. Disconnect and blank off pumping lines to source ball valve and ball valve actuator.
5. Open speedivalve on inlet 1 pump. The inlet 1 vacuum should rapidly recover to its normal value.
6. Remove ball valve actuator assembly and blank off port.
7. Remove complete front flange assembly.
8. Mount API interface (without the APCI source chamber) on source housing, using two dowels from original front flange.
9. Connect turbo pump backing line. Plug in turbo pump water cooling lines.
10. Install electrospray cradle, connecting the two wires (and gas line if Mk.4 cradle), and sliding the steel collar over the electrospray transfer lens.
11. Connect the cables from the electrospray trolley to the instrument side panel. These include ground, mains power, D-type and fibre optic connections.
12. Establish OPUS communication with electrospray trolley. Open DECterm window and type SIOSLOAD. At the ETH prompt type WB 681700 1. To return to $ prompt, press Ctrl Z twice. The message “ES unit present on optic loop” will appear in OPUS.
13. Connect electrospray rotary pump exhaust. Fit the APCI source chamber, and begin to pump out source housing using side probe lock. Open speedivalve under turbo pump. Switch on first stage rotary pump on electrospray trolley.
14. Complete all remaining connections to APCI interface. Connect high voltage cable to free corona needle plug, and ground cable to APCI nebuliser.
15. After first ensuring that the APCI nebuliser temperature is set to zero in the API Tune menu, connect the heater / thermocouple cable to the nebuliser.
16. When inlet 1 pressure is less than 0.2 mbar, open butterfly valve. Close pumping line to side probe lock.
17. Switch on turbo pump, using switch on trolley front panel. Select full speed via ES Vacuum menu in OPUS.
18. Connect sheath and nebuliser gas to interface. Connect 7 bar nitrogen supply to trolley.
19. Connect 14 mm waste gas line to APCI extraction pump under trolley, connect wide waste tube to suitable exhaust, and turn on the extraction pump.
20. When turbo is running at full speed, and vacuum pressures are satisfactory, pre-heat the APCI nebuliser with the sheath and nebuliser gas flows on, for about 15 minutes at 500° C.
Electrospray / APCI Vacuum Trouble Shooting Guide

1. **Regularly record the normal ESI / APCI operating pressure ranges:**
   - This provides a benchmark. Typical values are $3 - 8 \times 10^{-6}$ mbar for the source ionisation gauge, and $8 - 18 \times 10^{-2}$ mbar for the turbo pump backing line (inlets 1 pirani).

2. **If the source ionisation and turbo backing pressures are too low:**
   - Is the blanking sampling cone still fitted, after EI or LSIMS was used?
   - Is the sampling cone a long way off axis from the skimmer?
   - Is the sampling cone partially or completely blocked?

3. **If the source ionisation pressure is high, but the turbo backing is normal:**
   - Is the turbo pump running, and at full speed? (see further information below).
   - Fit an EI source and leak check the instrument housing etc. with helium.
   - The re-entrant system may be leaking (particularly if it is cold).
   - One of the re-entrant valves is open.
   - The seal in the ball valve blanking flange may be leaking.
   - The seal around the FFR1 collision gas line may be leaking (Mk.4 cradle).
   - One of the solenoids controlling the FFR1 gas cell may be leaking.
   - The Cs+ gun may have shifted out of position when the housing was vented.
   - The seal around the main API flange may be poor (is the lid in the way?).
   - The seals around the glass window, or the instrument lid, may be poor.

4. **If the turbo backing pressure is high, but the source pressure is normal:**
   - There is probably a leak on the roughing lines. Shut the KF-16 valve below the turbo pump, and leak check the lines. Old, used co-seals do not always reseal.

5. **If the turbo backing and the source ionisation pressures are both high:**
   - There is probably a leak on the API interface, rather than the instrument.
   - Is the first stage pump working normally?
   - Is the stainless steel KF-25 union on the pump inlet screwed on tightly?
   - Is the vent valve in good order?
   - Is the first stage line leaking? (see separate procedure below).
   - Is the atmospheric pressure chamber seated correctly, and the seal OK?
   - Is the sampling cone seal leaking?
   - Is there a leak between the first & second pumping stages? (see separate procedure below).
   - Is there a leak from atmosphere in to the second pumping stage?
6. **Checking the first stage line for leaks:**

- The first stage pumping line should never be disconnected by undoing the hose clamps, since they may be difficult to re-seal.

- Vent the interface. Remove the first stage line from the interface, complete with the pumping ports and the KF-25 tee. Seal the black plastic port with a suitable rubber bung, or press a KF-25 co-seal and blanking flange against the end. Connect the tee into the inlets 1 lines, and use the inlets 1 pirani to assist with leak checking.

7. **Checking for leaks between the first & second pumping stages:**

- Vent the interface. Isolate or switch off the first stage rotary pump. Remove the atmospheric pressure chamber.

- Make up a piece of tubing suitable for sealing the hole in the skimmer (care! the tip of the cone is extremely delicate! If damaged, the skimmer must be replaced!). Suitable candidates are short lengths of 6 mm OD x 4 mm ID blue polyurethane tube, or the silicone tube used on the water cooled probe. Block one end, and cut the other end square so that it will form a good seal around the skimmer cone when held against it.

- Evacuate the turbo pumped stage and the source housing using only the rotary pumps, and check for leaks using the turbo backing pirani gauge to monitor the pressure.

8. **Turbo pump trouble shooting:**

- No power to mains switch on trolley front panel - check that cable BR01 is connected to the instrument side panel, and check the operation of the relay in unit MA3485 (which should close when the butterfly valve is open). The relay can be temporarily bypassed for test purposes by connecting the mains plug EA04 directly to a suitable 240 V supply.

- Turbo pump ‘soft start’ - this can optionally be reprogrammed using a Varian hand held controller.

- Turbo pump regularly trips out - reset it via the ES Vacuum menu, and establish the cause; check the cooling water lines and the bore though the pump for debris.

- Failure to reach full speed, and / or particularly loud whining probably indicates bearing failure.

- If the pump comes to a complete stop after a high voltage discharge event, and cannot be restarted, it is likely that the microprocessor in the turbo controller has been adversely affected. This may be reprogrammable using the hand held controller.

9. **A note concerning Analyser leaks:**

Relatively small analyser leaks can result in the almost total loss of any multiply charged protein ions, but will probably not noticeably decrease the signals from low mass test compounds such as gramicidin S.
Quick Guide - General ESI Trouble Shooting

Section A, A Poor, Intermittent or Unstable Signal, Independant of Sample Mass

1. Solvent delivery, sample delivery or spray problems:
   - Very low frequency pulsing signal when using reciprocating pump - investigate check valve operation; purge the pump; refer to section 8-1-5.
   - Intermittent spray when using a syringe pump - investigate for blockage. Is a gas-tight syringe used? Has the barrel burst? Is there a leak? refer to sections 8-1-3 or 8-1-4.
   - Excessive injection peak tailing - refer to sections 4-5 and 8-4. Poor sample solubility may cause this - see chapter 5.
   - Poor sample injection technique, e.g. due to the wrong type of syringe, or a damaged injector - refer to section 8-4 and the literature supplied with the injector.
   - Is the probe in good order? - refer to sections 4-5 and 6-1.
   - Is the nebuliser working at full strength? - refer to Fig 4-5-G. (nebuliser gas is optional with the single hole counter electrode, but is essential with the pepper pot version).

2. Problems in atmospheric pressure chamber:
   - Is the signal obtained with a standard test compound down to half of the benchmark level? - clean the counter electrode and the sampling cone, and wipe any deposits off the inside of the PEEK chamber; refer to sections 6-2 & 6-3.
   - Is the spray good, but the signal very unstable? - stability is usually improved by operating with the chamber a few millimeters off axis from the skimmer, particularly at high flow rates. If this gives no improvement, check the sampling cone for a hair or particle; refer to section 6-3.
   - Is the heater switched on & working correctly? If it is cold and a high flow rate was selected, the chamber may need to be dried out.

3. Occasional discharging heard, or flickering voltage readbacks:
   - Is the spray needle too close to the counter electrode, or the needle voltage too high?
   - Is the turbo pump running at full speed?
   - Investigate for vacuum problems - see the Vacuum trouble shooting guide.
   - Operate with the atmospheric pressure chamber a few millimeters off axis from the skimmer for optimum signal stability.

4. Tuning & Control difficulties:
   - Sampling cone, skimmer lens and / or ring electrode controls do not optimise at about 15 - 30% on low mass test compounds such as gramicidin S or leucine enkephalin - the high voltage setup may be incorrect, refer to section 4-6 in appendix 1. (The high voltage setup must be carried out individually for both positive and negative modes; if not done properly, sensitivity in one or the other mode may be poor).
   - The above slider controls cease to have any effect; OPUS may also report “ES unit absent from optic loop”, and / or the readbacks behave oddly - reboot SIOS in the first instance. Also manually reboot the electrospray trolley microprocessor if this does not clear the problem (see note in Section C below).
   - Generally poor sensitivity - the output of the high voltage supply that floats the RF lens may need occasional adjustment, refer to section 4-6 in appendix 1. Obtain a strong signal, and slowly adjust the relevant pot by up to a half turn in either direction (approx. +/- 15 V), at the same time as retuning ion energy and ring electrode controls, to establish the optimum setting.
   - High frequency breakdown superimposed on the ion signal - this is normal, and can occur when the voltage differences between the skimmer, hexapole and ring electrode are not optimal. The frequency should change as the ion energy and / or ring electrode controls are adjusted, until a point is reached at which the oscillation ceases. (If the frequency remains constant, suspect an RF tuning or RF power supply problem; refer to section 4-7 in appendix 1).
• Skimmer lens hardware present, but no software slider for it - the options in the instrument configuration menu must be set up to match the hardware, otherwise it is probable that the skimmer lens will be held at a non-optimal voltage, thus reducing ion transmission. (A software slider without the associated hardware will cause no problems).

SECTION B, A POOR SIGNAL AT SPECIFIC MASS RANGES

5. Poor high mass signal:
• A small analyser leak can cause a total loss of signal from proteins, but may not alter the transmission of low mass test compounds. Check the ratio of singly and doubly charged ions from gramicidin S.

6. Poor low mass signal:
• Unsatisfactory tracking of RF amplitude with mass. The amplitude is much more critical at low than at high masses; if the tracking is not set up carefully, or has drifted, then solvent ions are the first to disappear - refer to section 4-7 in appendix 1.

• The instrument magnet reference signal (from which the RF control signal is derived) is slightly different in current and field control modes. Thus some sensitivity difference should be expected when switching between these modes, particularly at low masses. An unsatisfactory Hall probe calibration may exacerbate this difference.

• If the RF tracking is correct, poor low mass sensitivity may be an indicator that the hexapole rods require cleaning - refer to section 6-5.

7. No ions in middle of spectrum:
• Due to incorrect setup of RF tracking; RF reference signal probably drops to zero between two break points - see Fig 4-7-B.

SECTION C, NO BEAM CAN BE FOUND

8. A selection of causes (see section 6-7 for further details):
• Interlock circuit is open; no source high voltages are on. (This can be easily overlooked when attempting to tune in EI or LSIMS modes just after using the electrospray interface).

• An instrument problem (e.g. detector not working, or magnet tripped) - temporarily fit an EI or LSIMS source, refer to chapter 11.

• An OPUS problem (e.g. an incorrect selection in the instrument configuration menu, or an incorrect parameter in one of the instrument menus).

• Electrospray unit not present on the optic loop - refer to section 4-4-7. Are the fibreoptic links to the trolley in good order?

• A solvent delivery, spray, or nebuliser problem; the sampling cone is blocked or a hair is lodged on it; a vacuum problem is causing a steady high voltage discharge.

• Non-optimal electrospray and/or instrument tuning parameters are in use (refer to previous Checklists for good values).

• Failure of one of the high voltage units, or a high voltage cable is disconnected from the interface or from the supply, or a board is loose, or a supply has overheated and is giving a low output.

• High voltage supplies are not balanced correctly - see setup procedure, section 4-6 in appendix 1.

• Failure or overload of one of the low voltage power supplies in the trolley - check the fuses. Failure of the low voltage wiring in the trolley or to the interface.

• The low voltage control cable, BR01, has become dislodged from the instrument side panel - the turbo pump will stop, and no trolley high voltages will come on.

• There is loss of SIOS control of the trolley; perhaps some HT supplies are on while others are off, and the readbacks are behaving strangely - manually reboot the trolley microprocessor by unplugging the electronics unit for 2 minutes.

• Failure of the RF system to track correctly with mass - try to tune on an ion above m/z 500, rather than a low mass solvent ion, since this is much less likely to be affected. Refer to section 4-7 in appendix 1.

• Complete failure of the RF system - check for presence of RF by observing the neon indicator, refer to section 4-7 in appendix 1.
Section 1

Introduction And Overview
Section 1

Introduction And Overview
Introduction And Overview

Atmospheric Pressure Ionisation (API) incorporates the techniques of both Electrospray Ionisation (ESI) and Atmospheric Pressure Chemical Ionisation (APCI). Electrospray is ideally suited to the analysis of a wide range of compounds of both low and high molecular weight, including peptides, proteins, drugs, pesticides, dyestuffs and inorganics. APCI is suited to the analysis of low molecular weight compounds, particularly by LC-MS at conventional LC flow rates of around 1 ml/minute, and does not tend to produce the multiply-charged ion series characteristic of ESI spectra of biopolymers.

The scope of this manual

This manual covers both ESI and APCI, as used with the ProSpec, AutoSpec, and ZabSpec ‘X’, ‘S’, ‘V’ and ‘M’ series of high performance mass spectrometers. Separate manuals are, or will be, available for ESI and APCI as used on 70 and ZAB series instruments, and on any new series of AutoSpec family of instruments.
Figure 1.1 ElectroSpray Schematic - Typical Voltages for 4kV Accelerating Voltage Shown

- Spray Needles 8000V
- Hot Bath Gas 80°C
- Hexapole Lens
- Pepper Pot Counter-Electrode
- 5000V
- Ion Acceleration Region
- Skimmer Lens
- Sampling Cone 4050V
- Skimmer Electrode 4000V
- Ring Electrode 3980V
- Source Slit 0V
- Focussing Lenses
- Source Diffusion Pumps >10^-7 mbar
- Analyser Diffusion Pumps >10^-7 mbar
- Rotary Pump 1 x 10^-3 mbar
- Turbo Pump 3 x 10^-6 mbar
- To Mass Analyser
- Nebuliser gas

Section 1
Introduction And Overview
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1-1. Principles of Electrospray (ESI) Mass Spectrometry

Samples are introduced in solution, either by direct injection or via an appropriate separation technique such as LC or CZE. Typical positive ion solvents are 1:1 water : methanol or water : acetonitrile containing approximately 1% acetic or formic acid. The sample solution is delivered through a stainless steel or fused silica capillary needle held at ~3kV with respect to a counter electrode, Fig. 1-1.

As a consequence of the strong electric field between the end of the needle and the counter electrode the sample solution is dispersed into an aerosol of highly charged droplets - the electrospray. This typically takes place with a flow rate of 1 - 5µl/min. For flow rates up to ~500µl/min, a combination of electrostatic and pneumatic nebulization are used (alternatively called pneumatically assisted ESI, or Megaflow ESI). Pneumatic nebulisation also makes the analysis of samples in pure water feasible. These droplets, assisted by a warm flow of bath gas, diminish in size by evaporation until a point where individual, often multiply charged, solvent free ions are produced. Some of the ions pass through a small sampling orifice (the sampling cone) into the first of two differentially pumped intermediate regions between the electrospray atmospheric pressure chamber and high vacuum. The intermediate regions employ a hexapole lens designed to optimise ion transmission whilst removing most of the neutral species. The primary ion acceleration subsequently takes place at high vacuum (typically ~3 x 10^{-6} mbar) in the source housing of the mass spectrometer where ion/neutral collisions are sufficiently infrequent to allow good transmission of large, multiply charged ions.

These multiply charged ions are characteristic of electrospray especially in the analysis of high molecular weight compounds like proteins.
FIGURE 1-2. AUTOSPEC ELECTROSPRAY SPECTRUM OF HORSE APO-MYOGLOBIN (Mr 16951.5)

Many compounds give a spectrum containing a series of multiply charged ions when analysed by electrospray. Each ion in the series usually differs by plus and minus one proton from each adjacent ion in the series. A typical positive ion spectrum from a protein, myoglobin, appears in Fig 1-2. The molecular mass is determined from the mass to charge ratios measured by the mass spectrometer for all the significant ions in the series.

The positive and negative ions have the general formulas:-

\[ [M + nA]^{n+} \quad \text{and} \quad [M-nA]^{n-} \]

where:

- \( M \) is the molecular mass of the protein (or other compound)
- \( n \) is an integer number of charges and
- \( A \) is the adduct, usually a proton (although other adducts such as Na may be observed).

The mass spectrometer measures the mass to charge ratio \( m \) of each peak given by:-

\[ m = \frac{(M + nA)}{n} \]

The molecular mass \( M = n(m - A) \) can then be readily calculated from the measured mass to charge ratio if \( n \) can be determined. Any two consecutive peaks in the series differing by one proton may be used to determine \( n \). Therefore, referring to Figure 1-2,

\[ m_2 = \frac{(M + nA)}{n} \]
\[ m_1 = \frac{(M + (n + 1)A)}{(n+1)} \]

Where \( m_2 \) and \( m_1 \) are the measured mass to charge ratios of two peaks with \( n \) and \( n + 1 \) adducts (usually protons) respectively. By solving these two simultaneous equations the charge \( n \) on \( m_2 \) can be determined by

\[ n = \frac{(m_1 - A)}{(m_2 - m_1)} \]

and \( n \) is then rounded to the nearest integer value. Since each peak in the series differs from the next by one charge it is simple to assign the charge state to each peak and calculate the molecular mass from every peak using \( M = n(m - A) \) and then average the molecular mass values. This process can be done using two different software packages available as part of the OPUS software, namely Transformation and Maximum Entropy.
1-3. Principles Of Atmospheric Pressure Chemical Ionisation (APCI) Mass Spectrometry

The APCI interface incorporates a heated nebuliser assembly with a 200 watt heater, and is capable of operating at solvent flow rates of ~0.2 to 2.0 ml/minute. Although the nebuliser heater may be run at 400 to 700 °C, the samples, entrained in the solvent vapour, rarely exceed a temperature of ~120 degrees. The nebuliser is held at ground potential, and ionisation is effected by means of the gas-phase ion/molecule reactions generated using a corona discharge needle held at high voltage with respect to the sampling cone, Figure 1-3.

The APCI interface utilises the same skimmers, pumping and ion transfer arrangement as the ESI accessory. Change-over between each technique is simple and straightforward.

The principle of positive ion formation is described below. The discharge created between the corona discharge needle and the sampling cone produces electrons which are then available to ionise moist air as the principal ionisation process. A sequence of ion/molecule reactions are then initiated, which results in the formation of hydronium ion - water clusters, H₃O⁺(H₂O)ₙ within a few microseconds (Sunner, J., Nicol, G., & Kebarle, P. (1988) Anal. Chem. 60, 1300-1307). A simplified reaction scheme is as follows.

\[
e^- + N₂ \leftrightarrow N₂^+ + (N₂, O₂) \leftrightarrow N₄^+ + O₂ \leftrightarrow O₂^+ \quad [1] \\
e^- + O₂ \leftrightarrow O₂^+ + O₂ \leftrightarrow O₄^+ \quad [2] \\
O₂^+, Oₙ^+ + H₂O \leftrightarrow O₂^+(H₂O) \quad [3] \\
O₂^+(H₂O) + H₂O \leftrightarrow O₂^+(H₂O)₂ + H₂O^+(OH) + O₂ \quad [4] \\
H₂O^+(OH) + H₂O \leftrightarrow H₂O^+(H₂O) + OH \quad [5]
\]

The hydronium ions will undergo successive clustering:

\[
H₂O^+(H₂O) + H₂O \leftrightarrow H₂O^+(H₂O)₂ \quad [6] \\
H₂O^+(H₂O)ₙ + H₂O \leftrightarrow H₂O^+(H₂O)ₙ₊₁ \quad [7]
\]

The hydronium ion - water cluster ions are the main reagent ions in positive ion APCI. These protonate with any molecule whose gas phase bacicity is greater than that of water, with very high efficiency because of the high (i.e. atmospheric) pressure:

\[
H₂O^+(H₂O)ₙ + B \leftrightarrow BH^+(H₂O)ₙ + (n+1-b)H₂O \quad [8]
\]

Cluster ions can also be formed by proton transfer to the solvent molecules, and also by clustering between the sample and solvent. The sample ions can be de-clustered by operating the nebuliser at a high temperature, resulting in hot nebulising gas to facilitate cluster removal. For negative ion formation, the principal reagent ion is O₂⁻, which may cluster to form O₂⁻(H₂O)ₙ. Ionisation of the sample is by charge transfer to compounds of higher electron affinity or by proton abstraction from strong gas phase acids, such as carboxylic acids, phenols and amides.
1-4. Other Sources Of Information

Details of the processing and manipulation of electrospray data, along with various experimental strategies that may be followed, are given in a separate manual, ‘Electrospray Data Processing’.

Detailed information concerning the low resolution electrospray behaviour of proteins can be found BioSpec numbers one, two and three, available from Micromass. Two application notes describe the AutoSpec electrospray analysis of native protein complexes in pure water at high m/z range (note no. 36), and high resolution accurate mass measurements carried out on small proteins (note no. 37).

Various other application notes published by Micromass are also relevant. Note no. 209, for instance, describes a capillary electrophoresis interface for a Platform. Note no. 212 details the Maximum Entropy method of processing multiply-charged electrospray data.

Complete sets of circuit diagrams should be supplied with each kit, and in addition the Varian manuals covering the turbo controller and turbo pump should be supplied. If any of these are missing, contact a Micromass representative to obtain copies.

1-5. Computer Animations

A number of procedures are currently available or will become available in the form of computer animations, suitable for PC’s with a colour screen. These can be particularly helpful during maintenance or cleaning operations, or for identifying and ordering individual components. Please enquire for further details.
Section 2

API Pre-Installation Requirements
API Pre - Installation Requirements

2-1. Instrument requirements:

1. OPUS software version 3.0 (or later).
2. Instrument fitted with HPLC wiring panel (standard on 'V' and 'M' series).
3. Instrument fitted with bench water sockets for turbo pump cooling (only absent on early 'X' series).

2-2. Gas requirements:

1. * A supply of high purity nitrogen (99.998% pure or better), regulated at 7 bar (100 psi) outlet pressure. Normal usage for continuous 8 hour/day operation is typically two standard lab cylinders per week (one cylinder contains approximately 10 m³ of N₂, 200 bar) when running electrospray at low flow rates, or somewhat higher if megaflow electrospray or APCI are being used.
2. * Suitable unions for connection of the N₂ supply to the 4 mm ID x 6 mm OD polyurethane inlet tube (6 mm or 1/4” Swagelok unions are satisfactory).
3. * Provision for safe venting of electrospray and/or APCI source exhaust gases (typically 2 - 6 litres/minute). A 5 m length of 4 mm ID x 6 mm OD tube is provided for removal of ESI waste gas, and a 5 m length of 1.5” hose is provided for removal of APCI waste gas. The customer is responsible for connection of this tube and/or hose to the outside atmosphere or to a fume extraction hood. Connection to the rotary pump exhaust lines is not recommended.

Nitrogen Supplies For API

There are three possible sources of nitrogen for use with electrospray/APCI, as follows:

Nitrogen Cylinders

This may be the most readily available source of N₂. However, frequent cylinder changes (every 1 - 3 days) will be required when using megaflow or APCI.

Please note there is probably no need to order ultra high purity N₂. Oxygen free N₂ (the most inexpensive grade commercially available) which is 99.998% pure is used successfully on the Quadrupole electrospray instruments.
**Liquid Nitrogen**

Obtaining gaseous N₂ from liquid N₂ boil-off gives a convenient, uninterrupted supply of pure nitrogen. However, some capital outlay is required in order to purchase the dewar and associated valves/pipework. It is recommended that users contact their local gas suppliers for quotations of installation and running costs. After initial installation costs, the price/volume of N₂ produced by this method will be significantly less than using gas cylinders.

**Nitrogen Generators**

Nitrogen generators are passive devices which employ a membrane to separate N₂ from O₂, CO₂ and other trace gases found in air. To operate they require a compressed air supply. Although N₂ generators will give a relatively inexpensive, continuous supply of N₂, the following should be considered before purchasing a system:

1. It should be ensured that the model being considered will deliver the required N₂ purity at the maximum N₂ flow rate required. (The purity of N₂ generated decreases with the flow rate).

2. It should be ensured that the generator will supply a pressure of at least 7 bar (necessary for ESI/APCI). This pressure must be present on the output side of the generator, i.e. an allowance should be made for the pressure drop across the generator itself. (Note: this may prevent the use of an existing lab supply of compressed air). A compressor sited in the laboratory will take up space and generate heat and noise.

3. In trials on Quadrupole electrospray instruments, a Balston 75-72 nitrogen generator (supplied by Whatman) was found to produce contamination peaks in both positive and negative ion electrospray and APCI. The contamination was primarily around m/z 100, with no significant contamination observed above m/z 200. The contamination appeared to have little or no effect on electrospray sensitivity above m/z 200, and thus should not present a problem for high mass electrospray work. However, in APCI, the presence of these contamination ions appeared to reduce the sensitivity of samples even above m/z 200. This may be due to the contamination ions reducing the number of available reagent ions.

**Oxygen in the Nitrogen**

The presence of oxygen in the nitrogen has not been observed to reduce the sensitivity on Quadrupole electrospray instruments, in either electrospray or APCI. A purity of 95% or greater is probably adequate in this respect.
2-3. Sample Delivery Requirements:

1. *A solvent delivery system capable of delivering steady, stable flows suitable for the technique. These are ~1 - 5µl/minute for pure electrospray, ~10 - 500 µl/minute for pneumatically assisted and megafow electrospray, and ~1 ml/minute for APCI. Some guidelines for selection of pumps are given in Section 8, while the minimum requirements for installation are given below:

   **~1 to 50 µl/minute:**
   A low pressure syringe pump such as the Harvard 22 pump (Harvard Apparatus Inc., 22 Pleasant St., South Natick, Mass., 01760, USA) is ideal. Select a suitable gas - tight syringe (Hamilton and SGE both market ranges of gas - tight syringes) for the required flow rate, e.g. a 50 µl syringe for 1 µl/min, or 250 µl for 10 µl/min, or 1000 µl for 50 µl/min.

   **~0.2 to 1 ml/minute:**
   Most good - quality, well - maintained reciprocating HPLC pumps are suitable, particularly if they incorporate some form of flow rate compensation. If the pump produces significant pressure pulses, it may not be possible to complete the installation, or subsequent sample analyses, satisfactorily.

2. *An HPLC injector (and appropriate injection syringes) with a suitable loop size for completing the APCI specifications; usually a 10 or 20 µl loop. The electrospray specifications do not normally require the use of an injector if a syringe pump is available, but if instead a reciprocating pump is to be used then a large (0.5 or 1 ml) loop can be advantageous.

2-4. Solvent And Sample Requirements:

1. The standard samples will normally be supplied by the Micromass engineer during the installation. For ESI, these include gramicidin S (this can be substituted with leucine enkephalin, since gramicidin S is becoming hard to obtain), hen egg lysozyme and raffinose. No other mass calibrants are required to achieve the standard specifications. Caffeine is used to evaluate the positive ion APCI performance, along with PEG-200 for SIR calibration. Since these samples can be used as benchmarks of the interface performance after installation, it is recommended that the customer also obtains supplies of them.
2. * The bulk solvents are as follows.

**ESI solvents:-**
1:1 water : methanol + 1% (v/v) acetic acid for positive ionisation and 1:1 water : acetonitrile either without additives, or optionally with 0.1% (v/v) ammonium hydroxide, for negative ionisation.

**APCI solvent:-**
1:1 water : acetonitrile, without additives.

3. * To assist in set-up of the APCI accessory, a continuous infusion (via the LC pump solvent reservoir) of a solution of 0.005% (v/v) of 3-picoline in water : acetonitrile may be extremely useful. (Pyridine is an alternative, but it is more hazardous). Since these compounds should not be carried on aircraft, they should also be provided by the customer.

* - All of these items are to be supplied by the customer.
Section 3

Safety Aspects
Safety Aspects

The electrospray and APCI interfaces present a number of potential hazards, as listed below. It is important that these are understood by all potential operators of the instrument.

If there is any doubt whatsoever concerning the safety of any procedure or operation, then contact the local Micromass representative for advice.

1. High voltages. Establish safe working practices when using the electrospray interface. Do not attempt any maintenance on the interface while the instrument is in 'operate'. Ensure that the electrospray probe microswitch is functioning correctly. Always double-check that the high voltages and RF supply are really off before commencing any maintenance work.

2. Be aware that several of the high voltage supplies are linked together (through resistor chains or through proximity) within the API housing. Do not try to fault-find unless competent to do so.

3. Since much of the API interface is constructed of plastic, it is important to ensure that all ground (earth) connections are satisfactory. In particular, the earth connection to the electrospray probe injector bracket, or to the APCI nebuliser, must always be fitted.

4. Be wary of working on the solvent delivery system when the instrument is in 'operate', because of the conducting properties of the solvents typically in use. The solvent delivery system should be adequately grounded (the syringe carriage on a Harvard 22 pump is insulating - the syringe will charge up!).

5. Clean up any flammable solvent spills promptly: avoid risk of fire caused by sparks (sparks are unlikely, but may occur if the API interface is misused).

6. Be aware of the biological and chemical hazards presented by the samples/solvents used. Wear gloves when carrying out certain maintenance operations. Ensure that the waste gas is safely disposed of.

7. The external surfaces of the APCI nebuliser and chamber may become very hot (particularly in the event of a gas supply failure). Allow sufficient time for these components to cool before carrying out any maintenance operations.

8. The gas lines are at high pressures (up to 7 bar) and it is important that all of the connections are properly made, and that the tubing is in good condition. Ensure that the gas supply is shut off at source and that the lines have been depressurised before any maintenance is carried out, to avoid the danger of disconnected lines lashing around.
9. The ESI and APCI interfaces have NOT been designed to operate with gas flows of pure oxygen. If oxygen is believed to be required to suppress a corona discharge from the ESI needle, then compressed air or SF₆ should be used instead. Similarly, if oxygen is required as an APCI gas, then air should be used.

**Environmental Requirements.**

Altitude up to 2000M

Temperature 5 - 40°C

Maximum relative humidity 80% for temperatures up to 31°C decreasing linearly to 50% at 40°C.

Mains fluctuation to be within the range 360V to 460V line to line.

Mains supply transient overvoltages according to installation category II of IEC 644.

Pollution degree 1 in accordance with IEC 664.

**Ventilation Requirement**

Suitable exhaust lines should always be fitted to the rotary pump in the bottom of the electronics trolley and to the bath gas outlet when the system is in use.

**Lifting Requirements**

If it is required to lift the electrospray electronics trolley, it should be lifted by at least four people one supporting each bottom corner.

**Electrical Information**

Fuse ratings MA3485 electrospray distribution unit.

FS1: Fuse 20 x 5mm 5A (T) HRC ceramic
FS2: Fuse 20 x 5mm 6.3A (T) HRC ceramic
FS3: Fuse 20 x 5mm 500mA (T) HRC ceramic.

**Disposal**

**WARNING:**

Do not incinerate electronic assemblies. Emission of noxious fumes may occur and metal cased capacitors may explode due to build up of internal pressure.

Oil from the vacuum pumps should be drained and disposed of appropriately.
Section 4

Installation And Operation Of The Electrospray Interface
Installation And Operation Of The Electrospray Interface

This chapter lists all of the procedures required to install and operate the API interface in Electrospray mode for the first time. It will not be necessary to repeat all of these procedures during subsequent uses of the interface on the same instrument.

4-1. Removal Of The Existing Interface

The electrospray interface is ready - assembled on its own source housing front flange, so to fit electrospray onto the AutoSpec family of instruments it is necessary to remove the existing instrument front flange, lock assembly and ball valve actuator handle. The differences between the various versions of instrument housing are detailed below. If there is any doubt about the API or instrument sensitivity or performance, it is wise to establish that the basic instrument is satisfactory, using EI or LSIMS, prior to fitting the API interface.

4-1-1. ‘X’ Series Instruments With Stainless Steel Housing

Slide off the standard source surround cover. Vent the source. Isolate the inlet rotary pump, and remove the ball valve actuator assembly. Blank off the port using a blanking flange with a flat on one side (use an O-ring from the ball valve actuator assy.). Blank off the 1/4" pumping line using the 1/4" plug. Refer to Fig. 4-1-A.

FIGURE 4-1-A FRONT LOCK REMOVAL FROM EARLY ‘X’ INSTRUMENTS WITH STAINLESS STEEL HOUSING, AND PREPARATION FOR ELECTROSPRAY
Disconnect the 1/4” line to the front vacuum lock at a convenient place, and blank it off using the cap. Remove the vacuum lock and guide assembly by undoing the two screws that attach the guide rod assembly to the housing. Remove the ball valve assembly from the housing by undoing the 4 hexagonal head screws.

4-1-2. ‘X’ And ‘S’ Series Instruments With Aluminium Housing

Slide off the standard source surround cover. Vent the source. Isolate the inlet rotary pump, and remove the ball valve actuator assembly. Blank off the port using a blanking flange (use an O-ring from the ball valve actuator assy.). Blank off the 1/4” pumping line using the 1/4” plug. Refer to Fig. 4-1-B.

Disconnect the 1/4” line to the front vacuum lock at a convenient place, and blank it off using the cap. Remove the front flange assembly complete, by undoing the 4 hexagonal head screws. Retain the two stepped dowels - they are needed for correct alignment of the API housing.

FIGURE 4-1-B FRONT LOCK REMOVAL FROM LATE ‘X’ AND ALL ‘S’ INSTRUMENTS HAVING ALUMINIUM HOUSING, AND PREPARATION FOR ELECTROSPrAY
FIGURE 4-1-C FRONT LOCK REMOVAL FROM 'V' AND 'M' SERIES INSTRUMENTS, AND PREPARATION FOR ELECTROSPRAY

- Dimension A should be 48 mm for compatibility with the Mk.3/Mk.4 cradles. Some 'V' series instruments may be fitted with a lid in which A is 78 mm - this should be replaced with M802251CD1 issue 3.

- Housing Lid

- Standard Left Cover (compatible with API)

- Standard right cover (Replace with API cover)

- Fit the blanking flange using M4 x 16 bolts

- Disconnect here and blank off

- Stepped dowels

- Screws, M8 x 25

- Disconnect here and blank off
4-1.3. ‘V’ and ‘M’ Series Instruments

Slide off the large and small sections of the source surround cover. Vent the source. Isolate the inlet rotary pump, and remove the ball valve actuator assembly. Blank off the port using a blanking flange (use an O-ring from the ball valve actuator assy.). Blank off the 1/4” pumping line using the 1/4” plug. Refer to Fig. 4-1-C.

Disconnect the 1/4” line to the front vacuum lock at a convenient place, and blank it off using the 1/4” cap. Remove the front flange assembly complete, by undoing the 4 hexagonal head screws. Retain the two stepped dowels - they are needed for correct alignment of the API housing. The large section of the source surround cover can now be refitted.

4-2. Mounting The Electrospray Interface

Before commencing, remove any pumping lines (in particular the first stage pumping tube) and electrical connections from the API housing - these trailing components can foul on the instrument, etc., making assembly difficult. Remove the ESI probe, or the APCI chamber, from the assembly. Remove the protective cap over the transfer lens, insert the two stepped dowels into the top holes in the main API flange, and offer up the assembly to the instrument housing (see below for specific details regarding the various instrument types).

This procedure can be carried out by one operator, with care and practise, but if in doubt get someone to help! Tip - when the dowels have located, use your body weight to hold it in place while the top two bolts attaching the API interface to the instrument housing are fitted. This is why the ESI probe should first be removed.

4-2-1.‘X’ Series Instruments With Stainless Steel Housing

This combination requires a special clamp flange assembly; refer to Fig. 4-2- A. Raise the two clamp bars so that they are vertical, and finger tighten the screws until these bars remain in this position. Offer up the assembly to the instrument housing, and when it is in position swing the clamp bars down so that they engage behind the lip of the instrument front flange. The interface is now held in place sufficiently well so as to be able to tighten the API flange at leisure.

Fit the other bolts that secure the clamp bars to the API flange, and tighten the screws to pull the flanges closely together. Fit the electrospray cradle in the source housing, referring to Fig. 4-2-B or Fig. 4-2-C for instruments having Mk. 1 or Mk. 2 optics respectively. Note that two electrical connections to the electrospray transfer lenses are required, as illustrated on Fig. 4-2-D for the Mk. 2 optics. The earth connection can be attached to any of the grounded lenses, as indicated. The steel collar has the function of pulling the lens stacks into a reasonable alignment. However, a discrepancy of even 1 - 2 mm at this point is not a problem, since the instrument lenses are capable of steering the beam to the entrance slit.
1. Lock the clamp bars vertically, by hand-tightening the screws.

2. Swing the clamp bars down so that they engage on the instrument flange.

3. Fit the additional bolts and tighten as necessary to pull the flanges together.
FIGURE 4-2-B. SIDE VIEW OF ELECTROSPRAY CRADLE, MK 1, FOR EARLY 'X' INSTRUMENTS HAVING STEEL HOUSINGS AND MK 1 OPTICS

This face should clear the Electrospray Transfer Lens Stack by between 1 and 3mm.

Numbers indicate spacer lengths, * - wavy washers may be added here to provide the correct overall dimensions.

FIGURE 4-2-C. SIDE VIEW OF ELECTROSPRAY CRADLE, MK3, 'V' AND 'M' SERIES INSTRUMENTS (AND EARLY INSTRUMENTS HAVING STEEL HOUSINGS AND MK 2 OPTICS)

Two pairs of Cut-away Lenses

This face should clear the Electrospray Lens Stack by between 1 and 3mm.

Numbers indicate spacer lengths, * - the 6mm spacer may be altered to provide the correct overall dimensions.

Whole assembly: M802740DC1 (Not including ring)

WIRING

<table>
<thead>
<tr>
<th>Source</th>
<th>Destination</th>
</tr>
</thead>
<tbody>
<tr>
<td>spring contact L1</td>
<td>Beam Centre (right)</td>
</tr>
<tr>
<td>spring contact L2</td>
<td>Beam Centre (left)</td>
</tr>
<tr>
<td>spring contact L5</td>
<td>Focus 1 (both plates)</td>
</tr>
<tr>
<td>spring contact L8</td>
<td>V_vcc: flying lead to skimmer contact</td>
</tr>
<tr>
<td>end of cradle</td>
<td>Ground; flying lead to exponential lens</td>
</tr>
</tbody>
</table>

Source: spring contact L1, spring contact L2, spring contact L5, spring contact L8, end of cradle

Destination: Beam Centre (right), Beam Centre (left), Focus 1 (both plates), V_vcc: flying lead to skimmer contact, Ground; flying lead to exponential lens

Wiring:
- spring contact L1: Beam Centre (right)
- spring contact L2: Beam Centre (left)
- spring contact L5: Focus 1 (both plates)
- spring contact L8: V_vcc: flying lead to skimmer contact
- end of cradle: Ground; flying lead to exponential lens
FIGURE 4-2-D VIEW OF TRANSFER LENS FROM SIDE, SHOWING ELECTRICAL CONNECTIONS

FIGURE 4-2-E. OFFERING UP THE API FLAT FLANGE ASSEMBLY TO THE HOUSING OF ‘X’ AND ‘S’ SERIES INSTRUMENTS WITH ALUMINIUM HOUSINGS
4-2-2. ‘X’ And ‘S’ Series Instruments With Aluminium Housing

The assembly is straightforward - refer to Fig. 4-2-E. The same M8 x 20 bolts that secured the source lock are also used to attach the API flange.

Fit the electrospray cradle in the source housing, referring to Fig. 4-2-F. Note that two electrical connections to the electrospray transfer lenses are required, as illustrated on Fig. 4-2-D. The earth connection can be attached to any of the grounded lenses, as indicated. The steel collar has the function of pulling the lens stacks into a reasonable alignment. However, a discrepancy of even 1 - 2 mm at this point is not a problem, since the instrument lenses are capable of steering the beam to the entrance slit.

4-2-3. ‘V’ And ‘M’ Series Instruments

The assembly is straightforward - refer to Fig. 4-2-G. Note that longer bolts, M8 x 35, are required to attach the API flange, because of the step in the flange.

Occasionally it will be found that the API flange fouls on the instrument lid; if this occurs then move the lid back towards the analyser.

Fit the electrospray cradle in the source housing, referring to Fig. 4-2-C (early design, having maximum sensitivity) or Fig. 4-2-H (a later design having a gas cell optimised for ESI and APCI). Note that two electrical connections to the electrospray transfer lenses are required, as illustrated on Fig. 4-2-D. The earth connection can be attached to any of the grounded lenses, as indicated. The steel collar has the function of pulling the lens stacks into a reasonable alignment. However, a discrepancy of even 1 - 2 mm at this point is not a problem, since the instrument lenses are capable of steering the beam to the entrance slit.

---

**FIGURE 4-2-F SIDE VIEW OF ELECTROSPRAY CRADLE, MK2, ‘X’ SERIES WITH ALUMINIUM HOUSINGS, AND ALL ‘S’ SERIES INSTRUMENTS**
FIGURE 4-2-G OFFERING UP THE API FLAT FLANGE ASSEMBLY TO THE HOUSING OF ‘V’ AND ‘M’ SERIES INSTRUMENTS

FIGURE 4-2-H. MK4 CRADLE, WITH INTEGRAL HIGH PERFORMANCE GAS CELL (OUTSIDE DIMENSIONS ARE IDENTICAL TO THE MK3 CRADLE).
4-3. Vacuum, Electrical And Gas Connections

All early electrospray systems (and all retrofits) are supplied with the API electronics and the first-stage E1M18 rotary pump mounted in a trolley. There are only minor differences between the kits for ‘X’, ‘S’, ‘V’ and ‘M’ series instruments.

4-3-1. Vacuum Connections

Fit any probe in the instrument side lock, as these vacuum lines are required to evacuate the instrument source housing. The turbo pump backing line will have been tee’d into the existing inlets 1 rotary pump line during initial installation by the Micromass engineer; see Fig. 4-3-A. This line can be permanently left in place when the electrospray interface is subsequently removed. An E2M18 rotary pump will have been fitted in place of the original E2M8 pump supplied with early instruments (all ‘M’ Series instruments will already be supplied with the larger pump, which is essential with the new API interface design).
FIGURE 4-3-B CONNECTION OF THE TURBO BACKING LINE TO THE INLETS 2 POSITION OF THE MANIFOLD ON A 'V' OR 'M' SERIES INSTRUMENT

Pirani 2 wiring:
Connect inlets 2 pirani into the vacuum control unit as follows:
- Connector BV13
  - Pin 3: red
  - Pin 6: green
  - Pin 12: blue
Then reposition Link 1 and set the atmosphere and vacuum levels.

FIGURE 4-3-C POSITIONING OF THE TURBO BACKING LINE ON 'V' AND 'M' SERIES INSTRUMENTS

Adjust the angle of the turbo pump so that the knob passes easily through the hole in the API source surround cover.
**Important:** if it is planned to use the instrument to carry out MS-MS studies etc. using the various gas cells, then the transient gas loads on the turbo backing pump, i.e. Inlets 1, when the cells are pumped out may be inconvenient to handle. The method involves temporarily shutting off the Speedivalve below the turbo pump until the Inlets 1 pressure falls. An alternative solution involves fitting a small pump on the Inlets 1 lines, while the large pump for backing the turbo is fitted on Inlets 2 (or vice versa). Please consult the Factory or your Micromass Agent for details. Note that the pumping line backing the turbo must incorporate a Pirani gauge (it is not acceptable to operate API without a pressure gauge), and that the length of the line must be kept to a maximum of ~3 metres (Fig. 4-3-B).

Connect the turbo backing line to the underneath of the pump using a KF-16 clamp and centring ring, ensuring that the valve extension knob (if present) will pass through the hole in the small section of the source surround cover, as shown in Fig. 4-3-C.

Connect the black plastic fitting on the first stage pumping tube assembly to the side of the electrospray housing using the six M5 x 20 screws provided.

Ensure that the wire attached to the resistor chain protrudes into the electrospray housing; connect the spade connector to the tag on the skimmer. If a skimmer lens is present, make sure that its feedthrough is uppermost, and connect the wire to the pin on the skimmer lens (make sure that the feedthrough end is fitted securely on the feedthrough pin). Tip - either remove the atmospheric chamber to fit these wires, or reach in through the pumping port).

The other end of the first stage pumping line should be connected to the E1M18 rotary pump mounted on the electrospray trolley, using the KF - 25 fittings provided, see Fig. 4-3-D. Connect the exhaust line from the E1M18 pump to the existing instrument exhaust system.

**To begin pumping down at this stage, do the following:**

Fit the atmospheric pressure chamber after making the skimmer and skimmer lens connections (refer ahead to Figure 4-3-H, section 4-3-4). Connect the API trolley power cable to the instrument power outlet (refer ahead to Figure 4-3-E, section 4-3-2), and begin pumping down. Suggested sequence: Begin by evacuating the instrument housing, then open the KF-16 valve below the turbo pump, then turn on the rotary pump beneath the trolley and open the KF-25 valve above the pump. When the inlets 1 backing pressure has fallen sufficiently low (~0.2 mbar; after ~5 minutes’ pumping), open the source butterfly valve, and turn the turbo pump on using the mains switch. Select full speed from the electrospray vacuum menu (refer ahead to Fig. 4-4-E, section 4-4). It may take up to 24 hours for the vacuum pressures to reach satisfactory levels the first time the interface is fitted.
FIGURE 4-3-D. THE FIRST-STAGE PUMPING LINE, SHOWN FOR THE SKIMMER LENS VERSION (GREY TUBE). THE SKIMMER-ONLY VERSION (CLEAR TUBE) IS SIMILAR.

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Note that in subsequent venting cycles, for instance during routine cleaning, the rotary pump beneath the trolley should be left running, and the KF-25 valve should be used to isolate the pump as appropriate.

4-3-2. Electrical & Optical Connections To The API Trolley

Connect the trolley power supply, the electrical link, and the optic links, as shown in Fig. 4-3-E. The earth wire must be connected! Route the optic cable in such a way as to avoid damaging it. Keep the short optic link and the 15 way D-type connector (the high voltage interlock bridges pins 3 & 5) - these must be replaced on the HPLC panel when the API interface is removed.

The power supplied to the instrument HPLC panel is routed through the contact breakers in the main distribution unit - it may be necessary to reset the breaker labelled ‘HPLC’.

The electrical sockets on the rear of the trolley may be supplied either from the instrument 240 volt circuits, or from a separate supply provided by the customer (e.g. 110 V). The wiring on ‘X’, ‘S’ and ‘V’ instruments includes a british 13A plug located at the back of the instrument. Remove this plug and connect as appropriate. On the ‘M’ series instruments, this cable is routed through the RY05 - RY06 loop. Simply plug an external supply in to RY06. If the power supply has been changed, then please label the electrical sockets on the trolley appropriately.

FIGURE 4-3-E. CONNECTIONS BETWEEN THE API TROLLEY AND THE INSTRUMENT HPLC PANEL.

To change the mains supply to the trolley sockets, plug the appropriate supply into the mains link (06), or replace and reconnect the 13A British plug, and label the trolley sockets as appropriate.
4-3-3. Gas Connections

An N₂ supply at a pressure of ~7 bar is required for correct operation of nebuliser assisted electrospray and of APCI (but a pressure as low as ~2 bar can be used if only pure electrospray without nebuliser assistance is to be carried out).

Make the gas connections as shown in Fig. 4-3-F. Note that, depending on the types of samples to be run, the waste gas from the source should be considered as ‘hazardous’, and should be disposed of appropriately. The red waste line should not be connected to the existing rotary pump exhaust lines, since it is likely that the oil vapours would blow back into the electrospray housing and also vent to the laboratory atmosphere when the electrospray probe is not mounted in the housing. The auxiliary gas line supplies only the nebuliser (not the bath gas), and may be selected using the control on the front of the trolley. Air, SF₆ or other gases may be used, and are reported by some researchers to inhibit corona discharges. Note however that pure oxygen should never be used, since the tubing and fittings have not been designed for this purpose.
FIGURE 4-3-G. ELECTRICAL CONNECTIONS TO THE ELECTROSpray PROBE AND API HOUSING

- RF power supply and control signals (ES03)
- RF PCB cover
- RF HV offset and ring electrode (and the skimmer lens cable if the lens is not fitted) (ES04)
- Sampling cone, counter electrode and heater (ES02)
- ESI probe
- Earth connection to injector bracket (ES05)
- Anaconda from trolley
- Skimmer lens (if fitted) (ES06, or unlabelled)
- Turbo pump cable (grey, unlabelled, not in anaconda)

Note: The earth link between one of the turbo brackets and the flange MUST be fitted.

Note: Keep all wires clear of this hose to avoid discharging from the resistor chain.
FIGURE 4-3-H. INTERNAL CONNECTIONS TO THE SKIMMER AND SKIMMER LENS (IF FITTED)
Slacken / Tighten ONLY this Thumbscrew when removing the whole Atmospheric Chamber, if a beam has already been obtained.

Orientate the Atmospheric Pressure Chamber so that these two holes in the gas baffle are vertical.

Remove these three screws to gain access to the Counter Electrode

Orientate the plastic body in the housing so that the sampling cone feed through and waste gas port are at the bottom

FIGURE 4-3j. ELECTRICAL AND GAS CONNECTIONS TO THE ATMOSPHERIC PRESSURE CHAMBER

Counter Electrode/Heater (wires with 1 mm cambion pins; connect either way round)

Sampling Cone (wire with 1.6 mm quickmate pins; connect to the central pin in the socket)

Blue Tube (Inlet)

Red Tube (Waste)

Atmospheric Pressure Chamber, shown off-axis for clarity

Socket

TOP

Pepper Pot

Thumbscrews

Section 4 Installation And Operation Of The Electrospray Interface
4-3-4. Electrical Connections To The API Housing

Hook the anaconda from the trolley on to the bracket beneath the electrospray housing. Make the electrical connections as shown in Fig. 4-3-G. Note that all of these cables, with the exception of the skimmer lens supply, carry a source high voltage interlock circuit. Thus, if any one of ES01, ES02, ES03 or ES04 is disconnected, or if the electrospray probe microswitch is open, the source and trolley high voltage supplies will remain off (even though the Operate buttons on the data system and on the instrument remain green).

Care must be taken to ensure that the high voltage skimmer lens cable, ES06, is fitted at all times, since this cable carries no interlock protection. Note that the turbo pump must be earthed to the instrument housing. Failure to ground the pump may result in the EPROM in the turbo controller being wiped clean by any major high voltage discharge (in which case the pump will stop).

Figure 4-3-H shows the internal connections to the skimmer and the skimmer lens. It is possible to make both connections via the pumping port without removing the atmospheric chamber, although it is usually easier to gain access by removing the chamber, as shown. Failure to connect either of these two wires will most likely result in reduced signal stability (but not complete loss of signal).

Refer to Fig. 4-3-J for connection of the internal sampling cone and counter electrode heater connections. The two wires for the heater can be connected either way around. Because the pin sizes are different, it is not possible to accidentally cross-connect the heater supply to the sampling cone.

Note the orientation of the atmospheric pressure chamber in the housing, Fig. 4-3-J. It is positioned such that the red exhaust tube is at the bottom of the assembly, so that in high solvent flow applications any solvents that condense in the chamber will be blown out. (In contrast, in the old electrospray interface the exhaust line was positioned at the left hand side).
4-3-5. Vacuum Trip Levels & Turbo Interlock

When the API interface is fully pumped down, the line backing the turbo pump will be at around 0.1 - 0.2 mbar, and the source ionisation gauge will register around $3 \times 10^{-6}$ mbar. During initial pumping down, the turbo backing pressure will be higher, and this may mean that the Inlets 1 trip level must be adjusted, as follows.

**Brief instructions for early instruments with a manual, analog vacuum control unit (M640):**

**WARNING - The Vacuum Unit Contains Dangerous High Voltages. Only A Qualified Engineer Should Make T his Adjustment.** Swing the unit out, and remove the lid. Set the internal switches to ‘set trip level’. Adjust the Inlets 1 trip level pot (RV6) at the bottom of the unit so that the meter reads 0.3 mbar. Reset the switches to the ‘read’ position, and refit the unit.

**Brief instructions for instruments with an integrated vacuum control unit (MA3515):**

Adjust the Inlets 1 trip level to 0.5 mbar on the data system. (If the maximum allowed is 0.2 mbar, then a more recent vacuum control microprocessor, possibly with a new version of software, may be required - contact the Factory for advice).

The Inlets 1 pressure must drop below the trip level before the source butterfly valve can be opened. If this presents a problem, then the KF-16 speedivalve below the turbo can be temporarily closed while the butterfly valve is opened. The mains power for the turbo pump is interlocked such that pump can only be switched on when the butterfly valve is open.

4-3-6. Turbo Pump Speed, Soft Start And Water Cooling

The Varian turbo pump controllers are supplied with two types of EPROM. With the original type, the pumps are accelerated from stationary to full speed in a matter of minutes, regardless of the condition of the pump bearings.

The current type of EPROM supplied includes a ‘soft-start’ feature, which is designed to increment the speed at a rate dependent on a sensor in the bearing. The pump will take approximately 45 minutes to reach full speed. The ‘soft start’ can be deselected using a hand held programmer if it is required.

It is important that the turbo speed request, (Figure 4-4-A, section 4-4) is set to ‘Full Speed’, and that the API high voltages are never switched on when the speed readback reports that the pump is running at 90% or less. Ideally the interface should only be switched to Operate when the speed is above 95%. (The only exception to this rule is when the API interface is present, but another ionisation technique is being used. In this case no voltages are applied to the API system, thus discharges cannot occur).
FIGURE 4-4-A. DECLARING THE PRESENCE (OR ABSENCE) OF THE ELECTROSPRAY UNIT, IN THIS CASE WITHOUT A SKIMMER LENS (V3.1 SOFTWARE)

FIGURE 4-4-B. SETTING UP THE CORRECT ACCELERATING VOLTAGE IN THE INSTRUMENT MENUS (V3.1 SOFTWARE)
There are a number of different water cooling fittings that may be present on the turbo pump. The most satisfactory is a brass tube which passes right through the body of the pump. Other types of fittings can fur up, depending on the quality of the cooling water. It is important to check the water flow occasionally, and to remove any deposits from the system. The pump will automatically shut down if the temperature of the bearing exceeds 63° C.

4-4. The Data System

4-4-1. Configuring & Re-booting

When the API trolley has been connected to the HPLC panel and powered up, the presence of the trolley must be declared in the Instrument Configuration menu as shown in Fig. 4-4-A. Select 'ES/API Unit', and select 'Skimmer Lens' if one is present. Save these parameters, then leave OPUS and re-boot the SIOS, by typing SIOSLOAD RB at the $ prompt.

The message ‘ES unit (version xxx) present on optic loop’ should be seen when OPUS is restarted. If this is not seen, then check that the optical connections are satisfactory, by observing the flickering red light that is transmitted through the optic circuit. Very occasionally, the microprocessor in the electrospray trolley will fail to reset itself correctly, and in this case it must be manually re-booted by unplugging the power supply cable underneath the trolley (cable EA06, ‘ESP Control’) for about 2 minutes (refer to section 6-7 for detailed instructions).

4-4-2. Instrument Setup - Accelerating Voltage

The ESI/APCI interface must be operated at an accelerating voltage of 4 kV, for three reasons. Firstly, potentially damaging high voltage discharges may occur if higher voltages are selected. Secondly, the electrospray needle and APCI corona discharge high voltage supplies have a maximum rated output of 8 kV, and these components need to be around 4 kV above accelerating voltage. Thirdly, the hexapole RF supply is set up to track with a magnet reference signal, which itself depends on the accelerating voltage.

It is possible to decrease the accelerating voltage to 2 or 3 kV in order to extend the m/z range of the instrument, but this will compromise ion transmission through the hexapole below m/z ~500.

Note that the value for the accelerating voltage must be entered in two places: both in the first ESA box on the instrument picture, and in the voltage limits, as shown in Fig. 4-4-B. When data acquisition is started, it is the voltage value in the Limits menu that is loaded. If this is left at 8000, then damage may occur.

It is useful to save the instrument parameters, and then they can be recalled at a later date (or after a crash) without needing to set up the accelerating voltage again (Fig. 4-4-B).
**FIGURE 4-4-C. THE ELECTROSPRAY TUNING MENU; IN THIS CASE A SKIMMER LENS IS PRESENT (V3.1 SOFTWARE).**
4-4-3. Recall Of Other Instrument Menus - Warning

No harm can result from browsing through other Instrument files in which EI, LSIMS, etc. sources are called up, as long as the instrument is in Standby mode at the time.

Be aware, however, that whenever a particular source is selected in the instrument menu, then the voltages and functions relating to that source are activated and others are turned off. If, for instance, a LSIMS source is selected when ESI is fitted and the high voltages are on, the data system will immediately turn off all of the trolley high voltages (since ESI is now not required), but will leave the accelerating voltage on. In this circumstance, because the accelerating voltage supply is connected to the skimmer, in close proximity to the now-grounded components, some damage may result.

4-4-4. Electrospray Tuning Menu & Suggested Settings

Figure 4-4-C shows the electrospray tuning menu (in this case a skimmer lens is present). Refer also to Fig. 1-1 for an overall schematic.

The voltage readbacks are all with respect to ground. The values reported are not very precise, but nonetheless they are diagnostic of certain fault conditions. The individual components of the menu are detailed below.

**Needle Voltage.**

The needle voltage slider is set up so that 0% corresponds to 2.5 or 3.0 kV above accelerating voltage (for positive ionisation), i.e. 6500 to 7000 V with respect to ground. Changing the slider from 0 to 100% will increase the needle voltage by up to 2.5kV, i.e. a maximum of 9000 to 9500 V with respect to ground. The upper limit depends on the output characteristic of the particular high voltage supply used; in practice it is only necessary to achieve ~8500 V.

The needle voltage required when using nebuliser-assisted electrospray with the all-steel needle assembly and a chicane (pepper pot) counter electrode at medium flow rates is typically not very critical; set the slider to about 30% in the first instance. In contrast, at ~1 to 5 µl/minute with unassisted electrospray and using the triple layer fused silica needle assembly, the needle voltage required is extremely critical.

**Counter Electrode.**

The counter electrode is set up to be around 1000 V above the sampling cone, or around 5000 V with respect to ground. (In some cases the optimum voltage difference appears to be around 500 V). It is not adjustable from the data system (no tuning is required).
**Sampling Cone & Skimmer Lens.**

Two types of control are available depending on the modification level of the electronics. On earlier versions of the electrospray electronics the sampling cone and skimmer lens voltages are completely independent of each other. On later versions the skimmer lens linked to the sampling cone voltage so a fixed offset between sampling cone and skimmer lens can be set which remains the same when the sampling cone voltage is altered. This feature allows stepping or ramping of the sampling cone voltage to be performed during acquisition. The two modes of operation are described below.

**Sampling Cone & Skimmer Lens with Independant Control.**

The sampling cone and the skimmer lens sliders are both set up so that 0% corresponds exactly with the accelerating voltage. The range of both sliders is 250 V, so that the voltages on these components can be varied between 4000 and 4250 V with respect to ground. Typically, the optimum voltage difference between these two components is 10 V, with the skimmer lens higher than the sampling cone (for positive ionisation).

The sampling cone/skimmer lens voltages are very compound and mass dependent. Typically, for optimal transmission of low mass molecular ions such as doubly charged gramicidin S at m/z 571 or singly charged leucine enkephalin at m/z 556, slider values of ~20% (i.e. ~50 volts above the skimmer) will be required. In order to fragment such compounds, or to transmit the multiply-charged ions from proteins, slider values of ~40 to 80% may be required.

**Sampling Cone & Skimmer Lens with Linked Control.**

The sampling cone lens slider behaves exactly as described above, and when both the sampling cone and the skimmer lens sliders are set to 0% the voltage on both these components is set to accelerating voltage. However when the skimmer lens slider is increased the voltage on the skimmer lens increases from the voltage set on the sampling cone to a maximum of 20V above the voltage set on the sampling cone. When the sampling cone is varied from 4000 to 4250V the offset between the sampling cone and the skimmer lens remains constant.

Typically the skimmer lens optimises at about 10V above the sampling cone voltage ie 50% on the skimmer lens slider, once the optimum is determined this lens position rarely needs to be changed.
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**Skimmer.**

The skimmer is held at accelerating voltage (assumed here to be exactly 4000 V). It is not adjustable from the electrospray tuning menu, but is controlled using the ion energy slider or potentiometer (depending on the instrument type). The range of the control is set up to be +/- 1% of the optimal ion energy, i.e. an 80 V swing. On instruments with the manual pot box and oscilloscope (‘X’, ‘S’ and ‘V’ series), it is usually easier to find a positive ion electrospray beam with the ion energy pot turned a little to the left (giving a higher voltage output) compared to the optimum for other ionisation techniques. On ‘M’ series instruments with data system tuning, the slider should be set initially a little to the right, because the convention is reversed.

**Hexapole Offset.**

The hexapole offset (DC) voltage is held at about 10 V lower than the skimmer (for positive ions). It is not adjustable from the electrospray tuning menu, but instead is set up using the trim pots on the appropriate high voltage board in the trolley. It does not normally require any further manual adjustment once it has been set, because there is a sufficiently wide ion energy window to compensate for small drifts simply by tuning the ion energy.

There is no data system control or readback of the hexapole RF voltage.

**Ring Electrode.**

The ring electrode slider is set up so that 0% corresponds exactly with the accelerating voltage. The range of this slider is 100 V, and it swings downwards, so that 100% corresponds to a voltage of 3900 V with respect to ground. The optimal voltage for most compounds and under most conditions is ~10 V below the hexapole offset voltage, which corresponds to a slider value of ~20%, i.e. approximately 20 V below accelerating voltage, or 3980 V with respect to ground (for positive ions). Once set, the ring electrode slider rarely requires much adjustment.

**Bath Heater.**

This controls the temperature of the bath gas, counter electrode and sampling cone. It is a current-controlled circuit, without any readback system: 0% and 100% on the slider correspond to ambient temperature and approximately 100 °C respectively. The heater should normally be left on continuously at 80% on the slider, which corresponds to approximately 80 °C (it will take about 1 hour to reach the equilibrium temperature starting from cold). If high flow rates are to be used, for instance 200 µl/minute, then set the slider to 100%.
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Installation And Operation Of The Electrospray Interface

FIGURE 4-4-E. THE API VACUUM MENU (V3.1 SOFTWARE).

FIGURE 4-4-F. SIOS REQUESTS AND READBACKS TO VIEW THE TABLE, TYPE M 681700 <RETURN> AT THE ETH PROMPT IN THE MAIN MENU.
Sampling Cone Ramp

If this option is available, select ‘No Ramp’ in the first instance; ramping is only required for certain types of complex experiment, and is not necessary when tuning on individual ions.

Solvent Trip

This is the trip level of the source ionisation gauge. Leave it at a high value when operating in electrospray or APCI modes.

4-4-5. Programming The Sampling Cone

The sampling cone/skimmer lens combination can be programmed in a number of ways, for instance using different voltages in alternate scans, or by ramping or stepping the voltages within one scan. This enables a number of complex experiments to be performed (refer to the Data Processing Manual).

The programs required can be written in the OPAL language, or will become available as part of the OPUS package (full details not currently available).

4-4-6. API Vacuum Menu

Figure 4-4-E shows the API vacuum menu, used for setting the turbo pump speed and for resetting the pump/controller after an event causing shutdown (e.g. overheating of the pump bearing). The pump should always be operated at full speed, to keep the turbo pumped chamber at a pressure below the discharge range when ESI or APCI are fitted. Speed readbacks of less than 100% will normally indicate a pump problem (which may be accompanied by undue high-pitched noise).

4-4-7. Interface Diagnostics

The SIOSLOAD tables can provide valuable diagnostic information to assist in problem solving. Figure 4-4-F. Call up a new DEC window and type

SIOSLOAD <return>

(note no R or RB) at the dollar prompt. The main menu can be used to access status information on the source, vacuum, electrospray, beam control, etc, depending on the type of instrument.

The electrospray unit addresses be seen in detail by typing

M681700 <return>

at the ETH> prompt in the main menu. A value of 81 in the top left-hand corner of the table indicates that the electrospray unit is present on the optic loop. A value of 01 indicates that the unit was present at one time, but is not currently present on the loop. This is usually caused by a local microprocessor crash. A value of 00 implies that the unit was not available at the time that SIOS was booted. Control-Z can be typed at the ETH> prompt to either return to the main SIOSLOAD menu, or to exit to the $ prompt from the main menu.
FIGURE 4-5-A. THE VARIOUS NUTS AND FERRULES USED WITH THE ELECTROSPRAY PROBE FITTED WITH THE DOUBLE LAYER STEEL NEEDLE ASSEMBLY (ALL DIMENSIONS ARE APPROXIMATE)
4-5. Building Up The ESI Probe, With Double Layer Steel Needles

The electrospray probe is designed such that it can be built up for several different applications, for low flow ESI, high flow ESI, or capillary electrophoresis coupling using double or triple layer needle arrangements. The standard arrangement, utilising a double layer steel needle optimised for medium and high flow electrospray (~10 to ~500 µl/minute), is described below, since it is one of the simpler and more robust versions, which is therefore ideal for use during the installation and commissioning phases of operation. Certain of the other arrangements will be detailed in a later chapter.

4-5-1. Components Required

1. M805244AD1 Type 34 gauge needle, 250mm long; must be cut clean and square. Polish shiny, square and burr-free with 600 or 1200 grade abrasive paper if necessary, taking care not to close over or block the I.D.

2. M805245AD1, Diameter 1.6 x 0.25 mm blue PEEK tube 210.5 mm long; must be cut clean and square, and must have reference mark 20.5 mm from one end. The reference mark should be very small, so that the tube is not weakened.

3. Code 6070211, Valco union with 0.010" through hole to make connection to sample lines (Valco ZU1C).

4. Code 6436005, Diameter 1.6 x 0.125 mm red PEEK tubing to connect between the ESI probe and the solvent delivery system (length to suit the siting of the solvent delivery system; usually ~1 metre).

4-5-2. Assembly instructions

1. Understand the differences between the various types of nut and ferrule used with the ESI probe, Figure 4-5-A.

FIGURE 4-5-B. SCHEMATIC VERTICAL CROSS SECTION OF THE ELECTROSPRAY PROBE.
2. Poke the blue PEEK tube through the probe shaft, inserting it from the handle end towards the needle tip end, with the ref. mark nearest the tip, Figure 4-5-B. (Alternatively, it can be done the other way, if a torch is used to help guide the naturally curvy PEEK tube into the concealed hole).

![FIGURE 4-5-C.](image)

3. Add the Rheodyne short nut and then the Upchurch LiteTouch double ferrule to the needle tip end of the PEEK tube, and gently tighten it so that the tube is gripped and the Ref. mark on the tube aligns (+/- 0.5 mm) with the reference marks inscribed on the probe body, Figure 4-5-C.

4. Find a good eyeglass and inspect both ends of the Type 34 st. steel tube: they must be clean, square, burr-free, and round (not squashed or oval). Reject or clean up needles as appropriate. A high-quality finish is essential. (Tip: support the Type 34 needle in either a Type 24 or 21 needle while polishing or cleaning it).

5. Insert the Type 34 st. steel needle through the PEEK tube, from the handle end, Figure 4-5-C. It should slide through easily, so long as the LiteTouch ferrule at the front was not over tightened.

6. (If it jams at the ferrule, disconnect the union and pop apart the two halves of the ferrule using either a LiteTouch removal tool or a thin-bladed screwdriver. This should remove the compression, and the needle should slide through).

![FIGURE 4-5-D](image)

7. Fit a teflon ferrule and a Waters nut to the probe handle end of the PEEK tube (Valco or Rheodyne nuts or LiteTouch or steel ferrules are unsuitable here!) and gently tighten it onto the back of the probe body, Figure 4-5-D.
8. Slide a Rheodyne nut and the two halves of a LiteTouch ferrule over the PEEK tube.

9. Adjust the position of the Type 34 needle so that it is exactly flush with the end face of the PEEK tube, Figure 4-5-D,— a zero dead volume connection here is essential.

10. Fit the Valco 0.010” union, and tighten it firmly (making sure that the PEEK tube remains firmly butted up against the internal flange). Check that the Type 34 needle tube is now gripped firmly in this union. Judiciously tighten the fitting a little more, Figure 4-5-E.

11. Note: if the needle tubing is still free to move, it is possible that it may pass into or through the 0.010” hole in the union. To prevent this possibility, remove the union and repeat this procedure from step 9.

12. Now tighten the Waters nut onto the probe body, and tighten the Rheodyne nut at the probe tip end, Figure 4-5-E.

13. Inspect the tip of the Type 24 nebuliser needle using a good eyeglass. It must be clean, square and burr-free, and should be slightly radiused to reduce the possibility of corona discharge. Clean and shape it as necessary using fine abrasive paper.

14. Fit it to the front of the probe using a LiteTouch ferrule and a Rheodyne nut (these nuts have a good surface finish that is unlikely to support a corona discharge). The Type 34 needle should protrude by about 1 to 2 millimetres through the Type 24 needle, Figure 4-5-F.
Strong turbulence should be able to be felt about 6" from the probe tip; with experience the strength of the nebuliser can be judged fairly accurately using this method.

Method 1

Method 2

Electrospray probe held vertical, with needle tip at the liquid surface

Gas jet pushes down 15-20mm below the surface

FIGURE 4-5-G. TESTING THE STRENGTH OF THE NEBULISER. (NOTE: USE ONLY METHOD 2 IF THE SOLVENT OR SAMPLE FLOW IS ON, AND THERE IS ANY POSSIBILITY THAT THE COMPOUNDS ARE HAZARDOUS).

GOOD SPRAY.

Flat (dark) surface

ACCEPTABLE—Some of the spray is on axis

NOT ACCEPTABLE—Adjustment is required

NOTE: the spray cone is narrower using the nebuliser than it is without (ie, "pure" electrospray).

FIGURE 4-5-H. TESTING THE STRAIGHTNESS OF THE NEBULISED SPRAY.
15. Check that the needles are essentially on-axis by viewing the probe end-on. Bend them into position if necessary. It is helpful to check that the probe shaft is also straight at this stage. (The axial position of the Type 34/24 needles may need to be slightly adjusted at a later stage to give the maximum ion currents possible when using the pepperpot counter electrode assembly).

16. Reports suggest that the two needles should be exactly coaxial for optimum performance, so that the nebuliser gas flows symmetrically around the inner needle. This is best adjusted at a later stage.

17. Connect the sample line to the rear of the Valco union, Figure 4-5-F. The main requirement here is that the tube is insulating. The choice of line depends very much on the intended applications, but here are some suggestions. The red PEEK tube is the most robust, and is ideal for continuous infusion or large (20µl or more) loop injection work required during installation & testing, but is clearly not ideal for low volume injections at low flow rates because of the relatively large dead volume.

(a) red 0.005" PEEK tube.

(b) 50 or 75µm ID/150 µm OD fused silica in teflon sheath.

(c) 50, 75 or 100µm ID/375µm OD fused silica gripped with 0.020" PEEK.

(d) microbore PEEK tubing gripped with 0.020" PEEK.

18. Check the sample flow; use a commercial HPLC filter union between the pumps (if used) and the injector (if present) or the probe, to protect against blockages. If the needle does block, first try to clear the obstruction using high pressure from the HPLC pumps (do not try this with a low pressure syringe pump, otherwise the syringe may burst). Alternatively, try to clear it by ultrasonication or with a thin wire.

19. Check the nebuliser gas flow. With 5 to 6 bar on the regulator and the nebuliser flow valve fully open, the flow meter should register about 8-12 litres/hour. As a rough guide, a strong turbulence should be felt on the palm of one's hand when it is held ~20 cm in front of the probe tip. Alternatively, the gas jet should be strong enough to poke ~15 to 20 mm into a beaker of water - refer to Figure 4-5-G for details. Investigate for gas leaks at the Legris (4mm polyurethane tube) coupling's as well as the two 1.6 mm fittings at the probe tip.
20. With both the solvent flow on (at preferably 30 to 100 µl/minute) and the nebuliser gas flow on, check the appearance of the nebulised spray, Figure 4-5-H. (HT is off at this stage!). The gas jet should be essentially on-axis. Hold the probe tip 3 to 5 centimetres away from a dark shiny surface (such as black plastic or dark paint) to observe the position of the solvent spray (at flows of less than 10 ul/minute this can be hard to see). If the spray is veering to one side check the exact shape of the needle tips, and adjust the distance between them until the spray is improved.

21. The probe should now be ready to use.

4-5-3. Procedure To Replace The Type 34 Needle

1. Ascertain first that it is the needle that is blocked. The narrow-bore PEEK or teflon tubing that is often used for the solvent delivery lines can become sealed if the unions are over tightened. If a 1/16” through - bore union was used instead of the recommended 0.010” bore union (Figure 4-5-F, step 17), there is a good possibility that when the sample line is tightened in the fitting its end will seal the needle tube. The needle itself may possibly become blocked if concentrated samples or buffers were left in the system overnight.

2. Remove the nebuliser needle from the probe tip.

3. Remove the Valco 0.010” union from the blue PEEK tube that runs through the probe body.

4. Undo the Waters nut that holds the PEEK tube in the probe body, and undo the Rheodyne nut at the probe tip, but do not try to remove them from the tubing at this stage.
5. The blue PEEK tube can now be pulled back towards the probe handle to expose both of the LiteTouch ferrules, but the Type 34 needle will probably still be firmly gripped within the PEEK, Figure 4-5-J.

6. Both of the LiteTouch ferrules should be popped apart to release the compression on the Type 34 needle; the needle should slide out easily, and can then be replaced with a new one. All other components should be reusable.

7. Tip for popping the ferrules apart: use a LiteTouch ferrule removal tool (available from Upchurch in the USA or Anachem in the UK, part code LT-300) or a thin-bladed screwdriver, Figure 4-5-J. Because of the confined space and the flexibility of the PEEK tubing, it is helpful to push against a flat surface, such as a small spanner. Alternatively, gently grip the steel ring with pliers and hold it steady while applying a twisting force to the removal tool or screwdriver.

8. Proceed to install a new type 34 needle as described in the previous section, using the existing blue PEEK tube and fittings.
4-5-4. Nebuliser/Pepperpot Compatibility

Note that if the standard chicane (pepperpot) counter electrode is fitted, then it is imperative that the nebuliser is used at all times. If the nebuliser is not used, or is used with insufficient gas pressure, or becomes blocked, then ions will not be blown through the L-shaped passages. The annulus between the nebuliser needles can become occluded for instance by a build up of sample if the solution dribbles slowly through the probe when it is not in use.

If the nebuliser is not working and cannot be immediately repaired, then it is possible to completely remove both the front and the back halves of the pepperpot and to spray directly against the sampling cone in order to continue operation.

With the nebuliser working correctly, there will be almost no difference in sensitivity with and without the pepperpot. In contrast, in one case in which the nebuliser was not working, a 2000-fold sensitivity difference was measured with and without the pepperpot present.

A counter electrode having the earlier straight-hole design is also available as an option, which may be more suited to low flow (~1 µl/minute) applications (see Chapter 7). When changing between the two designs, the stop screw must also be changed to prevent the probe tip hitting the Pepperpot. The stop screws are M5 x 25 and M5 x 35 mm respectively.

4-6. High Voltage Setup (see Appendix A if required)

There are six separate high voltage supplies in the electrospray trolley, and in addition the instrument accelerating voltage supply energises the skimmer.

The high voltage supplies in the trolley will be set up by the engineer during the initial installation, following the procedure detailed in Appendix A. The setup is unlikely to drift with time, but if sensitivity decreases unexpectedly and no other cause can be identified, the setup may need to be checked or adjusted. The setup procedure is fairly straightforward, although since it involves direct measurement of high voltages it should only be carried out by competent personnel. If in any doubt whatever, then obtain the help of a service engineer.

Note that if one of the electrospray trolley high voltage power supplies fails and is exchanged for a new unit, then this will have to be set up according to Appendix A before use. If the instrument accelerating voltage supply is replaced, it is advisable to check the electrospray high voltage setup afterwards.
4-7. Hexapole RF Setup (see Appendix A if required)

The hexapole RF signal is produced by the boards mounted on top of the API interface. The RF amplitude is scanned with mass, and this scan function is controlled by the electrospray control pcb mounted in the trolley. The RF supply itself and the scan function will be set up by the engineer during the initial installation, following the procedures detailed in Appendix A. Refer to the Quick Guide, section 4-7-1 in Appendix A, for an overview of the hexapole lens.

The RF setup may be subject to slight long-term drift; if a significant drift has occurred then the most common symptom is a partial or complete loss of low mass sensitivity. It is therefore advisable to carry out simple checks of the performance at low mass at regular intervals (perhaps 3-4 monthly). Since the setup procedure is fairly complex, it is recommended that it be carried out by a trained engineer.

In the event of the top one of the two RF boards on the housing being exchanged, or the control pcb in the trolley being replaced, it will be necessary to carry out the full setup procedure.

4-8. Finding a Beam

Prior to installing electrospray for the first time, it is useful to check that the instrument is operating at full sensitivity at 8 kV in EI or LSIMS, in both positive and negative ionisation modes, and that it tunes to high resolution (20,000 RP) satisfactorily.

It is also helpful to check that operation is satisfactory at 4 kV accelerating voltage (the sensitivity should be half that at 8 kV). Make a note of the pot positions for 4 kV tuning (‘X’, ‘S’ and ‘V’ series instruments) or save the source and lens parameters (‘M’ series instruments) to give an initial set of values for electrospray. Also make a note of any discrepancy between the actual mass and the requested mass of ions around m/z 500 - 600 when tuning, to make it easier to locate the electrosprayed ions when looking for a beam. Alternatively, use the Hall probe readback to correctly position the magnet.
4-8-1. Positive Ions - Full Procedure To Find A Beam

1. Check that the vacuum pressures are acceptable, and that the turbo pump is running at full speed.

2. Check that the atmospheric pressure chamber is located centrally in the housing. This will provide the best starting point for finding a beam.

3. Check that the interface is ready to use.

4. Connect up a high purity nitrogen supply at ~7 bar (~100 psi, or ~700 mPa) to the gas inlet at the back of the trolley or the back of the instrument. Also connect the red waste tube to a suitable exhaust system (refer to Figure 4-3-F).

5. Connect the sample line to a solvent delivery pump capable of delivering ~10 to 50 µl/minute (e.g. a Harvard 22 pump with a 100 or 250 µl gas-tight syringe, or a good-quality reciprocating HPLC pump).

6. Make up a solvent system containing 1:1 water : methanol with 1% (v/v) acetic acid, and 10 ng/µl gramicidin S or 10 ng/µl leucine enkephalin (make up ~100 to 500 ml; both of these solutions can be kept for months at room temperature without noticeable degradation, and are useful for characterising the interface performance).

7. Fill the gas-tight syringe or prime the HPLC pump with this solution (or alternatively fill a ~1 ml loop with it), and start the flow, at 10 to 50 µl/minute. Observe the flow at the electrospray needle tip. Check for leaks.

   If there is no flow at the tip of the steel needle, then remove the red PEEK tube where it connects to the needle and check for flow here.

   (a) If there is no flow, then there is probably a blockage in the PEEK tubing. Deformation of the ends of the tube can easily occur if the fittings are too tight.

   (b) If there is flow, then there is probably a blockage in the needle. If a high pressure pump is available, then try blasting the blockage out using a flow of ~0.2 to 0.5 ml/minute; otherwise replace the needle.

8. Turn on the nebuliser gas (refer to Figures 4-5-G and 4-5-H), and aim the spray at a suitable object, so as to observe the angle of the spray. It should be reasonably central, and should appear quite uniform, without many large droplets and without spitting (the black acetal provides a convenient surface on which to observe the moisture).

   If necessary, adjust the relative positions and angles of the inner type 34 and outer type 24 needles so as to optimise the spray.

   Note: it is not necessary to make these adjustments with the high voltage on, or by using the spray tester, at these flow rates.
1. Adjust the position of the stop block on the measurement rod to give the distance indicated (High Voltage OFF)

2. Adjust the tophat screw as indicated
9. Set the distance between the electrospray needle and the counter electrode to between 8 and 10 mm. Use the measurement rod, as shown in Figure 4-8-A, and then adjust the stop screw so that the needle tip can come no closer than ~4 to 5 mm from the counter electrode.

10. Insert the probe into the source.

11. Turn on the nitrogen bath gas and adjust the flow meter on the front of the electrospray trolley to give a flow of approximately 300 l/hour.

12. Check that Electrospray at 4000 V is selected in the data system Instrument menu, and switch the instrument into Operate. Check the source readbacks to ensure that the HT is on.

If the HT is not on, then it is likely that one of the safety interlocks is not fully in. Refer to Figure 4-3-G.

If significant sustained discharging is heard, switch back to Standby immediately. Refer to Section 4-6, high voltage setup, unplug all of the high voltage cables, and proceed to check that all of the electrospray supplies are coming on correctly.

13. Set the bath heater to 80 °C (it will take about 1 hour to reach temperature, although it will be useable after 15 minutes). Set the electrospray source tune sliders to:
   needle voltage = 30 - 50%
   sampling cone = 20%
   skimmer lens = 25%
   ring electrode = 20%
   as a starting position, and save these values in a new parameter file.

14. Open the slits fully. Set the tuning pots to suitable positions for 4 kV operation, or to the central position if the optima are not known (‘X’, ‘S’ and ‘V’ series instruments). Recall any previously - determined lens parameters (‘M’ series instruments), or set the sliders to the middle positions.

Note: be careful when trying to view any previously - obtained focus 1, beam centre and focus 2 values on ‘M’ series instruments, since these are listed/saved in the Source Tune menus. To view for instance the EI values of these parameters, First Turn Off The HT, then call up the EI source and make a note of the values, then reselect the electrospray source and enter the new values, and finally turn on the HT again. Refer to Section 4-4-3 for further information.

15. Check that the magnet is on (it may have been switched off when SIOS was re-booted), and open the analyser valve.
16. Check that the hexapole RF voltage is present, by selecting a mass of ~1000 or more so that the neon RF indicator is illuminated (refer to Section 4-7). Then select an appropriate magnet mass corresponding to 571 (doubly charged gramicidin S) or 556 (singly charged leucine enkephalin); at these masses the neon indicator should not be lit up. If the RF diode function generator has not been set up, then check that the input signal to the RF board on top of the housing is ~1.5 volts.

17. It should now be straightforward to find a beam by tuning the electrospray and instrument lenses. Refer to Section 4-4-4 for a description of the electrospray tune menu; aim to keep the sliders near to the values suggested above. If absolutely no flicker of a beam can be found after an hour of looking, then it is likely that there is a fundamental problem somewhere.

4-8-2. Optimisation Procedure

(The following points are not necessarily in order - the best sequence will be determined by the behaviour of the system).

1. Tune the various electrospray and instrument lenses as appropriate. Aim for a sufficiently strong and stable signal to be able to work with. Note that if the ion energy or ring electrode controls are some way from their optima, then a high frequency breakdown will be generated in the hexapole region - this is normal behaviour.

2. Check that the hexapole HT is optimal, by tuning the ‘+ve o/p adjust’ trimpot on the high voltage supply PCB. (The ion energy window is big enough so that if the initial high voltage set-up was carried out correctly, any adjustment should be small; only carry out this check occasionally).

3. Loosen the two black knobs securing the top hat assembly on the housing, and adjust the three black thumbscrews so as to move the atmospheric pressure chamber to its optimum position in relation to the skimmer. (The best position is usually ~1 to 2 mm off-axis). The beam stability may be improved by off-axis operation at the cost of some sensitivity, as a result of reduced second-stage pressure.

4. After positioning the chamber, re optimise the instrument lenses.

5. Optimise the flow rate of the bath gas. (It is usually best to leave the nebuliser flow meter fully open).
6. Optimise the exact position of the electrospray probe, by both distance and axial position of the probe tip in relation to the counter electrode.

Move the probe in and out from its initial position 8 to 10 mm from the counter electrode, while keeping the microswitch closed. Note that if the probe is pushed too far in and the requested needle voltage is too high, then discharging may occur. This may crash the electrospray microprocessor, causing various optic loop and SIOS error messages. (Collisional damage to the needle tip is prevented by correct setting of the probe stop screw).

Wiggle the probe up, down and sideways in the housing to determine the position giving the best signal, and then carefully bend the needle tip to match this position when the probe is reinserted in the housing. The best position is usually a little off axis, so that the central jet of the electrospray is blown through one of the four holes in the pepperpot counter electrode.

7. The hexapole RF tracking program should be set up, if not already done. Check that the low - mass tracking is satisfactory by running some low mass samples (or by fragmenting leucine enkephalin using a high sampling cone/skimmer lens voltage to generate the intense m/z 120 ions, for example).

8. SAVE all of the tuning parameters for the source and instrument, using the Save options in the instrument menus.

It can also be very useful to note down all of the parameters on the Electrospray Checklist sheets, since this information can make subsequent fault diagnosis much easier.

9. It should be possible to obtain a signal of 0.3 to 2 volts on the unresolved doubly charged ion of gramicidin-S with the photomultiplier set to 300 volts.

10. Gramicidin-S or leucine enkephalin can be used as a benchmark for the performance of the interface, although it is also important to check that the interface meets its original installation specifications from time to time.

4-8-3. Negative Ion Operation

Once a positive ion beam has been optimised, it should be straightforward to obtain a negative ion beam, using the following guidelines.

1. Flush the solvent delivery system (or injector loop and lines to the probe, as appropriate) with a solution of 1:1 water : acetonitrile or water : methanol containing ~1% (v/v) ammonium hydroxide solution, to neutralise any acids present.

2. Make up a solution of 50 ng/µl lauryl sulphate (SDS) in pure methanol, or 100 ng/µl raffinose in 1:1 water : acetonitrile. Continuously infuse or inject this solution through the ESI probe as described above. (The SDS solution is stable for long periods at room temperature, but the raffinose may deteriorate).
3. Set the magnet to transmit the ions at m/z 265 (SDS) or 503 (raffinose). This should correspond to an RF reference voltage of ~0.5 to 0.7 V or ~1.5 V respectively.

4. Check that the high voltage supplies have been set up for negative ion operation (section 4-6). If the high voltages required for optimal transmission of positive ions from gramicidin S or leucine enkephalin were measured using a high voltage probe, then these values can be used to set the sliders in the ES Negative Setup menu so as to give the identical but inverted values. (For example, If the ring electrode and accelerating voltages were measured as +3.92 and +3.94 kV respectively, and the accelerating voltage is now -3.97 kV, set the ring electrode slider to give -3.95 kV).

5. Recall any instrument tuning parameters previously used for negative ionisation. (Warning - do not recall EI or LSIMS instrument menus whilst the HT is on!).

6. Do not alter the physical positions of the spray needle in relation to the counter electrode, or the sampling cone in relation to the skimmer, from the positive ion optimum positions.

7. Set the needle voltage somewhat lower than the positive ion optimum value (this can be achieved by offsetting the positive and negative supplies by ~500 V and selecting the same needle voltage slider position).

8. Switch the instrument into Operate, and proceed to search for the negative ion beam. Optimise the beam as described above for positive ions.

9. Save the ESI tuning and instrument parameters, and complete a negative ion Checklist for future reference.

4-9. Subsequent Removal And Re-installation Of The Electrospray Interface

4-9-1. Removal And Storage Procedure

1. Switch the instrument into standby. Turn off all solvent and gas flows.

2. Make sure that all relevant operating parameters have been saved or noted down, for instance using a Checklist, for future reference.

3. Gently tighten the three thumbscrews so that the atmospheric pressure chamber remains in its optimal position in the housing when the vacuum is released. Also tighten the two thumbscrews holding the top hat in place.

4. Flush the sample lines with a suitable storage solvent - pure methanol is suitable - to displace any material that may precipitate out of solution or permit microbial growth to take place.
5. If the nebuliser was not in use immediately prior to shutdown, it is good practice to turn on the nebuliser gas for a few minutes to displace any solvent that may have seeped back into the nebuliser lines.

6. Close the source butterfly valve and the analyser isolation valve. Wait for a few minutes for the turbo pump to slow down, then vent the source.

7. Turn off the first stage rotary pump.

8. Disconnect the solvent, gas and electrical lines to the electrospray probe. Remove the probe and store it in a safe place (for instance in the spray tester, or in the electrospray housing after it is removed from the instrument).

9. Disconnect the turbo pump cooling lines from the bench supplies, and the bath and waste gas lines and all of the cables to the electrospray housing.

10. Remove the electrospray cradle from the source housing.

11. Disconnect the first stage pumping line from the housing by removing the six screws (not by removing the tube clamp). Carefully lift away the pumping port and remove the wires attached to the skimmer and the skimmer lens.

12. Disconnect the backing line between the turbo pump and the KF-16 speedivalve.

13. The electrospray housing can now be removed from the instrument by one person (but if in doubt, get someone else to help!). Use a 6 mm hexagon ball driver or allen key to remove the two bolts at the bottom of the electrospray flange. Then hold the electrospray housing in place by using body weight against the top hat assembly, and remove the top two bolts securing the flange to the instrument.

14. Lift away the electrospray housing, taking care not to damage the exposed lens stack. Recover the two stepped dowels from the flange, and fit the protective black plastic cap over the lens stack. Store the housing in a cupboard to prevent ingress of dust.

15. Remove the blanking port from the left-hand side of the instrument housing.

16. Remove the optic and electrical cables from the instrument side panel, and fit the optic link (to complete the optical circuit) and the D-type blanking plug (to complete the high voltage trip circuit).

17. After disconnection of the inlet gas lines and rotary pump exhaust line from the trolley, it may be wheeled away from the instrument.
4-9-2. Re-fitting The Vacuum Lock

Refer to the relevant figures in section 4-1 for specific details. Re-fitting of the vacuum locks on all of the instruments with aluminium housings is basically similar, although the specific parts differ.

4-9-2-1. ‘X’ Series Instruments With Stainless Steel Housing

1. Remove the source surround cover.
2. Re-fit the guide rod assembly to the housing.
3. Re-fit the ball valve assembly to the housing, after checking that the ‘O’ ring is in good condition. Smear it lightly with diffusion pump oil if necessary.
4. Proceed through the instructions listed in the next section, from point 5 onwards.

4-9-2-2. All Instruments With Aluminium Housings

1. Retrieve the two stepped dowels from the electrospray flange, and insert them in the two top holes of the vacuum lock flange.
2. Remove the source surround covers as appropriate.
3. Check that the ‘O’ ring is seated correctly in its groove, and smear it lightly with diffusion pump oil. Check that the mating surface on the instrument housing is clean.
4. Offer up the vacuum lock flange to the housing, and attach it using the four bolts.
5. Connect the 1/4 inch roughing line from the vacuum lock to the instrument bench roughing lines.
6. Close the speedivalve on top of the inlets 1 rotary pump, and remove the blanking plug from the roughing line for the ball valve lever, on the left-hand side of the housing.
7. Fit the two sections of the ball valve lever assembly to the left-hand side of the housing. Push the inner section into the side of the vacuum lock assembly, and connect to the roughing line. Then fit the outer section, making sure that the lever engages correctly with the ball valve, and that the stop stud is in the correct position (orientated on the side nearest to the analyser housing). Finally, tighten the four screws to hold the lever assembly in place.
8. Mount an appropriate source (EI, LSIMS, etc.) in the housing.
9. Open the speedivalve on top of the inlets 1 pump, and proceed to pump the source housing down in the normal way. The KF-16 speedivalve on the turbo pump backing line should remain closed (there is no need to remove this line). Check for leaks.
10. If no beam can be found, check that the optic loop is intact and that the high voltage trip circuit is complete (the optic link and the D-type blanking plug should both be fitted on the HPLC panel).
4-9-3. ESI Re-installation Procedure

The electrospray re-installation procedure is considerably more straightforward than the original full procedure, because it will not be necessary to fit the turbo pump backing line, build the probe, adjust the high voltage supplies, or set up the RF tracking. However, after long-term storage a few points are relevant:

1. Check the water flow through the turbo pump cooling lines before use. If the flow is unsatisfactory, remove the connectors at the turbo pump and clear any obstructions.

2. A turbo pump controller with the ‘soft start’ option will bring the turbo pump up to the requested speed gradually after the pump has been stored. This may take 45 minutes. Do not switch the instrument into Operate until the turbo pump has reached at least 95% speed.

3. If the sample lines were not flushed with clean solvent prior to storage, the tubing may have become blocked due to precipitation of salts, or as a result of bacterial growth.

4. Similarly, if residual samples & solvents were not flushed from the nebuliser lines before storage, the annulus between the inner sample and outer nebuliser needles may have blocked, or the acids in certain solvents may have corroded the brass connector union where the 4 mm green plastic nebuliser tube attaches to the probe.

4-9-4. Transfer Of The API Interface Between Two Mass Spectrometers

The versions of API interface which include an API trolley can be transferred between two instruments reasonably straightforwardly. However, it is not possible to transfer an API interface from an ‘M’ series instrument having integral, bench-mounted API electronics to another instrument.

The following operations must be carried out when transferring one API interface between two mass spectrometers:

1. Complete an electrospray checklist in full prior to removal of the interface from the first machine. The high voltages, and in particular the magnitude of the differences between the sampling cone, skimmer lens, accelerating, hexapole offset, and ring electrode voltages, should be recorded (use a suitable high voltage probe and refer to Section 4-6).

2. Remove the interface, and exchange any hardware that differs between the two instruments (for instance, the API flanges and cradles if swapping between ‘S’ and ‘M’ series AutoSpecs).
3. When swapping the API interface between an AutoSpec family and a 70 or ZAB instrument, note that the function of one of the high voltage supplies changes. The skimmer lens supply on the AutoSpec becomes a slave accelerating voltage supply on 70's and ZABs. A special modification to allow the use of a skimmer lens on the 70 and ZAB series instruments is required (refer to the 70/ZAB API manual).

4. If swapping between an ‘M’ series instrument and any other type (‘X’, ‘S’ and ‘V’ AutoSpec family, or 70 or ZAB series instruments), then it is necessary to change the links on the electrospray control PCB, MA3606-200. The link positions are: ‘B’ for ‘M’ series instruments, and ‘A’ for all others.

5. If the mass ranges of the magnets on the two instruments are very different, it will also be necessary to either reprogramme the diode function generator on the MA3606-200 PCB that controls the RF tracking, or to exchange this PCB for another one having the correct setup. It is preferable to use two separate PCB’s, since the setup procedure is complex. (Magnets having a 4500 and a 5000 mass range are sufficiently similar that there is no need to adjust the programme).

6. Install the interface on the second instrument, and balance the high voltage supplies such that the same small differences are set between the sampling cone, skimmer lens, accelerating, hexapole offset, and ring electrode voltages. (Alternatively, set up the supplies to match any previously noted values obtained using the interface on this instrument).

7. It should now be a simple matter to obtain a beam on the second instrument.

4-10. Installation and Operation of the Crossflow Counter Electrode.

The crossflow counter electrode (CCE) has been developed to improve electrospray source lifetime when analysing ‘dirty’ samples e.g. plasma or urine with minimum cleanup. It is a direct replacement for the existing 4 hole counter electrode (pepperpot) on all AutoSpec family mass spectrometers. It produces ion currents which are approximately 50% of those produced by an optimised ‘pepperpot’.

The crossflow counter electrode is an option available for the standard electrospray API source.
FIGURE 4-10-A. SCHEMATIC DIAGRAM OF THE CCE

<table>
<thead>
<tr>
<th>CODE</th>
<th>DESCRIPTION</th>
<th>QTY</th>
</tr>
</thead>
<tbody>
<tr>
<td>M805207CC1</td>
<td>INNER BODY API SOURCE</td>
<td>1</td>
</tr>
<tr>
<td>M804870CD1</td>
<td>CROSS FLOW COUNTER ELECTRODE</td>
<td>1</td>
</tr>
<tr>
<td>M804870CD2</td>
<td>CROSS FLOW COUNTER ELECTRODE</td>
<td>1</td>
</tr>
<tr>
<td>5314043</td>
<td>SCREW M1.6x6 ST STL CH HD</td>
<td>2</td>
</tr>
<tr>
<td>5316035</td>
<td>GRUB SCREW M2x3 ST STL SLOTTED HD</td>
<td>2</td>
</tr>
<tr>
<td>5371002</td>
<td>DOWEL M2x6 ST STL</td>
<td>1</td>
</tr>
</tbody>
</table>
4-10-1 Principle of CCE operation

In essence, the CCE substantially reduces the amount of involatile material (from samples introduced into the source) deposited in the sampling cone orifice and hence reduces the gradual loss in sensitivity as the orifice becomes progressively smaller. In extreme cases, it also reduces the likelihood of a catastrophic loss of sensitivity as the orifice becomes blocked by a solid particle. The CCE addresses two major areas to effect this improvement. These are:

1. The electrospray is directed across a single hole in the CCE instead of at 4 holes in the pepperpot. In this way, the major part of the involatile material in the spray is deposited on the bottom of the ‘catcher’ and only those ions and small aerosols from the edge of the spray enter the hole in the CCE for transport by the drying gas stream to the sampling cone. With the Pepperpot the major part of the spray is deposited at the entrance of the four holes, ultimately interfering with the spray.

2. The gas stream containing ions and residual solid material is directed across the top of the sampling cone as it exits the CCE instead of being aimed directly at the orifice as with the pepperpot. In this way, the major part of the ions and the material in the gas stream pass across the top of the cone until a significant potential is applied between the CCE and the sampling cone, when essentially only ions are deflected into the hole in the cone. The standard counter electrode voltage of 1000V above the source accelerating voltage is normally optimum.

4-10-2 Installation and Use of the CCE.

Replace the existing pepperpot with the CCE using the two M2 x 3mm slotted grub screws provided. **Ensure that the exit hole in the CCE is facing downwards when the source is mounted in the instrument.** Install the source in the instrument.

Before introducing the probe, adjust the probe stop on the interface anticlockwise so that it enters the source approximately 10 - 15mm further away than it was with the pepperpot.

After switching to operate, signal from a sample peak should be monitored. Move the probe in and out by approximately 5mm to determine its optimum position. The instrument is now ready for operation with the CCE and other source parameters may now be adjusted or optimised as required.

A schematic diagram of the CCE is shown in Figure 4-10-A.
Section 5

A Guide To Running ESI Samples
The most commonly used solvent system for electrospray is a 1:1 mixture of methanol : water, containing 1% by volume acetic acid. This has been used successfully for a wide range of sample types.

1:1 acetonitrile : water + 1% acetic acid is also used if the sample to be run is insoluble in or incompatible with methanol.

It may be necessary to vary the ratio of methanol or acetonitrile and water to fully dissolve a particular sample. In addition, the acid content may be critical for certain compounds where solubility is pH dependent.

In general, observe the following as a guide:

• Use only high purity reagents and HPLC-grade solvents to avoid excessive background noise;

• De-gas all mobile phases with helium sparging, ultrasonication or vacuum filtration;

• Ensure the sample is fully soluble in the mobile phase (see section 5-2);

• A reliable spray is produced over a wide range of solvent compositions (100% water to 100% methanol or acetonitrile). However, for extremes >80% to <20% organic solvent in water, it is essential to assist spray formation with nebulizer gas;

• Certain compounds, eg. organometallics are extremely hydrophobic, and so aqueous mobile phases cannot be used. THF, chloroform, dichloromethane, toluene and ethyl acetate can be used, with nebulization gas. Where possible, methanol should be added to chlorinated solvents at ~20% to reduce the tendency to “freeze” the needle tip and maintain steady flow conditions;

• Dichloromethane and THF are incompatible with certain grades of HPLC components and tubing, in particular PEEK. Consult the relevant source if unsure;

• Avoid the use of salts and organic buffers (see below; 2 mM NaCl and/or 1-10 mM ammonium acetate may be used, but Tris or phosphate buffers must be avoided).
5-2. Sample Preparation: Solubility & pH

It is essential that samples are fully soluble in the mobile phase at the concentration to be run.

Emulsions or samples containing precipitates or insoluble particles should not be introduced to the electrospray probe, as blockages of the injector, tubing or capillary needle are likely to occur. Centrifuge or filter samples if necessary.

If a sample is insoluble in 1 : 1 MeOH : H₂O + 1% acetic acid, a more detailed investigation of solubility will be required.

The points below should act as a guide.

1. “Like dissolves like” eg. samples containing OH groups are generally soluble in alcohols, ie. try pure methanol.

Highly chlorinated compounds are usually soluble in dichloromethane.

2. Sample origin, ie. how it was isolated/purified is usually a good indication of solvent compatibility.

3. Substitution of acetonitrile for methanol may be useful when a compound is partially soluble in methanol or methanol/water (due to differences in polarity/proton affinity).

4. Use methanol, acetonitrile and water only where possible.

5. Proteins, peptides and other large biomolecules are usually soluble in some combination of water, acetonitrile and methanol, but are often highly dependent on acidity. A typical example is myoglobin which is only partially soluble in pure water, but fully soluble when ~1% acetic acid is added.

In general, for proteins/peptides, prepare a bulk solution in pure water adding acid stepwise to give full solubility. Once fully in aqueous solution, methanol or acetic acid can be added without precipitation. Dissolving proteins directly into methanol/water may be more difficult.

6. Once dissolved, it may be possible to prepare a dilution of a sample at low concentration in a solvent which is not a particularly good solvent for a bulk solution. If this is necessary then dilutions should be made in clear vials to enable precipitation to be observed, should this occur.

7. N.B. The mobile phase used as a carrier for loop injections of a sample should be the same as the sample solvent, or preferably a slightly better solvent for the sample to avoid precipitation within the loop/probe. If this is observed, memory or carry over effects will also be avoided.
8. The majority of compounds give a stronger signal in ES+ mode when the optimum acid content is used. This is usually between 0 and 5% acetic acid. Formic acid may be used in place of acetic acid, but due to its higher pKa value it is necessary to use less formic acid.

Trifluoroacetic acid is best avoided since above very low concentrations >0.05% v/v, TFA tends to suppress the signal from many compounds. Typically, peptides run in 1% acetic acid will give 5 to 8 times stronger signals than they would in 0.1% TFA.

9. For negative ion electrospray, it has been observed that the use of acetonitrile : water rather than methanol : water tends to give stronger and more reliable signals for many compounds, since the probe tip is less susceptible to glow discharge effects when acetonitrile is used.

Where possible, acid should not be used with negative ion samples, as low pH tends to suppress negative ion formation.

It may be necessary to purge the pump/probe with dilute (~0.1 to 1% v/v) ammonium hydroxide to remove excess acidity within the system. In certain instances, the addition of 0.1% to 1% v/v NH₄OH to the sample solution may improve signal strength.

5-3. Preparation Of Standard Samples

5-3-1. Gramicidin S And Leucine Enkephalin

Gramicidin S is usually used as a standard test compound during initial set up / tuning at a concentration of 10 ng/µl in 1:1 MeOH : H₂O + 1% acetic acid.

The predominant ion is [M+2H]²⁺ at m/z 571.36 (the monoisotopic ion). [M+H]⁺, at m/z 1141.7, is usually around 1 to 5% of the doubly charged intensity.

This solution should be used to optimise the system (see section 5-4). Currently, Sigma does not sell Gramicidin-S, and so if it is not available on site use a similar peptide such as one of the enkephalins (m/z ~500-600, singly charged) or bradykinin (m/z 530.79, doubly charged) at the same concentration. Leucine enkephalin (m/z 556) is a good choice, and is stable in the above solution at room temperature.
5-3-2. High Molecular Weight Standards

Once a satisfactory beam has been obtained for gramicidin-S, it is useful to examine the performance of the electrospray by running some high molecular weight standards.

Commonly used High Molecular Weight Standards:

a) Hen Egg Lysozyme, MW 14305 (observed as \([\text{M} + n\text{H}]^{n+}\) ions)
b) Horse Cytochrome-C, MW 12360 (observed as \([\text{M} + n\text{H}]^{n+1}\) ions)
c) Horse Myoglobin, MW 16951.5 (observed as \([\text{M} + n\text{H}]^{n+}\) ions)
d) Bovine Serum Albumin (BSA), MW ~66400 (often heterogeneous)

It is important that the supplier's storage instructions are followed carefully; all of the above are recommended by Sigma to be stored dessicated at -20°C. Typically, stock solutions should be made at high concentration (~1 - 10 µg/ml) in pure water, and these should be kept frozen except when making up working solutions. The lifetime of the stock solutions are affected by the number of freeze-thaw cycles and the time at room temperature, as well as the total storage time.

Failure to obtain good spectra of these may indicate a vacuum problem (refer to the Troubleshooting Guide, section 6-7).

In addition to being useful indicators of performance, the charge state distributions may be used for mass calibration (with the exception of BSA). They are also useful in gaining experience in the use of transformation and maximum entropy software parameters (refer to the ESI Data Processing Manual).

5-3-3. Observations On High Molecular Weight Standards

1. When the signal has been optimised for the major component (charge state) in a particular series, the other charge states should give an approximately normal distribution without any noticeable suppression at high or low mass. The distribution is highly dependant on the Sampling Cone / Skimmer differential voltage.

2. Adduct peaks should not be greater than 10-15% of the main peak for each charge state (assuming that the sample consists of one component). Excessive adduct formation may be due to:

   **Source Tuning:** unnecessarily high Sampling Cone voltage results in fragmentation. When reduced, a “cleaner” spectrum should be produced.

   **Gas Flows:** the relative intensity of the chemical noise peaks on either side of the sample peaks depends to some extent on the flow rates of nebuliser and bath gas.
**Sample Degradation:** lysozyme and cytochrome are stable in solution when refrigerated for at least two weeks, longer if in pure water. Myoglobin and BSA bulk solutions are less stable and should be refrigerated in pure water as bulk solutions, and diluted fresh into mobile phase.

**Electrical Discharge On Needle Tip:** this is usually caused by rough edges on the inner or outer needle. Remove using fine abrasive paper, or replace needle entirely as a last resort. Backing off the needle from the counter electrode and/or reducing needle voltage may reduce this effect.

**Original Type 34 All-steel Needle:** the braze dissolves slowly in acid solvents, giving copper and silver adducts. Replace these needles with the new steel/PEEK needles as described in section 4-5.

### 5-3-4. Low Molecular Weight Standards

Some commonly used positive ion low molecular weight standards are listed below:

- **Tetraethylammonium salts m/z 242, M⁺ ion**
- **Rhodamine-B m/z 443, M⁺ ion**
- **Gramicidin-S mw 1141.7, (predominantly 2⁺ ions; some 1⁺)**
- **Leucine enkephalin mw 556.1, (gives [M+H]⁺)***
- **Renin substrate mw ~1759 (porcine; gives 2⁺ to 4⁺)**
- **Mellitin mw ~2845**
- **Insulin mw ~5733 (porcine; gives 3⁺ to 6⁺)**

Renin substrate, mellitin and insulin are useful for tuning and optimisation when preparing to run unknown samples of low or intermediate (up to 5000 Da) mass.

Leucine enkephalin and rhodamine-B are well characterised for use in Cone Voltage Fragmentation (CVF) and B/E Daughters experiments (see the ESI Data Processing Manual).

Rhodamine-B at 10 ng/µl gives intense CVF ions at m/z 399 and 355. A concentration of 1-10 pmol/µl of the above compounds is a useful starting point for tuning, using 50:50 MeOH : H₂O + 1% acetic acid as mobile phase and sample solvent.

For B/E daughters on leucine enkephalin, 50 pmol/µl gives strong and reproducible spectra when the instrument/experiment is correctly set up.
5-3-5. Negative Ion Standards And Calibrants

1. **Myoglobin** can be run in ES- mode by adding 0.1% to 1% v/v NH₄OH (ammonium hydroxide) to a neutral pH solution in 1:1 MeOH : H₂O. Under these conditions a distribution shift towards higher m/z, lower charge state, is usually observed.

2. **Lauryl sulphate (SDS)/Gramicidin-S/Leucine enkephalin/Raffinose** All these low molecular weight standards run well in Negative Ion Electrospray, particularly if sample solution pH is raised to 7 by the addition of ammonium hydroxide (~0.1 to 1% v/v) and using 1:1 acetonitrile : H₂O as mobile phase/sample solvent.

If SDS is prepared at 50 ng/µl in pure methanol without adjustment of pH, then [Mⁿ + Naⁿ⁻]⁻ clusters can be observed up to mass ~4000 Da. By optimising source tuning parameters for one of the larger clusters a useful multipoint negative ion calibration can be obtained.

5-3-6. Calculation Of Sample/Standard Concentration

Use the calculation below to prepare samples from bulk solids. For preparation of 1 millilitre of standard solution:

\[
\text{(weight taken (mg) / molecular wt.) x } 10^{-6} = \text{moles/µl}
\]

eg. 1.43 mg of lysozyme in 1 ml H₂O:

\[
1.43 / 14305 \times 10^{-6} = 10 \times 10^{-10} \text{ mol/µl} = 100 \text{ pmol/µl}.
\]
5-3-7. Quick Guide To ESI Calibrants

Table 5-3 summarises typical mass calibrants used for electrospray (see the ESI Data Processing Manual for full details).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Useful Mass Range</th>
<th>Normal Base Peak</th>
<th>Ion Distribution</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>SDS</td>
<td>90--4000</td>
<td>265</td>
<td>Exponential</td>
<td>Neg. ion</td>
</tr>
<tr>
<td>Ivory (commercial AES mix)</td>
<td>90--800</td>
<td>265</td>
<td>Exponential</td>
<td>Neg. ion</td>
</tr>
<tr>
<td>PEG 600 Diacid</td>
<td>~200--1200</td>
<td>~700</td>
<td>Bell</td>
<td>Neg. ion; main ions 14Da high</td>
</tr>
<tr>
<td>PEG (200 to 1000)</td>
<td>varies</td>
<td>varies</td>
<td>Bell</td>
<td>Neg. ion; peak assignments difficult</td>
</tr>
<tr>
<td>PEG (200 to 1000)</td>
<td>varies</td>
<td>varies</td>
<td>Bell</td>
<td>usually as H⁺ and Na⁺ adducts</td>
</tr>
<tr>
<td>PPG (425, 1000,2000,3000,4000)</td>
<td>~200-4000; ions to ~8000</td>
<td>varies</td>
<td>Bells; 1+,2+ and 3+ series*</td>
<td>Na⁺ forced by 2mM NaCl; also H⁺</td>
</tr>
<tr>
<td>CsI</td>
<td>133--7000</td>
<td>Similar to LSIMS</td>
<td>Exponential, with break points</td>
<td>1/50 diluted solution is OK to run</td>
</tr>
<tr>
<td>Gramicidin S</td>
<td>571.36; 1141.7</td>
<td>571</td>
<td>1142 usually ~2 to 10% of 571</td>
<td>Calibration check</td>
</tr>
<tr>
<td>Lysozyme</td>
<td>~1100-1800; up to ~3000*</td>
<td>1431 (10+), 1590 (9+)</td>
<td>protein</td>
<td>Not many peaks unless denatured</td>
</tr>
<tr>
<td>Myoglobin</td>
<td>600--1600; up to ~3000*</td>
<td>1060 (16+), 1131 (15+)</td>
<td>protein</td>
<td>616 = heme; well spaced peaks</td>
</tr>
<tr>
<td>Trypsinogen</td>
<td>~1000--3000*</td>
<td>...</td>
<td>protein</td>
<td>Peaks widely spaced</td>
</tr>
</tbody>
</table>

* - high Sampling Cone voltages change the observed distributions toward lower charge states. This can be used to give high - mass calibrations from proteins, and to remove 3+/2+ ions from PPG spectra.

5-4. Source Tuning & Parameter Optimisation

Prepare a mobile phase containing 10 ng/µl gramicidin-S in 1:1 MeOH : H₂O + 1% acetic acid. Procedure using an HPLC pump and Rheodyne 7125 or similar injector:

De-gas thoroughly and purge through the pump used to supply the electrospray flow.

Ensure the flow is steady and pulse-free by running at high flow rate (~3 - 5 ml/min) with the inlet tubing disconnected from the Rheodyne injector (at port 2). Removal of air from the tubing and pump heads is essential.
Re-connect inlet tubing, fit a loop across ports 1 and 4 of the Rheodyne and pump solvent through the probe at \( \sim 100 \) \( \mu l/\text{minute} \) (or lower flow rates if narrow bore capillary tubing is in use). At this stage, check that there is no constriction from the tubing (excessive backpressure reported at the pump).

2. Set flow rate to 10 \( \mu l/\text{minute} \).

With no nebulizer gas, adjust relative distance of inner/outer needle to give a good even spray when viewed in the spray tester (Needle Voltage slider at zero; acceleration voltage 1000 V, giving \( \sim 4000 \) V on the probe with respect to ground).

Also check that there is no discharge from the needle tip, by viewing the spray in the tester with all light excluded.

A purple corona discharge is often formed if the needle is not correctly positioned, or from rough edges on the needle tip. This discharge must be removed for good results.

Turn on nebulizer gas at maximum (\( \sim 10 \) l/hr should register on the flowmeter, for 6 Bar inlet pressure) and increase the flow rate to 40 \( \mu l/\text{minute} \). A fine mist spray should be formed without any obvious spluttering or electrical discharge formation. The spray should be on, or close to, the probe axis.

3. Align the Atmospheric Pressure Chamber to be visually central within the source housing using the three knurled thumbscrews. Replace the endplate and heat the source to 80°C with a bath gas flow rate of 2-300 l/hr (it will take 10 - 20 minutes to reach operating temperature starting from cold).

Insert the probe within the housing (assuming the insertion distance is correct to produce the required distance from the needle tip to the counter electrode, usually 8 - 10 mm initially). Set the flow rate to 40 \( \mu l/min \) and continuously infuse the 10 ng/\( \mu l \) gramicidin solution as the mobile phase.

With the acceleration voltage at 4 kV, and the needle voltage slider set to 50\%, adjust the sampling cone and ring electrode voltages to optimise the beam for the 2+ ion at 571.36. Once set, the ring electrode voltage and ion energy should not need further adjustment; in contrast, the sampling cone voltage is highly dependant on the type of sample.

Once a signal has been obtained, each source/instrument tuning and alignment parameter should be adjusted until the ultimate sensitivity is achieved for a resolution of \( \sim 1000 \) on the 2+ ion.

(If a beam is not obtained rapidly, then refer to the Troubleshooting Guide, section 6-7, and section 4).
Parameters Affecting Signal Strength

1. **Source Alignment**

   Adjust with the three knurled thumbscrews.

   With the hexapole RF lens and the pepperpot counter electrode, spikes may be observed on the gramicidin signal due to discharge effects within the source. These should be possible to remove using source alignment with only minimal (<10%) reduction in signal from the optimum alignment position.

2. **Probe Insertion Distance**

   Move the probe towards the counter electrode using the adjuster screw, the signal will usually optimise a few mm before becoming erratic and eventually breaking down, as the needle becomes too close.

   Set the probe length slightly further out than the absolute optimum signal position for improved stability.

3. **Needle, Angle Of Impingement**

   A more intense signal is usually obtained when the needles are angled slightly, either vertically or horizontally, or both, by a few degrees to allow the spray to follow a path through the pepperpot counter electrode which produces the maximum ionisation efficiency.

   The optimum angle for the needles can best be determined by trial and error, observing the signal intensity change as the probe is ‘wobbled’ in any particular direction, and then the needle should be gently bent into position.

   If required, the spray path through the pepperpot counter electrode and onto the sampling cone can be assessed by spraying a solution of 100 - 500 ng/µl Rhodamine-B (or similar dyestuff) for a few minutes.

4. **Bath Gas**

   Slowly increase/decrease the bath gas flow to obtain the maximum signal intensity. A maximum is usually reached at ~400 l/hr; above this only a very slight increase in signal is usually observed. In the interest of increasing gas cylinder life, it is common to run the bath gas slightly lower than the optimum flow rate (say 200 l/hr).

   As the aqueous content of the mobile phase is increased, the optimum bath gas flow rate may increase due to lower rates of desolvation.
5. **Nebulizer Gas**

Usually, little variation in signal strength is produced by variation of nebulizer gas pressure. The spray may become erratic over a wide range of mobile phase composition unless nebulizer flow is sufficiently high.

6. **Mobile Phase Flow Rate**

For a pepperpot counter electrode/all steel dual layer needle arrangement the optimum flow rate is usually between 20 and 40 µl/minute.

Above this, little increase in signal intensity is observed relative to the increased sample consumption (although the maximum flow rate is ~1000 µl/min).

From 20 to 40 µl/min, signal intensity may decrease in a linear manner. At 4 µl/min or less, there is a more pronounced loss of signal. However, if the bath gas flow rate is increased, this loss of signal can be minimised, and 50% intensity relative to 10 µl/min maintained down to ~1.5 µl/min. At ~1 µl/min, the flow rate becomes too low for this particular needle arrangement.

If low flow rate rate (µl/min) is required, a very stable spray should be first set up at 10 µl/min, and preferably an aqueous content of 50% to 80% maintained in the mobile phase. Sensitivity can usually be regained by careful optimisation.

7. **Benchmark Parameters**

Once the optimum signal has been obtained, make a note of the tuning values for the ESI interface and the instrument. The sample/solvent conditions, gas flows, source voltage settings, vacuum pressures, the detector gain, slit settings and resolution and the signal intensity can be noted on the Electrospray Checklist, copies of which can be found at the front of this Manual. Some or all of the voltage values can also be saved as Instrument Parameter files.

This information is extremely useful if the electrospray is to be used infrequently, since a benchmark for satisfactory performance is produced. Similarly, any deterioration in performance for more frequently used electrospray sources can be spotted early and rectified. (See the Troubleshooting Guide, section 6-7). A gradual increase in intensity is often observed if the benchmark test is applied on a daily basis until the absolute optimum settings are achieved with ease.

**Note.** The benchmark optimisation described for Gramicidin-S should be performed on any other compound if it is more relevant to do so on account of the type of samples/conditions to be used on a regular basis.
5-5. Running Samples

5-5-1. Experiment Setup

The most commonly used Experiment settings are shown below:

<table>
<thead>
<tr>
<th>Setting</th>
<th>Setting Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scan speed</td>
<td>Usually ~10 sec/decade</td>
</tr>
<tr>
<td>Resolution</td>
<td>1000</td>
</tr>
<tr>
<td>Acquisition</td>
<td>Continuum mode (or 10 - scan MCA to save data space)</td>
</tr>
<tr>
<td>Filter</td>
<td>Off (it is better to smooth using the data system afterwards)</td>
</tr>
<tr>
<td>Scan range</td>
<td>Usually +/-100 Da of highest and lowest peaks of interest</td>
</tr>
<tr>
<td>Sector scan</td>
<td>Magnet</td>
</tr>
</tbody>
</table>

Variations will be necessary to allow different experiments to be performed at the optimum efficiency. A few examples follow (see the ESI Data Processing Manual for more details):

* LC-MS or CZE-MS analysis: The scan speed should be set to allow at least 3 to 10 scans across an eluting LC peak for statistical accuracy.

* For a long acquisition, eg. LC-MS requiring a wide mass range, it may be necessary to acquire in centroid mode to save disk space. If multiply charged ions are present, ensure that the Accurate data mode is selected when displaying the spectra.

* For accurate mass, a narrow range voltage scan is preferable to a magnet scan. The range should be set as narrow as possible, and the resolution set to give complete separation of the sample/calibrant isotopes from interferences, but no higher than necessary. (If MaxEnt Reconstruction is to be used, the value for Experiment resolution should be 2 to 3 times the real resolution).

5-5-2 Calibration

For nominal mass, low (1000) resolution investigation work, it is preferable to use the Hall Probe for calibration. (However, if proteins are to be analysed, the small errors will be multiplied by the charge states, so that the overall errors, expressed as Daltons, can thus be quite large).

The Hall probe can be set using a single lockmass on a well-defined peak, eg. gramicidin-S 571.36 [M+2H]^{2+} or 1141.7 [M+H]^+, or SDS at [M]̂ 265.14 for negative ions. In general, it is better to use a lighter mass ion than a lower mass ion as the lockmass.

A multipoint calibration can be set up using the charge state distribution of lysozyme, myoglobin etc, provided the peaks produced are free of interference from adducts/sample degradation and span the entire acquisition range required.
For low resolution calibration, PEG is not recommended if an alternative is available, due to its tendency to produce carryover effects within the injector and connecting tubing. Large quantities may also contaminate the source. The minimum concentration and quantity should be used to produce a clear spectrum for calibration. It is also helpful to reserve syringes etc. for use only with the PEG.

If PEG must be used, then it is often better to calibrate after the sample data has been acquired to avoid interference from carryover peaks. Similar carryover problems have been reported using PPG’s.

5-5-3. Accurate Mass

Electrospray accurate mass determination is best performed by a voltage scan over the narrowest range possible which incorporates the reference peaks (usually PEG or PPG), one either side of the sample. Resolution should not be any higher than absolutely necessary to give a clear isotopic separation and resolution of interferences.

N.B. Unless the pH is exceptionally low, a mixture of [PEG+Na]^+ and [PEG+H]^+ is produced. Usually PEG+Na is the dominant series.

This effect can be useful since two peaks from a series can be used for calibration and the peak in between from the other series used as a check of mass measurement accuracy since its theoretical mass is also known. Simultaneous acquisition of sample and calibrant gives the most accurate results, but it may be found that incorporation of calibrant into the sample solution suppresses the signal to a large extent. In this case, alternate loop injections of sample/calibrant can be made, and then the spectra averaged together.

(Refer to the ESI Data Processing Manual for more details).

5-5-4. Native Protein Complexes

It is necessary to use the nebuliser-assisted spray in order to obtain spectra from pure water. Some guidelines follow:

1. Analyse the protein in a denaturing solvent system to gain information on the level of heterogeneity, and on the individual subunits.

2. If significant levels of salts or buffers are thought to be present, then desalt the sample using an HPLC cleanup step or by ultrafiltration. Salts may well have a significant effect on the peak widths obtained under non-denaturing conditions.

3. The pH of the protein solution can be estimated by removing aliquots (~20 ul) and placing these on suitable pH indicator paper. Ammonium acetate at 10 to 50 mM will not affect the quality of the spectra, and the pH can be adjusted by judiciously adding dilute acetic acid or ammonia. Be aware that the incautious addition of strong acid or alkali, such that the pH swings unexpectedly high or low, may convert the protein permanently into an unwanted form.
4. Solutions of proteins in water at neutral pH may deteriorate quickly, due to the action of bacteria or contaminating proteinases, and thus should be analysed swiftly.

5. Native protein complexe are typically seen at much lower charge states than the corresponding denatured forms, and so a suitable survey scan range would be from m/z ~10000 to ~1000. Mass calibration can be achieved using an LSIMS ion source, or by running CsI by electrospray ionisation.

6. If the native protein is heterogeneous (for instance if the subunits are not identical, or have varying degrees of glycosylation), or if significant levels of salt adducts are present, definitive mass measurement may prove quite difficult.

5-5-5. Limits Of Detection

For the best results from limits of detection studies, it is necessary to ensure the source is clean (see Chapter 6).

Other parameters affecting absolute sensitivity limits are contaminants from the mobile phase. Solvent interferences tend to increase considerably once a mobile phase has been prepared more than a few days open to the atmosphere, particularly if pH modifications have been made with eg. acetic acid which itself introduces contaminants and peaks due to solvent cluster formation.

Background solvent ions can never be entirely removed, but the background noise should be limited by using only high purity HPLC grade solvents and reagents, preparing fresh mobile phase whenever possible and purging the LC pump heads/tubing on a regular basis.

The choice of plastic or glass vials, and the type of pipettes used for making dilutions, can be critical. Contaminant ions may leach out from plastic and glassware, either slowly or quickly, and samples may be lost by adhesion to the walls of containers. Special care should be taken to avoid these types of problem, particularly in the case of SIR experiments. If, for instance, a new type of vial is used during the course of an SIR experiment, it is quite possible that a different amount of Na⁺ might leach from it and the balance of ion current between say [M+H]⁺ and [M+Na]⁺ thereby changes significantly.
5-6. HPLC Applications

5-6-1. Setting Up/Sample Input

A wide range of HPLC-MS analyses have been performed by electrospray over a range of conditions.

HPLC separations are generally performed on 4.6 mm, 2.1 mm or 1.0 mm i.d. columns. Packed fused silica columns with i.d.’s below 1 mm are less widely used. The optimum flow rates for separation on these columns are given in the table below.

<table>
<thead>
<tr>
<th>Column i.d.</th>
<th>Flow rate for optimum separation efficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.6 mm</td>
<td>0.5 - 2.0 ml/min</td>
</tr>
<tr>
<td>2.1 mm</td>
<td>100 - 400 µl/min</td>
</tr>
<tr>
<td>1.0 mm</td>
<td>10 - 50 µl/min</td>
</tr>
<tr>
<td>0.32 mm POROS</td>
<td>10 - 50 µl/min (this packing allows relatively high flow rates)</td>
</tr>
<tr>
<td>0.20 - 0.32 mm</td>
<td>1 - 5 µl/min</td>
</tr>
</tbody>
</table>

For separations on a 1.0 or 2.1 mm i.d. column, the entire LC effluent can be introduced to the electrospray directly. For 4.6 mm i.d. columns, the flow should preferably be split to provide the optimum flow rate for electrospray (10 - 100 µl/min) while maintaining ideal HPLC operating flow rates. If required, the ESI source can accept up to ~0.5 ml/min, but there is usually little benefit in doing this.

Commercial post-column dynamic splitters are available, but a simple and effective device can be prepared from a low dead volume HPLC tee and some lengths of PEEK tubing as detailed in Figure 5-6-A.

**FIGURE 5-6-A. A SIMPLE FLOW SPLITTER**

The split ratio is determined by the relative constriction of the route to the electrospray probe tip (3) and down to the waste tube (2).
Initially attach approximately 20cm of 120um i.d. (or 0.005") PEEK (red) tubing to the waste and connect the probe to the tee with all plumbing the same as to be used during normal operation. As the length of the waste tubing is reduced (use a PEEK cutting tool to prevent accidental constriction of the sheared end), the flow rate through the probe will decrease. A rough split ratio can be determined by eye with experience, or measured directly using the barrel of a syringe connected to the end of the electrospray needle with tygon or teflon or similar tubing.

Once a split is set up, it should be largely independent of the mobile phase composition so gradient elution HPLC methods can be used (nebulizer assisted spray required).

The tubing from the splitter should be connected directly into the back of the needle via the low dead volume connector (bypassing the loop injector, as this would only increase the dead volume and cause peak broadening). When microbore columns are used, the entire flow can be fed into the electrospray directly.

5-6-2. General Procedure

It is usually necessary to run a loop injection analysis of the mixture to be analysed (either using a post-column injector, or by bypassing the column) in order to optimise the source tuning (mainly the Sampling Cone/Skimmer Lens). These source tuning parameters should be maintained throughout the LC acquisition.

The signal intensity for each individual component in a mix may be considerably weaker than the signal obtained from the electrospray analysis of the eluting peak from the HPLC column due to mutual suppression effects.

The source tuning is best set to the optimum obtained for a peak known to elute around the mid point of the LC separation using a mobile phase for the loop injection which corresponds to the mobile phase composition formed by the gradient at the mid-point of a run.

Example: if the gradient is from 20:80 to 95:5 methanol:water in 20 minutes, choose a component that elutes nearest to 10 mins to tune source on. Use ~60/40 MeOH/H2O as mobile phase for loop injection.

5-6-3. Mobile Phase pH Considerations

If possible, incorporate a small amount of acetic or formic acid into the mobile phase to provide a constant supply of protons for ion-formation (>0.05% should be sufficient).

However, if the pH of the separation is critical and acid cannot be added to the mobile phase - first try loop injection of sample without acid. If no signal is seen until acid is added then a second pump will be required to introduce acid post-column. This can be tee’d in between the column and needle without affecting the LC separation.
5-6-4. Accurate Mass, Scanning Experiments

For accurate mass LC, the internal calibrant used is usually a PEG or PPG mixture having an average mass nearest to the masses of the samples of interest. Voltage scan calibration requires at least 2 peaks, whilst magnet scans require four or more reference peaks.

Since the addition of PEG to the mobile phase is not possible due to the likelihood of affecting the separation, causing peak broadening and producing a large U.V. offset on the detector it should be added post-column via a Tee as described in section 5-6-3 above.

Supplying PEG solution via a second pump has the added advantage that the ratio of PEG relative to sample can be varied by changing the PEG pump flow rate. This means peak heights of calibrant and sample can be matched, giving more accurate results.

Although it is usual to pump in the calibrant continuously, it is also acceptable to make discrete injections at 10 to 15 minute intervals during the LC run. Using this method it is potentially possible to avoid a clash between the reference and sample ions, and it is easier to identify trace components, although the data processing may prove to be somewhat more complex.

In addition, modifiers such as proprionic acid/isopropanol designed to reduce the signal suppression effects caused by TFA can also be added in this way.

5-6-5. High Resolution SIR Experiments

In contrast to accurate mass scanning experiments, it is essential that a suitable ion that can serve as the lockmass is present continuously during high resolution SIR analyses. This can most easily be achieved using a post-column makeup flow.

Although for instance mixtures of PEG's or PPG's are an obvious choice for both the mass calibrant and the lockmass ion, it may be better to use PEG etc. only for calibration, and to choose a single compound having an appropriate mass for the groups of sample ions to be monitored. This approach has the advantage that, if the lockmass material is chosen carefully, the chemical background noise level can be very low because there is essentially only one ion in the spectrum. There is much greater control over interferences on the sample channels in this case.
5-6-6. Mobile Phase Buffers

In general, salts and organic buffers are incompatible with electrospray, since they form adduct peaks and degrade the signal obtained. Also nonvolatile buffers such as phosphates and Tris tend to accumulate within the probe, and may cause blockages.

It has also been observed that trifluoroacetic acid (TFA) tends to suppress the electrospray signal to a significant extent (the signals obtained from peptides are typically 5 - 8 times better with 1% acetic acid than with 0.1% TFA). However, at around 0.05% or less, this effect is less pronounced. The LC separation should be re-tried at a TFA level at, or preferably below this level if signal suppression is thought to be occurring. Alternatively, post-column addition of “TFA-fix”, a mixture of proprionic acid and isopropanol, may be helpful.

Ammonium acetate, ammonium hydroxide and other volatile buffers are compatible with electrospray. 1 - 10 mM ammonium acetate causes no complications, although higher concentrations tend to reduce the sample signals.

1 - 3 mM sodium acetate or sodium chloride is also useful to force the production of sodium adducts of PEG or polypropylene glycol used as calibrants.
Section 6

Routine Cleaning and Maintenance
Routine Cleaning and Maintenance Of The Electrospray Interface, And Trouble Shooting

This chapter describes the routine cleaning procedures for the electrospray interface. The intervals between cleaning are largely dependant on the types of samples and experiments that are being run. As a rough guide, the counter electrode and sampling cone should be cleaned weekly or bi-weekly, the skimmer and skimmer lens cleaned at 3 to 6 month intervals, and the hexapole rods cleaned at 1 to 2 year intervals.

At one extreme, lengthy high resolution accurate mass experiments involving repeated injections of large quantities of samples and reference materials may result in a deterioration in performance within a few hours. At the other extreme, LC-MS experiments involving occasional injections of low levels of samples and using high purity water/methanol/acetonitrile/TFA solvent systems will cause far less contamination.

The regular use of an appropriate benchmark to quickly evaluate any deterioration in the performance of the interface is recommended. Good candidates are gramicidin-S or leucine enkephalin (for positive ionisation), and lauryl sulphate (for negative ionisation), since these compounds are stable in solution for long periods. Myoglobin or lysozyme or cytochrome C are also useful for characterisation of the high mass performance, but these are not necessarily stable, and the complexity of the spectra can make direct comparisons difficult.

Refer to the trouble shooting guide at the end of this section for specific causes and effects, and indications as to when cleaning or maintenance is necessary.

6-1. Maintaining The Electrospray Probe

The quality of the spray is critical if optimal performance is to be attained. If the spray is being generated entirely by electrostatic means (i.e. 'pure' electrospray), then the precise geometry of the needle tip is absolutely critical - refer to Chapter 7 for full details. Similarly, if instead a sheath liquid flow is being used (for instance to provide electrical contact to the tip in a capillary electrophoresis experiment), then special attention will have to be given to this aspect, which is not covered here.
If the more robust nebuliser assisted spray is in use, as described in section 4-5, then the needle tip geometry is much less critical. Routine checking and maintenance of this system is described below.

1. Check at the start of each day that the solvent lines are free of any blockage - either by observing the droplets at the needle tip before the nebuliser is turned on, or by observing an ion beam.

2. Also check daily that the nebuliser is operating at full power (refer to Figures 4-5-G and 4-5-H). Partial blockages can occur if concentrated samples were left in the probe overnight, or if solutions have slowly dribbled through the lines.

Remove the nebuliser tip and squirt a suitable solvent through it to dissolve any precipitate, and wipe off any dirt from the outside of the inner Type 34 needle.

If acid-containing solvents or chlorinated solvents such as dichloromethane have seeped into the nebuliser lines, then there may be damage to the Legris union on the probe, and/or to the 4 mm green polyurethane tube where it enters this fitting. Replace the fitting and/or trim back the 4 mm tubing to cure this problem.

3. Routinely check that the spray is at the optimal orientation in relation to the pepperpot, by wobbling the probe up, down and sideways and observing the effect on the ion beam. Gently bend the needle tip to achieve the optimum position.

Take this action if the needle has been accidentally knocked, or if the sensitivity is unexpectedly low.

4. Occasionally (weekly) check for correct operation of the probe microswitch, by pulling the probe a short way out of the housing when the HT is on and observing that the source high voltage readbacks drop to zero. It may occasionally be necessary to slightly adjust the angle of the microswitch spring lever so that the switch remains shut when the probe is in the housing.

5. In the unlikely event of the probe being dropped, check that the metal shaft is straight, and replace any damaged components.
Remove these three screws (fit M3 x 12 screws having heads compatible with the tools available, eg. use cap hd. with a hexagon ball driver, or cheese hd. with a screw gripping screwdriver).

Disconnect the blue (inlet) tube at one of the LegisUnions.

Disconnect the two wires to the heater / counter electrode (they can be refitted either way around, but cannot be cross-connected to the sampling cone).

Disconnect the wire to the Sampling cone.

Care: The metalwork may be hot!

Lift out the counter electrode assembly by holding the plastic gas baffle.
6-2. Cleaning The Counter Electrode

The counter electrode will collect the bulk of any non-volatile matter, and hence is the component most in need of cleaning. However, since this component operates at atmospheric pressure, it can become surprisingly dirty before any loss of performance is noticed. The chicane (pepper pot) version will protect the sampling cone from getting dirty more effectively than the straight - hole alternative. Both designs of counter electrode can be removed and cleaned without breaking the vacuum; however, it is usually advisable to clean the sampling cone at the same time.

Cleaning is usually indicated by (a) heavy contamination visible when looking into the atmospheric pressure source through the probe port, or (b) a 2- or 3- fold decrease in sensitivity, or (c) a marked decrease in stability that cannot be attributed to a spray problem. The procedure for cleaning the counter electrode by itself follows:

1. Turn off the solvent and gas flows, switch the instrument to Standby, and remove the probe from the source.
2. Warning. Double - check that the high voltages are really off, since there is no safety interlock to turn them off when the top hat assembly is removed.
3. Remove the top hat assembly by undoing the three M5 screws, to expose the atmospheric pressure chamber.
4. Disconnect the blue bath gas line at one of the unions.
5. Remove the three high voltage link wires.
6. Remove the counter electrode assembly by undoing the three screws, Figure 6-2-A. Pull out the counter electrode assembly from the atmospheric pressure chamber by holding the gas baffle - be careful, because the metal components can be quite hot.
7. Remove the two screws holding the counter electrode on the end of the heater.
8. The counter electrode can now be cleaned, as appropriate. Warning. If there is any possibility that the samples or solvents that have been sprayed are in any way hazardous, appropriate protective clothing, such as gloves and eye protection, must be worn.
**Step 1:**
Swab or gently scrape off deposits from both faces of each half.

**Step 2:**
Scrape out deposits from the bores using the cleaning tool inserted from direction A, under running water.

**Step 3:**
Rinse away any remaining particles or lint, shake dry (or blow dry) and reassemble.

**NOTE:**
BE AWARE OF ANY CHEMICAL OR BIOLOGICAL HAZARDS POSED BY THE SAMPLES DEPOSITED HERE, AND TAKE APPROPRIATE PRECAUTIONS!

**FIGURE 6-2-B. CLEANING THE PEPPERPOT COUNTER ELECTRODE**

**STEP 1:**
Clean the front and back faces by swabbing or gently scrubbing with a solvent-soaked cotton bud or tissue, until all deposits are removed

**STEP 2:**
Rinse away any remaining particles or lint, shake dry (or dry using lint-free tissues, or blow dry) and reassemble

**FIGURE 6-2-C. CLEANING THE STRAIGHT-THROUGH COUNTER ELECTRODE**
**Chicane (Pepper Pot) Design:**

Separate the two halves by removing the two screws, taking care that the small dowels do not get lost, see Figure 6-2. Remove heavy precipitates from the accessible surfaces using a solvent - soaked tissue or cotton bud or similar, or by gently scrubbing under running water. Remove deposits inside the flow passages by sliding the pepper pot cleaning tool backwards and forwards through each hole in turn, under running water (Figure 6-2-B). Finally, rinse the components thoroughly to remove any lint, shake them dry, and reassemble the two halves.

**Straight-hole Design:**

Remove heavy precipitates from the front and back surfaces using a solvent - soaked tissue or cotton bud or similar, or by gently scrubbing under running water (Figure 6-2-C). Rinse thoroughly to remove any lint, and shake dry.

9. **Note:** sonication is not always effective at removing stubborn deposits from the counter electrode, and thorough drying is not necessary.

10. Reassemble the counter electrode on the heater, and refit in the atmospheric pressure chamber. Reconnect the three wires (the two heater wires can go either way around) and reconnect the bath gas line, Figure 4-3-J. Fit the top hat assembly.

The system will be ready to use after 5 to 10 minutes (this allows the counter electrode to warm up and dry off).

**6-3. Cleaning The Electrospray Sampling Cone**

It is good practise to clean the sampling cone whenever the counter electrode is being cleaned. Because this involves venting the vacuum system, it is best to complete this procedure as quickly as possible (the longer that the turbo pump is off, the more slowly the ‘soft start’ programme will bring the pump up to full speed).

Indicators that the sampling cone requires cleaning are: (a) the counter electrode was heavily contaminated, (b) deposits can be seen on the sampling cone when the counter electrode is removed, (c) a 2- or 3-fold decrease in sensitivity, (d) a marked decrease in stability that cannot be attributed to a spray problem, or (e) a sudden and dramatic loss of sensitivity and/or stability that might be due to a hair having lodged on the orifice.
Areas requiring cleaning:

Step 1: inspect the bore, using a good eyeglass or low power microscope.

Step 2: Swab off deposits from the front and back faces using a cotton bud or tissue.

Step 3: Squirt solvent through the bore, using a 1 ml disposable plastic syringe, or a glass syringe with a Rheodyne needle port cleaner pressed firmly over the hole (NOTE: WEAR EYE PROTECTION!). Alternatively, clean using a fine wire.

Step 4: Rinse and dry, then re-inspect the bore prior to reassembly.

FIGURE 6-3-A. CLEANING THE ELECTROSpray SAMPLING CONE

Appearances of the bore on close inspection:

- Clean and clear: circular, with sharp edges.
- Hair or particle trapped within bore.
- Trapped particle or burr from one of the faces.
- Hair across the hole: characteristic beam instability.
- Bore partially occluded: characteristic decrease in turbo backing pressure.
A slow buildup of contamination may be accompanied by a gradual decrease in vacuum pressures. A complete blockage by for instance a particle that has sloughed off the counter electrode will often result in a sudden large pressure decrease, possibly accompanied by high voltage discharges. This can occur because the pressure in the first pumping stage has fallen to the discharge range. In contrast, a hair lodged across the orifice rarely causes any noticeable change in the vacuum pressures. If it is suspected that the sampling cone requires cleaning, then the counter electrode should be cleaned at the same time.

1. In addition to the counter electrode cleaning procedure described above, do the following:

2. Check that the three thumbscrews are tight, so that the atmospheric pressure chamber will not slip from its optimal position when the housing is vented.

3. Vent the electrospray and source housing (the procedure is described in section 4-9-1).

4. Remove the red waste line, the blue bath gas line, and the three wires from the atmospheric pressure chamber, as necessary.

5. Loosen the top one of the three thumb screws, and lift out the atmospheric pressure chamber from the electrospray housing.

6. The first time the sampling cone is removed from the atmospheric pressure chamber, make a mark on it (for instance, scratch an ‘X’ near the wire connection) so that it can be put back in the same orientation.

7. The sampling cone can now be cleaned. **Warning. If there is any possibility that the samples or solvents that have been sprayed are in any way hazardous, appropriate protective clothing, such as gloves and eye protection, must be worn.**

8. Examine the sampling cone for contamination using a good eyeglass or a low-power microscope, Figure 6-3-A. There are three areas requiring cleaning, namely the front, the back and the bore, and it is critical that they are each cleaned thoroughly. The deposits on the front and back faces are usually easily wiped off using a tissue soaked in an appropriate solvent (i.e. something in which the electrosprayed samples are soluble), see Figure 6-3- A.

(Note - it should not be necessary to use any abrasives to remove deposits from the electrospray sampling cones, in contrast to the cleaning procedure for APCI sampling cones).

9. The bore can usually be cleaned by squirting about 1 ml of an appropriate solvent through it using a disposable plastic syringe. Alternatively, the teflon needle port cleaner supplied with various Rheodyne HPLC injectors can be used to form a seal between a metal or glass syringe and the sampling cone.
10. Blow out any liquid remaining in the bore, and examine the bore very carefully using a good eyeglass or a low-magnification microscope. All deposits, burrs, or small obstructions MUST be removed for optimum performance to be obtained. Stubborn deposits may be removed using the fine cleaning wires supplied with Hamilton syringes, or by judicious use of a length of 150 um OD fused silica, for instance.

11. Make sure that the sampling cone is free of any lint which might get sucked across the orifice, then reassemble it on the atmospheric pressure chamber, taking care to trap the wire under the nearest screw. Tighten the screws until the sampling cone is pulled down and in contact with the plastic chamber, but avoid over-tightening them.

12. Offer up the atmospheric pressure chamber to the electrospray housing, tighten the top thumbscrew that was loosened to remove it, and begin pumping down. Turbo pump controllers having the ‘soft start’ feature may take 45 minutes to bring the pump up to full speed, so that if the cleaning can be scheduled, it is useful to do it before lunch or at the end of the day.

13. Make the gas and electrical connections to the atmospheric pressure chamber, and re-fit the top hat assembly. The interface will be ready to use when the turbo pump has reached at least 95% speed.

6-4. Cleaning The Skimmer And Skimmer Lens

The skimmer and skimmer lens require only infrequent cleaning, one reason being that the optimum position of the sampling cone is typically ~1 to 2 mm off-axis. If dyes have been electrosprayed, then coloured spots around the side of the cone will be visible; the tip of the cone remains relatively clean.

It is worthwhile to occasionally inspect the skimmer and skimmer lens visually when the atmospheric pressure chamber has been removed in order to clean the sampling cone. As a rough guide, the skimmer assembly will require cleaning at approximately 3 to 6 month intervals. Indications that cleaning is necessary are reduced sensitivity and/or stability that cannot be attributed to spray problems or contamination of the counter electrode or sampling cone.

Note: Because of the long intervals between cleaning and the variety of samples analysed during these periods, it is safest to assume that the skimmer assembly is contaminated with traces of hazardous compounds, and it is suggested that suitable protective clothing be worn during cleaning procedures.
Avoid touching the cone; if the edge is damaged the skimmer should be replaced.

Skimmer, M805169CD1
(or M805271CD1 without the skimmer lens)

Solvent-soaked cotton bud

Remove contamination from all surfaces of all items (except the screws) by swabbing with solvent soaked cotton buds or tissues.
The skimmer cone tip is extremely thin and delicate, and rough handling can cause considerable damage. The edge must remain clean, sharp and round, without burrs or dents, to maintain optimal performance. Do not invert it on a bench top, or touch it with metal tools, for instance. If a skimmer is accidentally damaged, it is usually best to obtain a replacement rather than to try to correct such damage.

The skimmer lens cone tip is also very delicate, but it is rather less critical - if it is damaged it is usually possible bend the tip back to approximately the original shape without any loss of performance.

6-4-1. Cleaning Of The Skimmer - Only Design

The outside face of the skimmer can be cleaned without removal from interfaces lacking the additional skimmer lens, simply by swabbing up the contamination using a solvent - soaked tissue or cotton bud, as shown in Figure 6-4-A. Use a circling motion around the cone, and avoid exerting any pressure at or near its tip.

The inside of the cone of the skimmer can be gently wiped clean in a similar manner, after it has been removed from the lens stack. Note though that accidental damage is much more likely to occur when the component has been removed. There is usually no benefit gained by sonication as opposed to swabbing.

Be careful not to touch the tip of the cone when re-installing it in the lens stack (as scrapings of skin can easily lodge in the cone).

6-4-2. Cleaning Of The Skimmer Lens/Skimmer Design

It is not possible to clean this design whilst it is still mounted on the lens stack, since the skimmer cone is not accessible. Carefully remove the assembly, and separate the skimmer lens from the skimmer. Clean each item inside and out by gentle swabbing with a solvent - soaked tissue or cotton bud, using a circular motion around the delicate cones, as shown in Figure 6-4-B. There is usually no benefit gained by sonication as opposed to swabbing.

Also clean the beige PEEK spacers and dowels by wiping with a suitable solvent. Mount the skimmer lens back on the skimmer outside of the housing, and check that the two cones are visually concentric. Be careful not to touch the tips of either cone when re-installing the assembly in the lens stack.
Remove the RF PCB's and the feedthrough, and poke the three wires inside the housing end of transfer lens stack.

Small block of scrap material, eg, aluminum, held against transfer lens stack.

Tap lens stack out using a large spanner or a small hammer. TAKE CARE NOT TO DAMAGE THE COMPONENTS INSIDE THE HOUSING!

Aluminum handling rods, M802580AD1 (Screw into the acetal plate underneath the skimmer).

FIGURE 6-5A. REMOVING THE LENS STACK FROM THE HOUSING

Section 6 Routine Cleaning and Maintenance Of The Electrospray Interface, And Trouble Shooting
6-5. Cleaning The Hexapole Lens

It is unlikely that the hexapole lens will require routine cleaning more frequently than once every one or two years; however, it is possible that in the event of a major vacuum accident or a major sustained high voltage discharge it becomes contaminated.

The condition of the hexapole rods can be checked when the system is vented, both by removing the skimmer assembly and viewing the rods end-on with a torch, and by removing either the hexapole feedthrough or the turbo pump. Evidence of significant white, grey, brown or black films, or oil residues, on the rods or the plastic insulators, coupled with an otherwise unexplainable decrease in performance, suggests that cleaning is necessary.

6-5-1. Removing The Lens Stack From The Housing

1. Vent the interface. Remove the atmospheric pressure chamber from the electrospray housing, and the source cradle from the instrument housing.

2. Remove the skimmer lens/skimmer assembly from the lens stack, being very careful not to damage the extremely delicate cones, and store it in a safe place.

3. Unplug the two cables to the hexapole PCB's on top of the housing, and remove the cover. Unscrew the two bolts attaching the bottom PCB and the feedthrough flange to the housing (there is no need to separate the two boards to do this). Lift away the boards & flange, and disconnect the three wires.

4. Poke the three wires down inside the housing, such that they will not become trapped when the lens stack is removed.

5. Two methods to extract the lens stack may be used. After removing the four M3 x 30 screws securing the stack, firstly try to twist and pull it out using the two aluminium pillars provided, see Figure 6-5-A. The stack is often a tight fit in the housing, so the second method involves tapping it out using a light hammer or similar object - be extremely careful to avoid damaging the source optics or re-entrant! (If in doubt, remove the whole housing from the instrument before attempting this). Also be careful not to trap the three wires.
6-5-2. Cleaning The Hexapole Lens

Depending on the severity of the contamination, cleaning if the rods alone, or of all of the components, may be required. The rods alone can be cleaned without complete disassembly of the lens stack, and this is all that is likely to be necessary on a routine basis.

Note: Because of the long intervals between cleaning and the variety of samples analysed during these periods, it is safest to assume that the rods are contaminated with traces of hazardous compounds, and it is suggested that suitable protective clothing be worn during cleaning procedures.

6-5-2-1. Cleaning Only The Rods And Support Disks

1. Measure the gap between the inner ends of the hexapole rods and the ring electrode (it should be 0.5 or 1.0 mm, depending on the design).

2. Carefully note the orientation of the copper wires in relation to the rest of the stack, to aid with reassembly.

3. Remove the twelve screws or studs securing the rods to the plastic support disks - the specific methods of attachment vary somewhat, so note carefully how it has been done. The copper wires to the rods will have to be removed to enable access.

4. Contamination can be removed from the rods by wiping off with a solvent - soaked tissue, followed by sonication and rinsing in suitable solvents. Be very careful to handle the rods gently, to avoid bending them.

5. If one rod is accidentally damaged (bent) during handling, then it is best to purchase another set of six and to replace them all. Slight scratches, however, do not cause any deterioration in performance.

6. Using a solvent - soaked tissue or cotton bud, carefully wipe off any contamination (whether it is visible or not) from the inner faces of the hexapole support disks, and from around the orifice in the ring electrode. (If discolouration is visible around this orifice, it should reveal an even, symmetrical pattern. If the pattern is not symmetrical, it might suggest that the rods were not aligned correctly originally).

7. Reassemble the rods as appropriate, ensuring that the distance between the inner ends and the ring electrode is set to the original measured value, with a tolerance of 0.1 mm, by using a feeler gauge.

8. Use the feeler gauge to check that the rods are evenly spaced from each other after reassembly - the gap should be 0.45 to 0.55 mm at both ends and in the middle.

9. Re-fit the copper wires on the studs or screws nearest to the ring electrode, such that alternate rods are connected.

10. Check that the exponential resistors have not been disturbed or dislodged during handling, then proceed to re-fit the lens stack in the housing.
6-5-2-2. Cleaning The Whole Assembly

1. Measure the gap between the inner ends of the hexapole rods and the ring electrode (it should be 0.5 or 1.0 mm, depending on the design).

2. Carefully note the orientation of the hexapole wires, the resistors, the ring electrode screw holes, the accelerating voltage connection tag, and the spring contact for the skimmer in relation to each other, to assist in reassembly.

3. Remove the three (or for some designs six) cap head screws from the ends of the support rods, one of which has a spring contact on the side, from the skimmer support disk.

4. Lift off the skimmer support disk, then the metal spacers and the hexapole rod/support disk assembly, noting the order for reference during reassembly.

5. The ring electrode may also be removed for cleaning if required (note its orientation).

6. Clean the rods as described above. Thoroughly clean the hexapole support disks, and the various metal components.

7. After cleaning, attach the rods to the support disks loosely (so that they are able to move), and assemble them with the various spacers and the skimmer support plate on the support rods. Some early designs incorporate three long and three short support rods, while later designs have only the long rods. It is important that the hexapole support disks locate on the long, not the short, rods in the early designs.

8. Tighten the three (or six) screws in the skimmer support plate. It may also be necessary to hold or tighten the three screws at the other end of the lens stack. It is important that the lens stack is evenly compressed, so that all of the lenses remain parallel. To check this, measure the overall distance between the top lens plate and the skimmer support disk, and adjust the tension on the screws until it is within ~0.1 mm.

9. Adjust the distance between the hexapole rods and the ring electrode to that measured previously using a feeler gauge, to a tolerance of 0.1 mm, and tighten the screws or studs holding them.

10. Use the feeler gauge to check that the rods are evenly spaced from each other after reassembly - the gap should be 0.45 to 0.55 mm at both ends and in the middle.

11. Re-fit the copper wires on the studs or screws nearest to the ring electrode, such that alternate rods are connected.

12. Check that the exponential resistors have not been disturbed or dislodged during handling, then proceed to re-fit the lens stack in the housing.
(NOTE: it is not necessary to separate the two boards in order to remove or attach them to the API housing)

Holes for Allen key, for access to the M6 bolt heads

Connect FL3 and FL9 to J1 and J3 respectively, bending the wires so that they will not touch the PCB cover when it is fitted

Flying leads FL4, FL5 and FL6 each go to the nearest feedthrough pin as follows:
- FL4 - pin 4 - copper wire (Hex)
- FL5 - pin 2 - nichrome wire (Ring)
- FL3 - pin 3 - copper wire (Hex)

three wires: the copper ones go to the hexapole and the nichrome one goes to the ring electrode

FIGURE 6-5-B. CONNECTION OF THE RF BOARDS TO THE API HOUSING
6-5-3. Re-fitting The Lens Stack In The Housing

1. Check the condition of the ‘O’ rings around the ring electrode and within the groove behind the skimmer support plate, and smear them with a thin film of diffusion pump oil to ensure a good seal (this is particularly helpful for the sliding seal formed between the housing and the ring electrode).

2. Check the orientation of the lens stack - the exponential resistor series should be vertical on the left-hand side, and the three wires should be bent so that their free ends are at the top.

3. Offer up the stack to the housing, being careful that the three wires do not get trapped. Pull the three wires up through the hexapole feedthrough port, and tighten the four M3 x 30 screws holding the stack in place.

4. Re-fit the skimmer lens/skimmer assembly (care! the cones are very delicate!) and connect the two wires. Install the atmospheric pressure chamber and fit the top hat assembly.

5. Connect the two copper wires to the hexapole RF feedthrough pins, and the single nichrome (silver-coloured) wire to the ring electrode feedthrough pin, and secure the feedthrough and the two RF boards to the housing, Figure 6-5-B. Fit the cover, and reconnect the two RF control cables to the boards.

6. Fit the cradle in the instrument housing and pump down.

6-6. Cleaning The Exponential Lenses And Cradle

These lenses do not require any routine cleaning; however, if LSIMS has been used for a considerable period with the electrospray system fitted and without any shielding against the Cs+ beam, or if a major vacuum accident involving contamination with diffusion pump oil has occurred, the following procedures may be followed.

Indicators that the exponential lenses may require cleaning include: (a) observation of a significant whitish, grey, brown or matt black film on the plastic insulators, (b) oil residues on the insulators or lenses, or (c) consistently low sensitivity, which cannot be attributed to any other cause after exhaustive tests.

6-6-1. Cleaning The Cradle

Disassemble into individual components, taking careful note of the order. Clean as appropriate for contaminated metal and ceramics, and reassemble referring to the appropriate diagrams and parts lists.
6-6-2. Cleaning The Exponential Lens Stack

1. First remove it from the housing by following the procedure listed for cleaning the hexapole lens.

2. Remove the skimmer/skimmer lens assembly (care! the cones are very delicate!), and stand the lens on end with the exponential lenses uppermost.

3. Remove the small screw at the top end of the resistor wiring. Gently pull off the gold pins to free the resistors from the lenses, and finally disconnect the resistors from the ring electrode.

4. Remove the three screws holding the lenses in place, noting the position of the terminal to which the accelerating voltage is connected (i.e. to the rod that has a spring contact beneath the skimmer).

5. Lift off the lenses and insulators, noting the alternating positions of the tags that accept the gold pins.

6. The metal lenses can be cleaned in the usual way. The ring electrode and support rods can be wiped clean with a solvent such as methanol.

7. If the resistors are contaminated with oil, then rinse them briefly in methanol. Wipe off any other deposits.

8. If the plastic step insulators are coated with a white, grey, brown or black film then this must be removed manually (sonication is unsatisfactory). Firstly, investigate whether or not the film can be wiped off, using a tissue soaked in an appropriate solvent. If it is removable, then repeat the procedure with all of the insulators. Finally, rinse the cleaned insulators in a suitable solvent, such as methanol, and dry them. If the film is not removeable, the whole set of insulators must be replaced. If the contamination is due to diffusion pump oil, it will be able to be removed by sonication in solvents or detergents (without manual cleaning).

9. Reassemble the lenses and insulators, taking care to orientate the lenses correctly, and re-fit the resistor chain.

10. Re-fit the washers, accelerating voltage connection tag, and screws to the support rods. It is important that the lens stack is evenly compressed, so that all of the lenses remain parallel. To check this, measure the overall distance between the top lens plate and the skimmer support disk, and adjust the tension on the screws until it is within ~0.1 mm.

11. Install the lens stack in the electrospray housing following the instructions in the preceding section.
Section 6  Routine Cleaning and Maintenance Of The Electrospray Interface, And Trouble Shooting
6-7. Electrospray Trouble Shooting Guide

The observed symptoms are underlined, and are followed by possible diagnoses.

Suddenly Unstable Ion Signal:

1. Hair lodged on sampling cone - no change in operating pressures; spray is still OK; needle voltage may tune abnormally (especially when the nebuliser - assisted spray is not being used). It can be a significant problem if the laboratory is dusty, or carpeted, or if particles of lint were left in the atmospheric pressure chamber after routine cleaning.

2. Particle of dirt lodged on sampling cone. This may occur when the pepperpot is very dirty; if a flaky deposit occurs, particles can slough off and land on the orifice.

3. High frequency drop-out - does the frequency change as the ion energy and/or ring electrode are tuned? If yes, then the voltage differences between these and the hexapole is wrong.

4. High frequency drop-out; no effect of ion energy or ring electrode tuning - may be RF board is oscillating. Select a low mass, such as 500; does the RF neon indicator remain brightly lit? If yes, RF is oscillating. Contact an engineer.

5. Overheating or overload of the power supply components, in particular the 24 V regulator, in the electrospray trolley (a rare fault).

6. Earthed component (usually a wire) close to the first - stage pumping line; dim purple glow in the tube near the object. Move the object! The tube must remain clear of earthed objects, particularly at the source end! (This is rarely a major problem).

7. Other causes: gas supply has run out; unstable solvent delivery?

Gradually Less Stable Or Less Intense Ion Signal:

1. Buildup of dirt on sampling cone - gradual blockage. Vacuum pressures will fall; discharges in pumping line may be seen, particularly if the tube is tapped.

2. Contamination of skimmer lens, skimmer, or hexapole rods - this is a rare problem.

3. Contamination of the plastic spacers inside the instrument source - if LSIMS has been used while ESI is still mounted. The Cs+ beam is reflected from the glass lid onto the ESI lens stack; a film can be seen on the insulators. Clean them; wipe off the film and rinse in solvent. Shield the lens stack from the Cs+ beam.
Ion Current Pulses Slowly And Regularly (reciprocating H plc Pump Used):

It is fairly common that at low flow rates the output of a reciprocating design of HPLC pump will show significant pulsation. This can easily be seen from a TIC trace generated from a very narrow scan of a suitable solvent ion or continuously infused sample. If the pump incorporates a flow compensation facility, then this should be used.

If one of the pump check valves fails to operate, then the TIC trace may look like a square waveform, and the drop-outs should be able to be correlated with one or other of the piston strokes.

Ion Current Decreases Slowly (over Hours/ Days):

1. Source is dirty - clean it. Most often, it is only the pepperpot and sampling cone that require cleaning.
2. The test sample has degraded. Check the performance using a second reliable benchmark compound.

Ion Current Decreases Swiftly (After H t Turned O n):

1. Charging effects - dirty insulators ?
2. Spray is unstable; may be a ‘difficult' solvent system in use, or a high solvent flow rate, without sufficient nebuliser gas pressure/flow to fully stabilise it.
3. Current overload on one of the HT supplies - check the SIOSLOAD table for the accelerating voltage supply.
4. One of the HT supplies is faulty & produces decreasing output voltage as it reaches operating temperature. Most likely to affect the Needle supply, since all of the other supplies remain at 4 - 5 kV.
5. If the solvent flow rate is above ~20 µl/minute, it may be because the sampling cone orifice is getting wet if the heater is not working.

Consistently Low Intensity Ion Signals, Or No Beam:

1. One of the resistors in the exponential lens stack is the wrong value (a rare problem, normally detected during manufacture; might also occur as a result of an unusual high voltage discharge).
2. One of the resistors is damaged, or the gold pin is not making contact with the correct lens, or is shorting to a different lens (may occur as a result of careless handling).
3. Various other electrical continuity problems, for instance: accelerating voltage cable is not plugged in, spring contacts are failing to make contact, one of the wires to the atmospheric pressure chamber is not connected, the small wire to the sampling cone is not connected, or physical damage to one of the high voltage cables that results in breakdown.
**No Ion Beam Can Be Found:**

1. HT not on - check the readbacks. If all are off, then check that all trip switches are closed. (‘M’ instruments: M681554 ‘ff’=operate; ‘fd’=24V trip). Link out pins 3 + 5 at BR01 to temporarily bypass the trip circuit to test it (do not operate like this!).

2. Some HT supplies are on but others are off - check the HT boards are correctly seated in the units (they may become dislodged if the trolley is moved). Check the control wires are properly connected.

3. Some HT supplies are on but others are off - reboot SIOS and manually reboot the trolley microprocessor by either disconnecting the mains power (the plug labelled ‘ESP Control’ under the trolley) and waiting for **2 minutes** (see below) before reconnecting, or press the ‘reset’ switch on the MA3606-200 control PCB.

**No Beam (Some Other Occasional Causes):**

1. The magnet has tripped off (a water cooling problem?). This condition is not necessarily reported by the data system.

2. The instrument mode has been set to ‘Inversion’ accidentally.

3. The wrong source has been selected (for instance on an AutoSpec T).

4. The detector system has failed.

**No Beam Obtainable, Or No Control Of The Beam, With Unusual Readbacks**

The microprocessor in the MA3606-200 electrospray control PCB should be automatically rebooted whenever the SIOS is rebooted, but sometimes this does not happen and the microprocessor ‘locks’ completely. This fault is rare, but can take a long time to diagnose once it has occurred.

Symptoms of this include odd readbacks for the turbo pump speed and/or the electrospray high voltages. Some supplies may be reported as on while others are off, independantly of whether the instrument is in Operate or Standby mode. These readbacks may be changing of their own accord, or may be fixed at unusual values; they probably will not change when the turbo speed request is changed or when the electrospray tuning sliders are moved.

Shut down OPUS and turn off the SIOS, then manually reboot the trolley microprocessor by either disconnecting the mains power (the plug labelled ‘ESP Control’ under the trolley) and waiting for 2 minutes (to allow time for the capacitors in the power supply to discharge) before reconnecting, or press the ‘reset’ switch on the MA3606-200 control PCB. Then power up the SIOS and restart OPUS: the electrospray system should now behave normally.
Accelerating Voltage Is Sometimes Reported As Low, Or Overloaded:

The rise, overshoot and settling times of the three separate high voltage supplies used in the electrospray source must be reasonably well matched. If this is not so, then it is possible that the use of an unmatched supply will result in an unusual, possibly latching, source discharge. It usually occurs with the instrument accelerating voltage supply, although might also occur with the other supplies. It is caused by unexpectedly large voltage differences during the switch-on phase. Characteristic indications are:

1. There is no problem obtaining a stable beam at 2 or 3 kV, with sensible voltage readbacks, but only an intermittent beam, or no beam, is obtained at 4 kV. The signal is simply either absent or present (a weak or unstable signal indicates a different problem).

2. When there is no beam, the accelerating voltage readback in the Source Supplies SIOSLOAD table is low, and the current is at ‘Overload’ (254 µA is the maximum).

3. One of the three high voltage supply PCB’s is different from the other two. Perhaps the electrospray problem has appeared shortly after the instrument accelerating voltage supply PCB has been repaired, swapped or replaced? Note that there are two makes of the MA3268 high voltage boards, one is manufactured by Brandenburg and the other is manufactured by Micromass. These types should never be mixed, because the switch-on characteristics are different.

4. The problem will be cured by exchanging the unmatched high voltage PCB for one of the same type as the other two.

5. (The above problem has not yet been observed on any ‘M’ series instruments, presumably because the different types of supplies used for the accelerating voltage and the electrospray supplies are reasonably well matched).

Needle Voltage Is Low; Signals May Be Weak Or Nonexistent:

1. If a high ionic strength solvent system is used with a relatively wide-bore sample line, then the current drain to ground may be sufficient to overload the supply. This has been noticed when spraying 0.1% TFA solutions using the red PEEK sample lines. The problem is solved by switching to a narrower diameter and/or longer length of transfer line, or alternatively by decreasing the conductivity of the solution.

2. The needle may simply be too near to the counter electrode; to test this pull back the probe by ~3 to 5 cm, and manually close the microswitch.
3. There may be a fault with the synchronisation circuit, which is tuned to produce a ~40 kHz signal during factory setup of the MA3606-200 control PCB. If this circuit fails, the boards can usually produce ~4 to 6 kV, but not more.

4. The individual high voltage supply may be faulty. To test this, swap BOTH the high voltage cables and the control (D-type) cables between the other supplies.

**Unusual Mass Spectra Obtained (poor High Mass Performance):**

Gramicidin-S: intensity of the singly charged ion relative to the doubly charged ion is much higher than normal; usual ratio cannot be obtained by reducing the sampling cone voltage.

Myoglobin: multiply charged ion series is unusually weak, with very significant peak tailing; intense ion at 616 (and 648 methand adduct) from the heme moiety.

Low mass sensitivity (gramicidin-S or raffinose) is OK but high mass sensitivity (proteins) is dreadful.

... Check for vacuum leaks, as this is usually a pressure effect. A leak on the analyser should be suspected if nothing obvious shows up - measure both Analyser 1 and Analyser 2. An array window may suddenly leak!

**Poor Negative Ion Sensitivity/Stability:**

Can ozone be smelled when the probe is in the spray tester? Is a blue glow visible at the needle tip? - If yes, reduce the voltage gradient on the needle tip by (a) decreasing the voltage, (b) increasing the distance, and/or (c) carefully polishing the needle tip to remove any burrs or particles. A corona discharge is not wanted.

**Poor Low Mass Sensitivity:**

1. Solvent ions not seen, but signal is OK at higher m/z ranges - RF board not tracking properly with mass. Set up the diode function generator on the control PCB. Note that the control PCB is set up to allow operation at 4kV ONLY; ESI cannot be used at other accelerating voltages! The set up depends on the magnet type! The standard coils must be used!

2. Low mass ions sometimes seen (e.g. in a magnet scan) but are hard to find by manual tuning: the diode function generator does not process low-mass RF requests easily. If the magnet mass is decreased by **dragging** the number down from ~200 using the mouse, low mass ions should be present. In contrast, if the magnet jumps through zero, for instance if ‘50’ is typed in the magnet menu, the diode function generator may fail to send the correct signal to the RF board.

3. Low mass sensitivity is generally poor; do not overlook the possibility that a fault has developed on the instrument, as opposed to the electrospray interface.
**Poor Ion Stability (A Mild Problem):**

Faulty grounding of the bottom end of the resistor chain in the first pumping stage. (A purple glow may be seen). Touch a ground wire on the KF-25 fittings at the bottom of the tube to see whether the stability improves. (This is a very rare fault, but if it is found then the grounding of the rotary pump MUST be checked for safety reasons).

**Unstable Spray, Using Stable Solvent Flow And Nebulisation:**

Needles are built correctly and are clean; solvent delivery is proved to be stable (and within a reasonable range); a 'good' electrospray solvent, such as 1:1 water:methanol, is used; nebuliser power is satisfactory; however, the spray pulses erratically when the nebuliser is operating. Try placing a few millilitres of methanol in the 4 mm nebuliser tube so that it is squirted through the nebuliser lines when the gas is turned on. This will probably solve the problem. The cause is not known, but possible explanations include the presence of contaminating materials in the nebuliser assembly, which are dissolved or blasted out by the slug of methanol. The problem does not seem to reoccur once this has been done.

**Sampling Cone Tunes At Maximum For Low Mass Ions:**

If the voltages are correctly set then the sampling cone slider should be at ~15 to 30% for optimum transmission of gramicidin-S or raffinose or similar. If it unexpectedly optimises at 100% then the source may be cold (has a heater wire slipped off? has the source just been installed?). Note that the heater is off by default after SIOS has been rebooted, even though a recalled setup menu may indicate that it is on.

**Loop Injections Are Not Reproducible:**

1. Possibly due to wrong or poor injection technique, for instance the use of the wrong type of syringe for the injector. Consult the technical information on the injector.

2. A faulty injector. Pressure - test the injector, and check for leaks from all of the connections, and also unexpected dripping from the waste ports or the injection port. The rotor seal may require replacement.

3. Precipitates present in the sample are blocking the tubing to varying degrees.

4. Consider testing the injector in an HPLC system, or failing that perform simple tests such as the injection of suitable dyes, which can then be observed in the flow stream.
Turbo Pump Fails To Operate, Or Is Slow:

1. (Check that the cables are connected and that power is reaching the mains switch).

2. (Check that there is communication between the SIOS and the electrospray unit; the high voltage readbacks should be behaving normally).

3. The EPROM in the controller has a ‘soft-start’ feature, whereby it will come up to speed slowly, over a period of up to ~45 hours, depending on the storage time and storage conditions of the pump prior to start-up.

4. It is possible that in the event of a high-voltage accident, the EPROM in the controller will be wiped clean. If a discharge was heard, and then the pump stops, suspect this cause.

5. If the pump is very hot it will automatically switch off (when a sensor near the bearing reaches ~65 deg. Celsius). Check the water flow through the cooling lines if this occurs, then restart the pump using the data system ‘reset’ switch in the ‘ES Vacuum’ menu.

6. Varian has a service agreement by which they will swiftly replace components which have failed within the warranty period - contact Varian in the first instance, or contact Micromass for advice.

7. If the pump is making unusual noises, in particular a high-pitched whine, then it is possible that the bearing is in poor condition. Contact Varian for advice.

Spray Problems (Particularly If Nebuliser Not Often Used):

With ~5 to 7 bar gas pressure, and Type 34/24 spray needles, the nebuliser flow meter should register ~5 to 10 l/hour. If not ..... Unusually high gas flow: check Nebuliser setup. Check for gas leaks particularly at the Legris unions. Get used to the feel of the double junction in these unions characterised by two resistances, firstly when the tube enters the brass clamp-ring, and secondly when it enters the sealing ‘O’-ring.

Certain solvents (for instance dichloromethane) can soften or damage the polyurethane tubing or the O-rings in the Legris fittings, if they are allowed to seep back into the nebuliser lines.

Unusually low gas flow: check for blockages. These occur most often at the needle tip assembly. Solids may build up in the annular space between the two needles, particularly when the nebuliser has not been used for a while. Remove the Type 24 needle and flush with appropriate solvent. If the reference marks on the needle and probe body are not aligned, it is possible that the needle tubes can occlude the gas line in the needle tip assembly.
To check nebuliser power: hold probe vertically, and direct the needle tip downwards into a vial of water. With the needle approximately at the surface of the water, the gas jet should push down into the water by a minimum of ~15 to 20 mm. With experience, the power of the nebuliser jet can be judged by feel more easily. The jet should be able to be felt on the palm of ones hand, ~20 cm or 6 inches from the needle tip.

To check nebuliser angle: set a solvent flow of above ~20 µl/minute (anything less may be hard to see) and observe the angle of the spray using the spray tester or by spraying against a dark surface (for instance the black plastic of the ESI housing).

**Very Low Sensitivity, And Poor Stability; Nebuliser Working Badly Or Not Used:**

If a pepper pot counter electrode is fitted, then the nebuliser gas must be used! If the nebuliser is not used, or becomes partially or completely blocked, sensitivity and stability will suffer.

In one example, the nebuliser became partially blocked, and the gas jet went to a depth of only ~5 mm into water (see above), while the flow meter registered ~2 L/hour. The ion beam consisted only of a few single ion spikes. When the two halves of the pepper pot were removed from the atmospheric pressure chamber and the ion beam was compared, a 2500-fold stronger signal was obtained. The probe position and the voltages were not altered to do this; the electrospray was formed between the needle tip and the sampling cone.

**Collapsed Resistor Pumping Tube:**

If the first stage pumping tube collapses, it must be replaced & cannot be repaired. The collapsed region will gradually spread. This collapse is rare, and usually occurs as a result of mechanical damage, or possibly solvent damage.
Section 7

Alternative Electrospray Needle Configurations
Alternative Electrospay Needle Configurations

The way in which the needles and transfer lines in the electrospay probe should be constructed depends primarily upon the type of experiment which is to be carried out. The main considerations are the flow rate range, and the type of sample introduction to be employed. If for instance the probe is to be optimised for small volume injections at flows of ~1 - 5 µl/minute, then all dead volumes must be minimised by using small I.D. connecting tubing and by very careful construction of any unions. If instead the probe is to be used for LC-MS at 500 µl/minute then dead volumes are much less of a problem, and larger I.D. connecting tubing will be required.

The standard method of building the probe described in section 4-5, with the double layer steel needles and red PEEK connecting tubing, is ideal for flow rates in excess of ~10 µl/minute and injection volumes of ~10 µl or more. This chapter describes some alternative ways of building and using the electrospay probe.

7-1. Consideration Of Dead Volumes

Since the highest ESI sensitivity is obtained at the lowest flow rates, and since standard LC fittings (injectors, tubing, unions etc.) are primarily designed for use at around 1 ml/minute, an appreciation of the adverse effects of dead volumes on injection peak broadening and tailing is important.

Various excellent technical publications available from Rheodyne (P.O. Box 996, Cotati, California 94928, USA) detail the effects of dead volumes in injectors, tubing and unions, in particular Technical Note no. 9. Similar information can often be found in HPLC catalogs or publications.

To keep dead volumes to a minimum, particularly when working at low flow rates and with small injection volumes, rigorously observe the following:

1. Select connecting tubing between the solvent delivery system and the ESI probe having the minimum internal volume compatible with the application, for instance 50 µm I.D. fused silica capillary tubing. (The transfer line provides electrical isolation, and so the minimum length is approximately 0.5 m).

2. Minimise the number of unions between the injector and probe tip; for instance use the triple-layer needle assembly, in which the fused silica goes right through to the needle tip.
3. Ensure that all unions are constructed in such a way that dead volumes are kept to a minimum. For instance, all tube ends must be cut clean and square, the tubes should bottom in the fitting before tightening, and the fittings should not be subsequently swapped with other unions. Use types of nuts and ferrules that are compatible with the make of union.

4. If capillary tubing is used, then ensure that all sleeving satisfactorily fills any cavities. This applies to the termination between the type 34 needle and the blue PEEK tube used in the standard needle assembly (section 4-5) and to any unions made with fused silica or microbore PEEK tubing. The I.D. of the sleeving must closely match the O.D. of the capillary tube; for instance, use orange (0.5 mm I.D.) PEEK tube to sleeve 375 µm O.D. fused silica or 0.020" O.D. microbore PEEK tube.

5. Select a good - quality low dispersion injector which is designed for low volume, low flow rate applications. A Rheodyne model 8125 injector is significantly better than a model 7125 injector, for instance. Choose an appropriate loop size and loop filling method.

6. Site the injector as close as possible to the ESI probe, for instance on the probe injector bracket (a long length of tubing between the solvent delivery system and the injector is not important). If LC-MS is to be carried out without any UV detection, then consider mounting the LC column on the probe as well.

7-2. Operation At Low Flow Rates With The Triple - Layer Needle

This section describes the complete procedure for building the electrospray probe, using a triple layer needle with the nebuliser but without the sheath flow option. This configuration has the minimum possible internal volume, and is thus ideal if very small volumes of sample are to be injected at low flow rates. However, as a general guide, it is significantly easier to obtain a stable, robust spray using the double layer steel needle assembly, as described in section 4-5.

If used in ‘pure electrospray’ mode (that is, without the use of nebuliser assistance), then the solvent systems must contain around 50 to 100% of organic solvent, and the quality of the spray will depend largely on the precise geometry of the needle tips. Furthermore, the straight - hole counter electrode is required in this case, or alternatively the counter electrode can be removed altogether (the pepperpot counter electrode should not be used; see section 4-5-4).

The spray tester will also be required, to allow visual inspection the quality of the electrospray when using the triple layer needle assembly.
7-2-1. Building The Probe With The Triple - Layer Needle

1. Strip down the probe and remove the type 34 sample needle and type 24 nebuliser needle and associated fittings (see section 4-5), and store them in a safe place.

2. Check that the 150 µm O.D. fused silica can pass freely through the type 27 inner needle, item 69. Slide the PEEK ferrules with their steel backing rings on to the 1/16" part of the needles as shown in Figure 7-2-A.

3. Position the needles in a suitable HPLC union (item 81 for instance) one at a time, and gently tighten a bush to clamp the two parts of the ferrule (item 80) together, Figure 7-2-B. Do not overtighten! The ferrule should lightly grip the 1/16" part of the needle, so as to give a stiff, sliding fit. The aim of this step is to lightly ‘swage’ the ferrule on to the tubing - it can alternatively be carried out within the nebuliser tee.

FIGURE 7-2-A.

FIGURE 7-2-B.
4. Remove the fused silica gripper from the probe shaft if fitted, and slide the inner needle (item 69) with the ferrule into position in the nebuliser tee, Figure 7-2-C. Align the back of the inner needle with the reference marks on the probe shaft (item 1). Then gently tighten the Rheodyne short bush (item 79), checking that the position of the needle does not change. The compression of the steel ring on to the PEEK part of the ferrule will cause the ferrule to grip the needle tightly, so that the union can be made and broken many times without further adjustment.

![Figure 7-2-C](image)

5. Repeat steps 3 and 4 with the nebuliser needle, item 70. The swaging can be done easily in the nebuliser tee in this case. Adjust the needles so that the outer needle is 0.5 - 1 mm back from the inner needle, Figure 7-2-D.

![Figure 7-2-D](image)

6. Choose the fused silica with the most suitable I.D. according to the application and the solvent delivery system to be used. 50 μm ID x 150 μm OD is generally ideal, but if the flow will be driven by gas pressure then 75 μm ID x 150 μm OD may be better.

Cut the lengths of fused silica and teflon tube as shown in Figure 7-2-E. (use these as guidelines only - the overall lengths are not critical!). The fused silica should be approx. 0.25 - 0.3 m longer than the teflon.

![Figure 7-2-E](image)
7. Make a shallow nick through to the bore of the 0.006” ID teflon tube (item 77), 0.15 m from one end. Slide the fused silica through the teflon tube. Fit the HPLC union 0.20 m from the end of the teflon tube, 0.05 m from the nick. Slide the short lengths of wide-bore teflon over both ends of the tubing, Figure 7-2- F.

Principle of operation: the primary seal is made at point ‘A’, typically by clamping the teflon tube on to the fused silica. Since this seal is not easy to make, an external union (item 81) is also fitted. If the primary seal leaks, then solvent will escape through the nick and will not be able to pass the external union. The wide-bore teflon adds rigidity to the weakened area in the vicinity of the nick.

Although the use of the nick and the external union are not essential, they are very effective in preventing the electrospray probe voltage from being pulled down towards ground potential. This can occur if the primary seal, ‘A’, leaks and a continuous column of conducting solvent is formed in the space between the fused silica capillary and its teflon sheath.

8. Remove the perspex cover (item 4) from the probe to gain access to the probe chamber (check first that the instrument is in standby). Feed the exposed fused silica and 1/16” teflon tube through the hole in the rear wall of the probe body (item 3), and lodge the short length of wide - bore teflon in the hole, Figure 7-2-G. This provides extra support for the fused silica where it enters the probe body.
9. Taking care not to snap the exposed length of fused silica, slide a Valco - or Waters - type steel bush (item 75) and a 1/16" teflon ferrule (item 80) on to the teflon tube. Carefully feed the exposed fused silica through the central sample/solvent port, Figure 7-2-H. (If resistance is felt, pull it back slightly and try again). When the end of the silica is observed in the front access area, pull it through and guide it past the nebuliser tee. Slide the 1/16" teflon sheath (item 77) in to the port and tighten the steel bush until the tube is gripped (do not overtighten).

10. Slide the fused silica gripper and tygon tube (items 9 and 88) over the fused silica and rest it against the shaft as shown in Figure 7-2-J. Be careful not to snap the fused silica. (The screw, item 41, should be loose).

11. Slide two of the short lengths of tygon tube (item 88) over the ends of the sharp-pointed steel tweezers that are provided in the AutoSpec tool kit, Figure 7-2-K. This provides an ideal tool for feeding the fused silica through the needle assembly.
12. Grip the end of the fused silica gently but firmly between the protected points of the tweezers, and feed it through the inner steel needle (item 69). Figure 7-2-L. Ensure that the silica is held on-axis, and that the loop of silica is not unduly stressed.

![Figure 7-2-L](image)

13. When the loop of fused silica has completely disappeared, slide the gripper assembly into position and attach it to the shaft with the screw, item 32, Figure 7-2-M.

![Figure 7-2-M](image)

14. Using the tweezers with protected points, very gently ease the excess fused silica back in to the probe shaft, Figure 7-2-N. The silica may be held either side of the gripper assembly. Stop the silica from springing back by holding it between your fingers whilst re-positioning the tweezers.

Because the silica is very thin, it can twist and coil within the probe shaft and the rear access area. If the fused silica snaps during use, the coiled length can be re-fitted in the needle assembly without the need for complete replacement.

![Figure 7-2-N](image)
FINE ADJUSTMENTS TO THE TIP OF THE TRIPLE-LAYER NEEDLE REQUIRED TO ACHIEVE A GOOD ELECTROSPRAY

Silica clean and square-cut: no jagged edges. Polish with very fine abrasive paper and examine with a good eye-glass.

D (150µm)

L (~0.5 to 1mm)

(Adjust until it is equal to D; that is, 100-200µm)

Inner Needle clean and square-cut, with rounded edges as shown. Polish with very fine abrasive paper (e.g., 1200 grade) and examine with a good eye-glass.

Outer Steel Needle

TO CONNECT DIRECTLY TO THE PRESSURISED VIAL ASSEMBLY:

Assembly:

Snip off any excess Fused Silica

Poke to bottom of Vial

Fused Silica

Teflon Sheath (Item 77)

86 Teflon Ferrule

40 mm

Steel Bush (Item 75 or Similar)

Waste

Cut off a few millimeters of fused silica / teflon using a sharp scalpel or razor blade to give a clean, square cut.

Attach to the fitting using a teflon ferrule (Item 86) and an appropriate type of bush

FIGURE 7-2-Q
15. Pull the silica back until approximately 0.15 mm protrudes from the tip of the inner steel needle, item 69. When viewed with a good eyeglass the tip should appear square and clean, Figure 7-2-P. Hold the silica in this position with the tweezers and gently tighten the gripper screw, item 41, until the fused silica is held in place. Do not overtighten.

16. Ensure that the sheath flow port is plugged, using the special plug (item 73) and the long bush (item 12) provided.

17. Re-fit the perspex probe cover, item 4.

18. Make the connection to the solvent delivery system, or to an injector, as shown in Figure 7-2-Q.

19. Test for solvent flow through the probe. In particular, look for leaks in the region of the nick in the teflon sheath. Tighten the appropriate fittings if necessary.

20. If no flow is observed, it is likely that the inlet end of the fused silica is blocked. Either snip off a few millimeters of fused silica using a proprietary fused silica cutter and re-insert it in the pressurised vial, or slice off a few millimeters of teflon and fused silica and re-make the fitting (see point 18).

7-2-2. Partial Replacement Of The Fused Silica

If the fused silica breaks in or near the needle assembly, then the following short procedure can be used to get the probe back in operation.

1. Ensure that the instrument is in ‘Standby’. Remove the perspex probe cover.

2. Slacken off the screw (item 41) gripping the fused silica. Remove the broken length of fused silica, and assess exactly where the break is. If there is estimated to be 100mm or more of fused silica protruding from the teflon sheath (item 77), then the following procedure can be followed. If not, it will be necessary to completely replace the fused silica - refer to points 6 through to 20.

3. Slacken off the steel bush holding the fused silica in the sample/solvent port, and carefully withdraw the tubing from the probe shaft. If the teflon ferrule remains behind in the port, retrieve it using a suitable tool (a short length of 1/16” steel tube, or a small screwdriver, are usually satisfactory).

4. Re-fit the steel bush (item 75) and the teflon ferrule (item 86) on the teflon tube (item 77). The same ferrule can be re-used, if it is not too badly damaged. Pull the ferrule some way past the point where it had previously swaged on to the teflon tube.
FIGURE 7-2-R. THE SPRAY TESTER

FIGURE 7-2-S. VARIOUS TYPES OF ELECTROSPRAY OBSERVED WITH THE SPRAY TESTER

ALTERNATE SPRAY AND LARGE DROPLETS
Voltage too low / Distance too great / Solvent flow too high / Aqueous content too high.

GOOD SPRAY.
Stable spray, central / Symmetrical, Very fine droplets.

MULTI-POINT, MAYBE SHIFTING AROUND
Voltage too high and/or needle too close.

SINGLE JET, NO SPRAY AT ALL
Needle positions unsatisfactory?

GOOD SPRAY, BUT OFF TO ONE SIDE.
Probably a burr on one side of one of the needles - Uneven electric field.
5. Try to straighten out the teflon tube, removing the natural curve, for a distance of ~ 100 mm from its end, by gently bending it. Now feed the fused silica and teflon tube through the central sample/solvent port, so that the silica is protruding a little further than the needle tip. The end of the teflon tube will now be somewhere inside the probe shaft. Note that if the teflon tube is not carefully straightened then it may snag in the rear access area (see Figure 7-2-H). If this happens, there is a possibility that the fused silica will snap near where it exits the teflon tube.

6. Continue to build the probe as described in points 10 through to 20 in section 7-2-1.

7-2-3. The Spray Tester

In order to obtain high quality electrospray mass spectra, it is first necessary to obtain a high quality, stable spray. The spray tester is shown schematically in Figure 7-2-R.

The principle of operation is as follows. The electrospray probe is inserted into the tester, and the thumbscrew engages the microswitch, enabling the high voltage supplies to be turned on. The thumbscrew can be used to set an optimal distance between the needle tip and the grounded plate (usually ~10 mm). An accelerating voltage of ~1 kV is selected on the Instrument page, so that the needle voltage is variable between about 4 kV and 7 kV with respect to ground. (Select a lower accelerating voltage, e.g., 100 V, to drop the range to ~3 kV to ~6 kV). The quality of the spray is observed using the flexible torch. The voltage and the positioning of the needle(s) and/or fused silica at the tip of the electrospray probe is adjusted until a high quality, stable, fine spray is produced. Figure 7-2-S describes some types of spray commonly observed.

7-3. Capillary Electrophoresis Interfacing

The electrospray probe is designed to support capillary electrophoresis interfacing to suitable commercial capillary electrophoresis (CE) instruments, for instance the Beckman P/ACE system. Involatile CE buffers can be used if a sheath make-up flow is utilised (for instance 20 µl/minute of 1:1 water : methanol with 1% formic acid).

The Beckmann P/ACE capillary electrophoresis unit uses 20 to 150 µm I.D. x 365 µm O.D. fused silica separation capillaries, which require special steel needles in the electrospray probe.

The sheath flow liquid can either be added through the sheath flow port (with the appropriate combination of needles), or through a standard low dead volume tee situated under the perspex probe cover (using longer needles). Since the sheath flow port has a significant dead volume, it is better to use the additional tee. This also allows the introduction of appropriate tuning compounds when an HPLC injector is fitted in the sheath flow line.
An elegant alternative method of making the necessary electrical contact to the liquid inside the separation capillary involves poking a 25 μm gold wire into a 50 μm I.D. capillary, and attaching the other end to the nebuliser needle with a small blob of conductive paint. This may be better suited to CE systems in which there is a significant electroosmotic flow, and in which volatile buffers are used.

7-4. Megaflow Operation

This section describes the methods required to operate the electrospray probe at high flow rates, i.e. in the range ~200 to ~500 μl/minute. It should be pointed out that since the signal intensity on the mass spectrometer is essentially independent of the electrospray flow rate, there is actually little advantage in operating at these high flow rates, apart from convenience. If, for instance, LC-MS is being performed at 1 ml/minute using a standard LC column, then either a liquid split can be easily set up with a tee (section 5-6-1), or a spray or gas phase split will occur automatically in the high pressure part of the interface. If sensitivity is at issue, it is always better to work at the lowest possible flow rates, by for example switching from standard to microbore, or microbore to packed fused silica LC columns.

The electrospray probe should be built using the double layer steel needle arrangement (see section 4-5), with a suitable high-flow transfer line (such as red PEEK tubing) for megaflow operation. It may be necessary to carefully adjust the two steel needles so that they are concentric and a good quality nebulised spray is produced, but this adjustment can be quite difficult to do. Also adjust the distance by which the inner needle protrudes; usually the optimum will be around 1 mm.

The pepperpot counter electrode should be used, to avoid contamination of the sampling cone. The bath heater temperature should be increased to the maximum, i.e. 100 on the slider, to aid evaporation of the solvent. Allow time for the new temperature to be reached before starting the high solvent flow.

A higher than normal flow rate of bath gas will probably also be required. It is important that the probe is clipped in the housing, to avoid the possibility of it being blown out by the increased gas pressure. It is also important that there is no restriction in the red exhaust gas lines (e.g. no kinks) under these conditions, for the same reason.
The atmospheric pressure chamber must be orientated in the housing as shown in Figure 4-3-J, so that any solvent that condenses on the pepperpot or sampling cone will run down and be blown out of the exhaust gas line at the bottom. (This is in contrast to the earlier design of electrospray, in which the exhaust gas exited at the side of the atmospheric pressure chamber, which could therefore fill up with solvent).

When a beam is obtained, the various tuning parameters should all be optimised, as there may be differences compared to lower flow operation. In particular, the probe position, the nebuliser and bath gas flows, the sampling cone voltage, and the position of the atmospheric pressure chamber in relation to the skimmer should be checked.

Some problems which may occur specifically during megaflow operation follow.

**Sudden Loss Of Signal Or Loss Of Stability**

Check for condensation on the visible (rear) face of the pepperpot counter electrode. If it is very wet, turn off or reduce the solvent flow until it appears dry again. If the signal (or the stability) returns to normal, then condensation was the problem. Check that the heater is functioning correctly, and if necessary reduce the solvent flow and/or the water content as appropriate.

In extreme cases, the atmospheric pressure chamber may need to be dismantled and dried (this can be done without breaking vacuum as long as the sampling cone does not require cleaning; see sections 6-2 and 6-3).

**Loss Of High Voltages**

Condensation can cause certain high voltages, in particular the needle, counter electrode and sampling cone voltages, to flicker due to tracking. Condensation in the hexapole region is also possible. Since this region is not directly heated, it may take a while to warm up to a suitable operating temperature.

The needle voltage may also be pulled down in situations in which high conductivity solvents (for instance 0.1% TFA) are in use in conjunction with a red PEEK transfer line. If this occurs, increase the length of the transfer line, and/or switch to a smaller diameter line, such as 75 µm I.D. fused silica, as the flow rate considerations allow.

**Overheating Of The Atmospheric Pressure Chamber**

The heater circuit consists of a simple current - controlled supply without any temperature feedback regulation. The current should be limited by a resistor on the heater PCB, such that the temperature of the atmospheric pressure chamber should not rise above ~130 C (the black acetal melts at ~160 C). If a fault develops with the heater circuit, then overheating is possible. Characteristic indications include the probe beginning to jam in the housing, and an odour of hot plastic.
Section 8

Solvent Delivery Systems For Electrospray and APCI
Solvent Delivery Systems For Electrospray And APCI

This chapter describes a representative range of solvent delivery systems, including pumping systems and injectors, suitable for ESI and/or APCI applications. The list is not exhaustive; rather, it is intended to be used for guidance only. The choice of a particular system depends primarily on the range of intended applications, and it can prove quite difficult to select one single system that is suitable for all purposes. Table 8-0 lists some of the possible combinations.

<table>
<thead>
<tr>
<th>Application area</th>
<th>Parts</th>
<th>1 - 5 µl/min infusion</th>
<th>1 - 5 µl/min CE</th>
<th>5 - 50 µl/min infusion</th>
<th>5 - 50 µl/min CE</th>
<th>50 - 300 µl/min HPLC</th>
<th>50 - 300 µl/min HPLC</th>
<th>300 - 1000 µl/min HPLC</th>
</tr>
</thead>
<tbody>
<tr>
<td>50 / 150 fused silica triple layer</td>
<td></td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>34/24 double layer steel</td>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>sheath flow liquid</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>nebuliser</td>
<td>opt.</td>
<td>opt.</td>
<td>?</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>saltpot CE</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pepper pot CE</td>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X hot</td>
</tr>
<tr>
<td>50 / 150 or 50 / 375 fused silica transfer line</td>
<td></td>
<td>X</td>
<td>X</td>
<td>special</td>
<td>X alt</td>
<td>X alt</td>
<td></td>
<td></td>
</tr>
<tr>
<td>red PEEK transfer line</td>
<td></td>
<td></td>
<td></td>
<td>X alt</td>
<td>X alt</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>pressurised vial kit</td>
<td>X alt.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10, 100 or 250 µl low pressure syringe</td>
<td>X alt.</td>
<td></td>
<td></td>
<td>X alt.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HPLC high pressure syringe pump</td>
<td></td>
<td>X alt.</td>
<td></td>
<td>X alt.</td>
<td>X alt.</td>
<td>X alt.</td>
<td>X alt.</td>
<td></td>
</tr>
<tr>
<td>HPLC recipro-cating pump</td>
<td></td>
<td></td>
<td>X alt.</td>
<td>X alt.</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8125 injector (low inj. volumes, low flow rates)</td>
<td>X</td>
<td></td>
<td></td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>7125 injector (for standard HPLC flow rates)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X</td>
</tr>
</tbody>
</table>

Key: X = required; X alt. = viable alternatives; X opt. = optional
**Syringe pump**
(Harvard model 22 plus gas-tight syringe)

**HPLC Syringe pump**
(Carlo Erba Phoenix 20)
(Applied Biosystems 140 series)
(ISCO)

**HPLC Reciprocating pump**
(Pharmacia-LKB; Rheos 4000)

**Waste**

**Splitter**

**Insulating, narrow-bore line**
such as:
50-75μm ID, 150μm OD fused silica
50-75μm ID, 375μm OD fused silica
microbore PEEK
0.005" ID × 1/16" OD PEEK
(ID to suit application)

**Capillary electrophoresis separation systems**

**LC separation systems**

**Gas pressure driven flow**
(simple system.)

**Reciprocating pump**
(operating at high flow rates)

* These systems are ideal for delivering the sheath flow if required

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**FIGURE 8-1-A. SOLVENT DELIVERY SYSTEMS FOR ELECTROSPRAY**
8-1. Low Flow Solvent Delivery Systems For Electrospray

The electrospray probe is designed to be used in the following 'flow modes', also summarised in Figure 8-1-A:

- Continuous sample introduction mode.
- Loop injection mode (with various injectors).
- LC-MS mode (packed fused silica, microbore and standard columns).
- Capillary electrophoresis mode (CE-MS).

The aim of this section is to describe the practical aspects concerning the use of the probe in the low flow continuous sample introduction, loop injection and LC-MS modes. These methods may also be adapted to provide the sheath flow for CE-MS applications.

8-1-1. Flow Driven By Gas Pressure

In this solvent delivery system the nitrogen (or auxiliary gas) supply for the electrospray trolley is utilised to drive a sample/solvent solution, or a sheath flow solution, continuously through the electrospray probe. It is also possible to mount an HPLC injector in-line, as described in section 8-1-2.

The nitrogen supply pressure is fixed, at around 5 - 7 bar, and the flow rate can be controlled by means of a small line regulator. The sample/solvent mixture, or the sheath flow solvent, is contained within a pressurised vial similar to that used with the Dynamic LSIMS accessory.

The length and internal diameter of the transfer line required (usually fused silica) should be determined using the Poiselle equation:

\[ V = \left( \Delta P \cdot \pi \cdot d^4 \cdot t \right) / \left( 128 \times 10^{-11} \cdot \eta \cdot L \right) \]  \[ \text{[1]} \]

Where:
- \( \Delta P \) = pressure difference across capillary (bar)
- \( L \) = length of capillary (metres)
- \( d \) = internal diameter of capillary (metres)
- \( \eta \) = viscosity of the bulk fluid (1.08 for water; assume ~0.75 for methanol and ~1.5 for 1:1 water : methanol; the 10-11 provides a correction to make the units compatible)
- \( t \) = time for which the pressure difference is applied (seconds)
- \( V \) = volume of liquid moved into or out of the capillary during time \( t \) (litres).

For example, a pressure difference of 2 bar accross a 1 metre length of fused silica should give a flow of pure water of 1.7 \( \mu \text{l/minute} \) if the I.D. is 50\( \mu \text{m} \), or 8.6 \( \mu \text{l/minute} \) if the I.D. is 75\( \mu \text{m} \). Although pure methanol has a lower viscosity than that of water, 50 : 50 mixtures have a higher viscosity than that of pure water.
FIGURE 8-1-B. FLOW DRIVEN BY GAS PRESSURE

FIGURE 8-1-C. CONNECTION OF THE PRESSURISED VIAL ASSEMBLY TO THE INJECTOR BRACKET.
The gas driven flow accessory has the major advantage that no syringe pump or HPLC pump is required: it is therefore ideal for sample/solvent delivery during the initial installation, and for sheath flow delivery during complex LC-MS or CE-MS experiments. Detailed instructions for use follow - refer also to Figures 8-1-B and 8-1-C.

1. Switch the instrument to ‘standby’ and turn off the supply of nitrogen (or auxiliary gas) to the trolley.

2. Install the 6 mm tee and the line regulator (Figure 8-1-B). The regulator bracket can be conveniently mounted using one of the four screws which attach the stainless steel top to the trolley. Turn the regulator to zero (fully anticlockwise) (note the function of the red locking ring).

3. The gas supply can now be turned on again.

4. Connect the length of PEEK tube to the vial assembly gas port using a steel bush and steel ferrule.

5. Connect the Omnifit valve to the PEEK tube, using all three O-rings (2 large + 1 small).

6. Connect the 4 mm blue polyurethane tube to the Omnifit valve using the two large O-rings in the fitting. Pass the end of the blue tube through one of the holes on the injector bracket, and then connect the blue tube to the regulator. When not in use the gas connections can be conveniently disconnected/reconnected at point ‘A’ shown on Figure 8-1-B.

7. Fit the appropriate length and I.D. of fused silica to the electrospray probe. It is advisable to sheath the fused silica within a teflon tube, as shown. Terminate the fused silica near the bottom of the vial, but do not allow the teflon sheath to dip in to the solution in the vial. Clamp the teflon sheath on to the fused silica by means of a steel bush with a teflon ferrule.

8. Fill the glass vial with the appropriate solution, and hand-tighten the perspex vial shield so that a satisfactory seal is formed.

9. Note. Check carefully that the glass vial and the perspex vial shield are in good condition before opening the line regulator valve.

10. Open the Omnifit valve, and increase the pressure using the line regulator until the desired flow rate is achieved. The flow can conveniently be measured using 5 or 10 μl capillary tubes, or the barrel of a glass syringe.

11. To stop the flow, shut off the Omnifit valve and loosen the steel bush holding the PEEK tube in the vial holder assembly to release the pressure.
Steel Bush And Teflon Ferrule

PEEK Tube

In-Line Filter (Optional)

Stainless Steel or PEEK Tube

This union may be slackened off in order to flush the tubing and filter with fresh solvent. (CARE !)

Fused Silica within Teflon Sheath

Ground

External Union

Nick

Transfer line to probe (several options exist)

Pressurised vial assembly (schematic)

Gas In

Ground

Solvent In Vial

Injecto Bracket (Part)

HPLC Injector (Loop and waste connections not shown)
12. If the electrospray probe is to be removed from the instrument for maintenance purposes, first close the line regulator and then disconnect the blue tube at point ‘A’, shown on Figure 8-1-B.

13. If the flow stops unexpectedly, it is probable that the fused silica has become blocked. The problem can usually be solved by cutting off 3 - 5 mm of the silica from the end protruding in to the vial. It is advisable to filter the sample/solvent solution used.

14. If required, the pressuried vial assembly can be connected directly to a separate regulated 0 - 10 bar gas supply. In this case, dispense with the blue plastic tubing, the in-line regulator, and the Omifit valve. Use high pressure tubing and swagelok or similar unions to make the connections to the new gas supply. Ensure that the perspex vial shield and the glass vial are in good condition before operating the vial assembly at high pressure! Do not exceed 10 bar.

8-1-2. Flow Driven By Gas Pressure, With Sample Injection

It is possible to connect an HPLC injector between the pressurised vial assembly and the fused silica lines, as shown in Figure 8-1-D. Suitable injectors are the Rheodyne 7125 or 8125. The injector bracket is designed to accept a range of both Valco and Rheodyne injectors.

The use of an in-line filter is recommended - many types are commercially available. Valco in-line filters (Valco part no. ZUFR1) can be obtained from Micromass, order code 6368200.

Careful attention should be paid to the injection technique, and to the cleanliness of the samples, since any particulate matter can easily block the transfer line. The low gas pressure required to drive the flow may well be insufficient to clear the blockage. (In contrast, similar blockages will tend to be shifted by the pressure rise that subsequently occurs in a syringe - or HPLC - pumped system).

An explanation of the nick and the external union on the fused silica/teflon sheath is given in section 7-1. If instead the double steel needle assembly is in use, then an unsheathed length of 50 or 75 µm I.D. x 375 µm O.D. fused silica can be used as a transfer line.

8-1-3. Flow Driven By Low Pressure Syringe Pump (Harvard Model 22)

Several small, accurate low pressure syringe pumps are available commercially. The Harvard model 22 pump (Harvard Apparatus Inc., 22 Pleasant Street, South Natick, Massachusetts 01760, USA ; tel. 508 - 655 - 7000) is quite commonly used for generating the low flow rates required for electrospray ionisation, and consequently its use is described here.

These pumps are only suitable for continuous infusion or loop injection studies; they are not suitable for chromatographic applications since they cannot develop the high pressures required.
FIGURE 8-1-E. CONNECTION TO A SGE MICROLITER SYRINGE, OR A PLASTIC SYRINGE, FOR USE WITH A SYRINGE PUMP

Part of SGE Syringe Barrel

0.5 mm OD Syringe Needle (Domed or Square - Cut Tip)

Syringe Nut (drill out nut to 1/16" if necessary)

0.5 mm ID x 1/16" OD PEEK Tube, protruding inside syringe nut.

1/16" capillary Union with 0.25 mm bore (Waters or Valco)

Steel Bush

Fused Silica

Fused Silica

1/16" Teflon Ferrule

Steel Upchurch Fingertight Nut and PEEK Ferrule (VG Code 6367400) or LiteTouch ferrule + Rheodyne nut

CONNECT / DISCONNECT THIS UNION

Transfer line (various options exist)

External Union

Luer fitting

Part of plastic disposable syringe

To Probe

Compress Spring

hypodermic needle, with domed tip

sheathing tube to match needle O.D.

Part of plastic disposable syringe

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Solvent Delivery Systems For Electrospray And APCI
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Separate gas-tight syringes are required for use with the Harvard 22 pump. 100µl or 250µl syringes with dome-tipped needles are ideal for the purposes of test and installation. Syringes having relatively large capacities (2 ml and above) can also be used, but because of the relatively slow travel of the plunger some considerable time may elapse before a stable flow rate is achieved. The Scientific Glass Engineering (SGE International Pty Ltd, 7 Argent Place, Ringwood, Victoria 3134, Australia) order codes and internal diameters of some suitable syringes are listed below. These syringes have 0.5 mm diameter tapered needles. The sharp point can be cut square, or dome-tipped needles can be obtained (SGE order code 038310).

<table>
<thead>
<tr>
<th>Volume (µl)</th>
<th>SGE order code</th>
<th>Internal diameter</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>100R - GT</td>
<td>1.46 mm</td>
</tr>
<tr>
<td>250</td>
<td>250R - GT</td>
<td>2.30 mm</td>
</tr>
</tbody>
</table>

Syringes from other manufacturers (Hamilton Company, P.O. Box 10030, Reno, Nevada 89520-0012, USA, for instance) can also be used, although it may be necessary to construct slightly different fittings to those described below.

Disposable plastic syringes can also be used for electrospray, but there is a risk that plasticisers, etc. will leach out of the syringe or plunger, resulting in an increase in the background noise at low mass. Select syringes manufactured from materials compatible with the solvents to be used. Polypropylene syringes with black rubber plungers appear to be satisfactory with water/methanol/1% acetic acid.

1. Enter the diameter of the syringe to be used by pressing Set and Diam simultaneously on the syringe pump control pad.

2. Enter the flow rate required by pressing Set and Rate simultaneously. (Refer to the pump instructions if in doubt).

3. Position the collar on one of the guide rods so that the pump will stop before the syringe plunger hits bottom.

4. Construct a union between the syringe needle and the transfer line as shown in Figure 8-1-E for a fused silica transfer line. Drill out the syringe nut so that it will accept the PEEK tube, if necessary. Do not overtighten the unions.

5. To re-fill the syringe, slacken off the fingertight nut (there is no need to remove it completely), and withdraw the needle from the fitting.

6. Note that the syringe is not grounded through the Harvard 22 pump, since the pusher block and syringe holder are plastic. The instrument should therefore be switched to ‘Standby’ before touching the syringe or the union. It is recommended that a grounded crocodile clip or similar is left permanently attached to the steel bush or the capillary union (Figure 8-1-E) to prevent this problem.
Principle Of Operation:

If the short length of PEEK tube is cut so that it is long enough to protrude inside the SGE syringe nut, then it will relieve the strain on the syringe needle. It may first be necessary to drill out the syringe nut with a 1.7 mm or 1/16” drill to make this possible. (This is not possible with some other makes of syringe, for instance those with fixed needles).

The 0.25 mm bore capillary union is used to provide a permanent seal on to the fused silica, using a teflon ferrule swaged on to the teflon sheath. The nick through the teflon sheath and the external union, are explained in section 7-2. The 0.5 mm diameter syringe needle butts up against the 0.25 mm bore. Note that if a much thinner needle or a union having a larger bore are used, then the needle may be able to pass right through and damage the fused silica. This is likely to result in a blockage.

The PEEK tube and the PEEK LiteTouch or Fingertight ferrules combine to form a robust zero dead volume seal around the needle. If this union is not over-tightened, the seal can be re-made hundreds of times before replacement is necessary.

Some Problems That May Occur:

1. The SGE removable needle syringes described here have plated brass fittings. The acids in typical positive ion ESI solvents can leach out the copper, which if it finds its way into the barrel will give characteristic copper adducts in the spectra.

   This occurrence can be avoided in several ways. Check that the needle seal is in good condition. Fill and empty the syringes slowly, so that liquids are not forced past the needle seal by the high pressures that can be developed. Do not dip the metal parts of the syringe in the solvents. Alternatively, use fixed needle syringes, or Hamilton removeable needle gas - tight syringes having stainless steel fittings.

2. If the transfer lines to the ESI probe become blocked, then the syringe may burst, since there is no over-pressure protection with this type of syringe pump. If an injection valve is fitted in line, the syringe can easily burst if the valve is left in the closed position. Larger diameter syringes or fixed needle types will burst more easily; the smaller removeable needle syringes tend to leak around the needle seal instead. It is worthwhile to obtain several spare syringes! If breakages are a problem, then disposable plastic syringes should be evaluated.

3. The actual flow rate may be uncertain, and may take a long time to reach an equilibrium value, either if there is an air bubble in the syringe, or if the ratio of the syringe volume to the requested flow rate is very large. This is because it takes some time to reach an equilibrium pressure. As a guide, choose a syringe and flow rate such that a full syringe will be emptied in 30 to 60 minutes, and be careful to exclude air bubbles from the barrel.
8-1-4. Flow Driven By High Pressure Syringe Pump

Various makes of high pressure isocratic or gradient syringe pumps are available, and almost all are suitable for delivering solvent isocratically at the flow rates required for ‘pure’ electrospray. However, some systems are easier to use than others, and many are very bulky, requiring their own trolleys. The Carlo Erba Phoenix system is not easy to program manually, although it can be controlled from the OPUS data system.

Most makes of high pressure syringe pump have a pre-pressurisation option, which brings the pressure rapidly up to a preset target level so that the flow rate quickly stabilises. However, the pressure developed in the Phoenix system rises very high very quickly, and the resulting pressure shock can destroy relatively delicate LC columns such as packed fused silica columns. To protect the column, it is necessary in this case to close an injector valve sited between the pump and the column.

These pumps are designed to work well against a reasonably high solvent back-pressure, and therefore the performance can often be improved by adding an old LC column between the pump outlet and the sample injector/analytical LC column.

Gradient high pressure syringe pumps are generally not capable of forming good quality, reproducible gradients at flow rates of less than around 20 to 50 µl/minute unless special care is taken in the design of the micro-mixer.

8-1-5. Flow Driven By Reciprocating HPLC Pump

In general, most reciprocating HPLC pumps are not capable of delivering sufficiently steady, stable flows at around 10 ul/minute to be useable for electrospray. There are, however, a few exceptions. The Pharmacia Biotech (Pharmacia Biotech Europe, Procordia EuroCentre, Rue de la Fusee 62, B-1130, Brussels, Belgium) model 2150 and 2148 pumps work well at 10 µl/minute and 1 µl/minute respectively in isocratic mode. Rheos 4000 (Flux Instruments AB, Lnsmansgrden, Box 134, S-18212 Danderyd, Sweden.) pumps are also satisfactory for isocratic work at low flow rates.

If a particular pump is being evaluated, then monitor the signal from a continuously infused strong ion, for instance gramicidin S, at the minimum flow rate using either a narrow scan experiment with a fast cycle time, or an SIR experiment, and examine the trace for any regular events that can be correlated with the piston stroke volume.

In general, none of these reciprocating pumps are good for running gradients at flow rates of less than ~50 - 100µl/minute.

When using a reciprocating pump system for low flow rate electrospray solvent delivery, it is important that the pump is kept in good order and serviced regularly. If the pump has a flow compensation option to maintain a stable flow while the pistons are changing direction, then this should be used.
The most likely fault to occur with a suitable reciprocating pump is that one of the check valves fail to function properly, either because of an air bubble, or due to dirt lodged in the valve. If this is suspected, purge the pump (following the instructions provided with the pump) and/or clean or replace the suspect check valve. A faulty check valve can easily be identified by monitoring a strong solvent ion or a continuously infused sample ion such as gramicidin S using a narrow scan experiment. Regular dropouts in the TIC or RIC trace should be able to be correlated with the piston stroke volume and the requested flow rate. Regular fluctuations of the pump pressure indicator are also diagnostic of this problem.

8-1-6. Flow Driven By HPLC Pump With Pre - Column Splitter (LC Packings Accurate)

One approach for forming good quality, reproducible gradients at very low flow rates involves using ordinary HPLC syringe or reciprocating pumps at normal, optimal flow rates (~1 ml/min) coupled with a fixed ratio splitter situated before the LC column. This may be a cost - effective solution for either isocratic or gradient work if the high flow LC system is already available. A splitter called Accurate is available from LC packings (Dufourstrasse 30, 8008, Zurich, Switzerland). LC Packings also manufacture a wide range of packed fused silica micro - HPLC columns, as well as special U - or Z - shaped capillary UV cells.

8-2. Medium Flow Solvent Delivery Systems For Electrospray

Operation of the electrospray interface at flow rates in the range of ~20 to 50 µl/minute, using the double layer steel needle assembly with nebuliser assistance, will give optimum short term and long term spray stability and reproducibility, and this range is therefore ideal for quantitative studies. The flow is sufficiently high that it is quite easy to generate, leaks are sufficiently large as to be easily detectable, and there is reasonably good sensitivity.

Low pressure syringe pumps fitted with relatively large volume gas - tight syringes can be used for continuous infusion or loop injection (but not chromatographic) studies. For instance, a 2 ml syringe would last 40 minutes at 50 µl/minute. There would be little benefit in operating a low pressure syringe pump above this flow rate, since there is no gain in long term stability or reproducibility, and there is increasing loss of sensitivity.

Almost all makes of high pressure syringe pump can also be used, and the gradient types should be capable of forming good quality gradients for LC- MS at flows of around 50 µl/minute.

Not all reciprocating pump systems are capable of operating satisfactorily in the medium flow rate range (around 50 to 100 µl/minute), and thus should be selected with care. Although these systems may be able to form good gradients at 1 ml/minute, they may be unreliable or unsatisfactory at medium flow rates.
8-3. High Flow Solvent Delivery Systems For APCI And Electrospray

The APCI interface is designed to operate at flows of around 0.5 to 1 ml/minute, but if complex LC-MS accurate mass or high resolution SIR experiments are to be performed, this flow may be provided by some combination of separation system and make - up flow, Figure 8-3-A. The requirement is thus for a pumping system (or systems) that performs satisfactorily at ~0.2 to 1 ml/minute; this is best provided by good quality reciprocating HPLC pumps. It is very important that the pump produces a pulse - free flow, since small pressure and flow variations will affect the operation of the APCI nebuliser and will show up as regular noise on TIC or RIC traces.

It is recommended that any pump used for APCI is kept in good working order, and that if the pump has a flow compensation facility then this is used. Any regular noise on the TIC traces should be compared with the pump piston movements to ascertain whether it is due to the pump. The use of a commercial pulse damper may be beneficial in some cases.

The pump requirements for high flow electrospray operation are somewhat less stringent than for APCI, although significant flow rate fluctuations will also show up as regular noise superimposed on TIC traces.

8-4. Selection Of HPLC Sample Injectors For APCI And Electrospray

Selection of a suitable HPLC injector is critical for optimum performance of the API interface. Because of the range of flow rates and experiments that are possible, it is hard to select one single injector that will suit every purpose. The injector catalogs and other literature available from Rheodyne (P.O. Box 996, Cotati, California 94928, USA) are a valuable source of detailed information on selection, use and maintenance of injectors.

The injector bracket on the electrospray probe handle is designed to accept a wide range of both Rheodyne and Valco injectors. Since APCI is often used as an LC-MS interface, it is usual to mount the injector in the LC system.
<table>
<thead>
<tr>
<th>Injection Valve Model</th>
<th>Description</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Valco C14W (with VISF-2 loop filler port)</td>
<td>Internal loop 0.06 to 1.0 µl interchangeable rotors. Designed for microbore applications.</td>
<td>A minimum of ~8µl is required to flush the loop, so this valve is not recommended if sample is limited. Chromatographic peak shape is excellent.</td>
</tr>
<tr>
<td>Rheodyne 7125, 7725 and 9725</td>
<td>External loops, 2 or 5 to 5000 µl. (9725 has inert flow passages)</td>
<td>No sample wastage. 1µl injections can be made, although injection technique is more critical than with the C14W and chromatographic peak shape is slightly worse.</td>
</tr>
<tr>
<td>Rheodyne 8125</td>
<td>Similar to 7125 but with smaller flow passages and 0.020 inch ports - designed for microbore applications.</td>
<td>No sample wastage. 0.1 to 1µl injections can be made. Chromatographic peak shape is better than with the 7125.</td>
</tr>
<tr>
<td>Rheodyne 7520</td>
<td>0.2, 0.5 and 1 µl internal loops. Designed for microbore applications.</td>
<td>Minimal sample wastage (0.3 µl dead volume). Since between 2 and 5 volumes should be loaded to flush the loop, the actual volume injected needs to be 1 to 3 µl.</td>
</tr>
<tr>
<td>Rheodyne 7410 with 7012 loop filler port</td>
<td>0.5, 1, 2 and 5 µl internal loops.</td>
<td>The tubing connecting the filler port to the valve contains 7 µl, so this valve is not recommended if sample is limited. Performance is probably similar to the C14W.</td>
</tr>
</tbody>
</table>

NOTE: All of these injectors require a 2 inch, #22 gauge needle with a square-cut tip.

TABLE 8-4. A COMPARISON OF INJECTION VALVES SUITABLE FOR LOOP INJECTION ANALYSES.
8-4-1. General Considerations

If sample quantities are limited, then it is important to select an HPLC injector having a minimal or zero dead volume between the end of the injection syringe and the loop. In the case of electrospray, it is also best to run at the minimum possible flow rate that can be conveniently and reliably generated.

If flexibility in injection volume is required, then injectors with interchangeable external loops are a good choice. (Some of the Valco and Rheodyne internal loop models are designed to allow fairly swift changeover to a different loop volume, although the ranges are limited).

If quantitative studies are required, then the highest injection precision will be obtained using complete loop filling methods. For quantitative electrospray, aim to work at around 20 - 50 µl/minute, and use the nebuliser-assisted spray and steel needles, since the best stability and reproducibility is usually obtained under these conditions.

If both low and high flow rate studies are to be carried out (e.g. if both ESI and APCI are in use), then it is advisable to select an injector that meets the requirements at the low flow rates required; it will then most probably also perform satisfactorily at the higher flow rate as well.

8-4-2. Injectors For Low Flow Rate Electrospray

If it is planned that low volume injections are to be made at flow rates of around 5 µl/minute, then the injector must be chosen with care. Most commonly available HPLC injectors are designed for use at around 1 ml/minute, and will have relatively wide flow passages that may cause noticeable peak tailing when working well below this flow rate. Some comparative information on a selection of injection valves is given in Table 8-4. It is best to choose a valve that is described by the manufacturer as suitable for microbore applications. Careful attention must be paid to the minimisation of all dead volumes (section 7-1) in order to achieve optimal performance when working at these flow rates.

If relatively large volume injections (~20 to 50 µl) are required at flows of around 5 µl/minute, then peak tailing may be much less of a problem since the injection peak will be flat topped. In this case, less stringent criteria may be applied for selection of a suitable injector.

At flow rates in the region of 50 - 200 µl/minute, there will probably not be very much noticeable difference in the performance of a microbore and a standard injector (for instance a model 8125 compared to a 7125), even with quite small injection volumes, in loop injection studies.
FIGURE 8-4-A. ATTACHMENT OF A RHEODYNE 8125 INJECTOR TO THE ELECTROSPRAY INJECTOR BRACKET (7125, 7725 AND 9725 INJECTORS ARE SIMILAR)

NEEDLE GUIDE
VALVE BODY
INJECTOR BRACKET
#8-32 MOUNTING SCREWS
SET SCREWS
KNOB

(Mounting Hole for 7012 Loop Filler Port If Rheodyne 7410 Valve is Fitted)

PORTS (Note-Use Only Rheodyne Ferrules and Bushes)

LOOPS

Pump
Column
Vents
Needle Port
Loop
LOAD
INJECT
8-4-3. Injectors For High Flow Rate Electrospray

In general, any good quality HPLC injector will probably be suitable for electrospray loop injection experiments carried out at flow rates in excess of 200 µl/minute.

Since the ESI sensitivity at these flow rates is relatively low (section 7-4), it is likely that the spray will only be operated at these high flows to facilitate convenient LC-MS interfacing. In this case, the choice of injector should be determined primarily by the LC system. For instance, if isocratic LC is to be performed using a microbore column, then it is probably best to use a good quality microbore injector. If, however, gradient LC is to be used, the injection volume can be very large as long as the sample solvent has a low elutropic strength, without causing any deterioration of the chromatography.

8-4-4. Injectors for APCI

Since flow rates for APCI are in the 0.5 - 2 ml/minute range, any good quality injectors may be used. The Rheodyne model 7125 is an excellent general purpose injector, and can be fitted with loops from 5 µl to ~5 ml.

The APCI interface is particularly sensitive to the pressure and flow variations that normally occur when these injection valves are actuated, and this can result in unwanted injection spikes on TIC traces, which may be troublesome in the case of loop injections of samples at levels near the detection limit. (There is no problem with LC-MS applications, since the injection spike occurs well before the samples elute). It is well worth considering the use of Rheodyne model 7725 injectors having special make - before - break flow passages if pressure - related injection spikes are a problem.

8-4-5. Connection To Rheodyne Injectors

Figure 8-4-A shows the correct way to connect the Rheodyne model 7125, 7725, 8125 and 9725 injectors. Note that the two vent tubes should be adjusted so that the height of their outlets is the same as the needle port. This prevents syphoning of waste solutions through the needle port when the syringe is withdrawn.

Rheodyne also recommend that the needle port is flushed with clean solvent at intervals of once every ten injections, using the needle port cleaner.

Manufacturers of HPLC injection valves normally provide excellent documentation on their products, which should be consulted in case of any difficulty.
8-4-6. Injection Technique And Loop Filling Methods

It is vital that injection syringes having the correct needle type are used, otherwise the injection valve may be damaged, and/or repeat injections may not be reproducible. The Rheodyne and Valco injectors all require syringes with 2” or 50 mm long dome - tipped 22 gauge (0.41 mm O.D.) needles.

The manufacturer’s literature supplied with new injectors usually describes injection methods in greater detail than the brief notes provided below:

**Injection Technique (Rheodyne 7125 or 8125):**

1. Flush any previous sample out of the syringe (this may require 10 - 15 rinses, or more if ‘sticky’ compounds such as PEG’s have been used).
2. Fill the syringe with sample, taking care to exclude air bubbles.
3. Switch the injector knob to the left- hand position (labelled ‘Load’), insert the syringe until it bottoms out, and empty the contents into the loop (A light resistance should be felt just before the needle bottoms, indicating that the needle seal is satisfactory).
4. Leaving the syringe in position, switch the injector knob to the right - hand position (labelled 'Inject'); this moves the contents of the loop into the flow stream.
5. The syringe can now be withdrawn.
6. Leave the injector in the ‘Inject’ position at least until all of the sample has exited the loop, taking the effect of mixing as a consequence of laminar flow into account (it is usually simplest to leave it in this position until another injection is required).

**Partial Loop Filling:**

As a rough guide, injections made using zero sample wastage models of injector will be quantitative for volumes up to half that of the loop volume. For example, injections of 1 to 10 µl into a 20 µl loop on a 7125 or 8125 should give a quantitative response. In the case of injections that are between 50% and 100% of the loop volume, a certain amount of sample will be flushed out of the loop due to laminar flow.

If the injector has a significant dead volume between the end of the needle and the loop, it is normally not possible to reliably partially fill the loop.
**Complete Loop Filling:**

If the injector is of a zero sample wastage design, then as a rough guide it is necessary to inject between 2 and 5 times the loop volume in order to completely fill the loop. For instance, ~50 µl should be injected to reliably and reproducibly fill a 20 µl loop on a 7125 or 8125 injector.

If instead a Valco CI4W or a Rheodyne 7410 with a 7012 loop filler port (Table 8-4) are being used, then the dead volume between the end of the needle and the loop must also be taken into account; it may be necessary to inject between 25 and 40 µl in order to completely fill a ~1 µl loop.

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**8-5. OPUS Control Of HPLC Systems**

Certain makes and models of HPLC system can be programmed from the OPUS data system (this will depend on the specific version of software available).

The HPLC UV trace can be recorded during MS data acquisition on one of the auxiliary input channels using a remote switching unit (consult your local Micromass representative for details).
Section 9

Installation and Operation of the APCI Interface
This chapter describes the installation and operation of the APCI accessory on the AutoSpec family of instruments, including the ‘X’, ‘S’, ‘V’ and ‘M’ series.

It is assumed that electrospray has already been successfully installed, according to the instructions given in Chapter 4. In the event that the electrospray option has not been provided, then it is possible to follow a combination of the instructions in chapter 4 and in this chapter in order to complete the initial installation using APCI. It will be necessary to select and run some suitable compounds, such as PEG 200 and 600, or caffeine and reserpine, in order to complete the hexapole RF setup (section 4-7).

9-1. Nebuliser Heater Power Supplies

Early instruments require a stand - alone power supply for the APCI nebuliser, which is driven from the beam control unit. On later instruments the existing heater supply PCB is used. The performance of the two versions is essentially identical.

9-1-1. Stand - Alone Power Supply, ‘X’ And ‘S’ Series Instruments

Figure 9-1-A shows the stand - alone power supply, MA3566, and the connections to the beam control unit. Note that the thermocouple wires are to be connected as follows: white to ‘+’ and blue to ‘-’.

The power supply is fitted with a 24 V circuit breaker, which is in the tripped condition when the red part of the switch is visible. If this breaker trips then the power to the heater will be shut off, but there are no other external indications that this has occurred.

The light emitting diodes on the power supply indicate the status of the unit, including the presence of the +15, -15 and +5 V supplies, and whether or not the heater is currently on.

The temperature readback may cycle by up to 50 - 60 °C, particularly whilst the nebuliser is approaching operating temperature, because of a lag in the control loop (control is effected via the optic loop using a software comparator). This is normal, and should not cause a problem during sample analysis.
Connect ES05 (the ground cable) to the nebuliser.

Tighten the three thumbscrews—note: the APCI chamber is only secured in place by these thumbscrews and the vacuum!

Plug in ES01 to the free socket, and clip it to the side of the APCI source chamber.

Plug in ES02 to the side socket.

Corona needle assembly

FIGURE 9-2-B. CONNECTIONS TO THE APCI NEBULISER

APCI source chamber

Part of nebuliser handle

Heater and thermocouple connections; plug in BB36 when safe to do so, i.e., having first ensured that the heater is off.

Sample inlet (via an Upchurch A-318 in-line filter); hold the flats when attaching a transfer line.

Slider block

Nebuliser gas inlet

Brass spade connector for earth wire, ES05

Sheath gas inlet (connect to the Bath Gas outlet)

FIGURE 9-2-A. FITTING THE APCI ACCESSORY TO THE API HOUSING
9-1-2. Integral Power Supply, ‘V’ And ‘M’ Series Instruments

When APCI is installed for the first time on a ‘V’ series instrument, the cable to the nebuliser (BB36) will be fitted by the engineer, and some of the components on the heater supply PCB will be altered to accommodate the high power requirement, and to enable data system control.

All ‘M’ series instruments already have the nebuliser cable (BB36) and necessary circuitry in place.

Since feedback control of the temperature is carried out by the circuits on the heater board, relatively little temperature cycling should occur with this version of APCI power supply.

9-2. Fitting The APCI Source

9-2-1. Removal Of The Electrospray Source

1. Vent the interface.

2. Shut down the electrospray sample and gas flows. Disconnect the sample line from the electrospray probe, and disconnect the nebuliser gas line from the panel on the back of the trolley.

3. Remove the high voltage lead, ES01, and the ground cable, ES05, from the electrospray probe.

4. Remove and store the electrospray probe (for instance place it in the spray tester to protect the needle tip). Remove the top hat assembly and the atmospheric pressure chamber.

5. Unplug ES02, undo the three M5 x 55 screws and remove the make - up piece from the end of the API housing.

9-2-2. Electrical And Gas Connections For APCI

1. Fit the APCI source chamber in place of the ESI atmospheric pressure chamber, and secure it in place with the three black plastic thumbscrews, Figure 9-2-A.

2. Plug in cable ES02 to the socket on the side of the chamber, and connect the electrospray needle voltage cable, ES01, to the trailing socket. Clip the union in to the spring clip.

3. Connect the nebuliser line (a 4 mm polyurethane tube) from the trolley to the left - hand Legris fitting on the nebuliser handle below the 1/16” sample inlet, Figure 9-2-B.

4. Connect the electrospray bath gas line (which now becomes the APCI sheath gas) to the right - hand Legris fitting on the nebuliser handle.
FIGURE 9-2-C. THE APCI EXHAUST SYSTEM

FIGURE 9-3-A. THE APCI TUNING MENU
5. It is best not to connect the sample line to the cold nebuliser at this stage, because if liquid dribbles slowly through the LC system then the ceramic putty used in its construction may soak it up.

6. Connect cable BB36 to the electrical socket, after ensuring that the heater is switched off and set to zero in the APCI tuning menu (refer ahead to section 9-3). Observe the data system temperature readback for a minute or so after making this connection. The readback should change from above 1000 °C with the lead disconnected (meaning that it is open-circuit) to a value near ambient temperature, but should not rise above this. If the readback is not correct, check the continuity of the circuit between the nebuliser (remove the cover over the handle) and the power supply.

9-2-3. The APCI Exhaust System

1. Connect the 14 mm O.D. blue nylon tube between the fitting at the base of the APCI chamber and the fan mounted on the leg of the API trolley, see Figure 9-2-C. (Since this tube is inflexible and is prone to kinking, it may be convenient to add a 14 mm Legris elbow near the fan).

2. Connect the 1.5” clear reinforced tube to the fan outlet using the Jubilee clip, and route the other end to the outside atmosphere or to a fume hood. **Note. This Exhaust Line Must Not Be Connected To The Rotary Pump Exhaust System.** The APCI solvents will be carried away through this line; since the solvent flow is relatively high it is important that it does not vent into the laboratory.

3. Turn on the fan by plugging it in.

4. Check that the exhaust system is operating by feeling for the draft through the APCI chamber, or at the outlet of the 1.5” tube.

9-3. The Data System

The instrument configuration and the setup of 4 kV accelerating voltage are the same as for electrospray, see sections 4-4-1 to 4-4-3. Like ESI, the APCI accessory must be operated at 4 kV rather than at 8 kV.

9-3-1. APCI Tuning Menu & Suggested Settings

If electrospray has already been installed, then make a note of the optimum ESI tuning parameters in the **ES Pos Tune** menu, Figure 4-4-C, that were suitable for low mass compounds such as gramicidin S. Then change the source to APCI (actually called ‘API’ in the OPUS menus), and type in the electrospray optima to provide a starting point for finding a beam. Figure 9-3-A shows the APCI tuning menu.
The voltage readbacks are all with respect to ground. The values reported are not very precise, but nonetheless they are diagnostic of certain fault conditions. The individual components of the menu are detailed below.

**Needle Voltage.**

The needle voltage slider is set up so that 0% corresponds to 2.5 or 3.0 kV above accelerating voltage (for positive ionisation), i.e. ~6500 - 7000 V with respect to ground. The range of the slider is 2.5 kV, thus with the slider at 100% the requested output is ~9000 - 9500 V with respect to ground. The upper limit depends on the output characteristic of the particular high voltage supply used; in practice it is only necessary to achieve ~8500 V.

The corona needle voltage required when using APCI is typically not very critical, although is very dependant on the positioning; set the slider to about 30% in the first instance.

**Sampling Cone & Skimmer Lens.**

The sampling cone and the skimmer lens sliders are both set up so that 0% corresponds exactly with the accelerating voltage. The range of both sliders is 250 V, so that the voltages on these components can be varied between 4000 and 4250 V with respect to ground. Typically, the optimum voltage difference between these two components is 10 V, with the skimmer lens higher than the sampling cone (for positive ionisation).

The sampling cone/skimmer lens voltages are very compound and mass dependant. Typically, for optimal transmission of the relatively low mass molecular ions produced by APCI, slider values of ~20% (i.e. ~50 volts above the skimmer) will be required. In order to fragment such compounds, slider values of ~40 to 80% may be required.

**Skimmer.**

The skimmer is held at accelerating voltage (assumed here to be exactly 4000 V). It is not adjustable from the APCI tuning menu, but is controlled using the ion energy slider or potentiometer (depending on the instrument type). The range of the control is set up to be +/- 1% of the optimal ion energy, i.e. an 80 V swing. On instruments with the manual pot box and oscilloscope (‘X’, ‘S’ and ‘V’ series), it is usually easier to find a positive ion APCI beam with the ion energy pot turned a little to the left (giving a higher voltage output) compared to the optimum for other ionisation techniques. On ‘M’ series instruments with data system tuning, the slider should be set initially a little to the right, because the convention is reversed.
Hexapole Offset.

The hexapole offset (DC) voltage is held at about 10 V lower than the skimmer (for positive ions). It is not adjustable from the APCI tuning menu, but instead is set up using the trimpots on the appropriate high voltage board in the trolley. It does not normally require any further manual adjustment once it has been set, because there is a sufficiently wide ion energy window to compensate for small drifts simply by tuning the ion energy.

There is no data system control or readback of the hexapole RF voltage.

Ring Electrode.

The ring electrode slider is set up so that 0% corresponds exactly with the accelerating voltage. The range of this slider is 100 V, and it swings downwards, so that 100% corresponds to a voltage of 3900 V with respect to ground. The optimal voltage for most compounds and under most conditions is ~10 V below the hexapole offset voltage, which corresponds to a slider value of ~20%, i.e. approximately 20 V below accelerating voltage, or 3980 V with respect to ground (for positive ions). Once set, the ring electrode slider rarely requires much adjustment.

Heater On / Off.

This switch has no function with ‘X’ and ‘S’ series instruments having the stand-alone power supply; in this case the heater slider must be set to zero to turn off the heater.

The switch only functions on the ‘V’ and ‘M’ series instruments.

Nebuliser Heater.

This controls the temperature of the APCI nebuliser, and by means of indirect heating, also the sampling cone. 0% and 100% on the slider correspond to ambient temperature and 1000°C respectively. The temperature readback from the thermocouple should remain reasonably steady during normal operation.

In order to successfully nebulise a flow of 1 ml/minute, a temperature of between 500 and 700 °C is usually required. Select ~500 for 100% methanol or acetonitrile in the first instance, or ~650 – 700 for 100% water. A slightly lower temperature may be required for lower flow rates.

Note that there is no protection against overheating, and that serious damage may be done to the nebuliser and/or the APCI source chamber if the heater is left on at a high temperature without any gas flow.
FIGURE 9-3-B. THE SIOSLOAD DIAGNOSTIC TABLES FOR THE APCI NEBULISER FITTED TO 'V' AND 'M' SERIES INSTRUMENTS.

Type SIOSLOAD, and then view the heater diagnostics by typing D6 at the ETH> PROM PT, or alternatively type M 681000 at the ETH> PROM PT to view the lower table.

<table>
<thead>
<tr>
<th>HEATERS</th>
<th>REQUEST</th>
<th>READBACK</th>
<th>COMMENTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Re-entrant</td>
<td>ON</td>
<td>99°C</td>
<td>99°C</td>
</tr>
<tr>
<td>Septum</td>
<td>ON</td>
<td>159°C</td>
<td>158°C</td>
</tr>
<tr>
<td>Cap Line 1</td>
<td>ON</td>
<td>24°C</td>
<td>58°C</td>
</tr>
<tr>
<td>Cap Line 2</td>
<td>ON</td>
<td>99°C</td>
<td>99°C</td>
</tr>
<tr>
<td>Cooled Probe</td>
<td>ON</td>
<td>30°C</td>
<td>478°C</td>
</tr>
<tr>
<td>Aghi Re-entrant</td>
<td>ON</td>
<td>24°C</td>
<td>478°C</td>
</tr>
<tr>
<td>Aghi Band</td>
<td>ON</td>
<td>199°C</td>
<td>478°C</td>
</tr>
<tr>
<td>Aux 1</td>
<td>OFF</td>
<td>0°C</td>
<td>478°C</td>
</tr>
<tr>
<td>Aux 2</td>
<td>OFF</td>
<td>0°C</td>
<td>478°C</td>
</tr>
<tr>
<td>(APCI)</td>
<td>ON</td>
<td>250°C</td>
<td>245°C</td>
</tr>
</tbody>
</table>

Note that the APCI request and readback values reported in this table are half that of the true values.
Sampling Cone Ramp

If this option is available, select ‘No Ramp’ in the first instance; ramping is only required for certain types of complex experiment, and is not necessary when tuning on individual ions.

Solvent Trip

This is the trip level of the source ionisation gauge. Leave it at a high value when operating in electrospray or APCI modes.

9-3-2. Interface Diagnostics

The SIOSLOAD diagnostic table shown in Figure 4-4-F, section 4-4-7, is common to both electrospray and APCI.

Information on the APCI nebuliser is contained in a different SIOSLOAD table. The address of the stand-alone heater fitted on ‘X’ and ‘S’ series instruments is M681200, while that of the integral heater on ‘V’ and ‘M’ instruments is M681D00, as shown in Figure 9-3-B.

9-4. Obtaining A Beam On Atmospheric Water Cluster Ions

Positively charged water cluster ions can usually be found using just the corona needle on its own, without the nebuliser. It is hard to predict which ions might be intense; usually one or more of the clusters at m/z 55, 73 or 91 can be found, and in some cases clusters up to m/z ~1200 may be detectable. These can be useful for mass calibration if the spectra are fairly reproducible from day to day. However, if water ions cannot be easily or reproducibly found, it does not necessarily indicate that there is anything wrong with the interface.

1. Position the corona needle so that the tip is approximately on axis with the sampling cone orifice, and 5 - 10 mm away from it. The adjusting screws allow in/out and horizontal movement of the needle, but vertical adjustment should be made manually, i.e. by gently bending the needle up or down.

To do this, first ensure that the high voltage is off; also unplug the high voltage connector to interrupt the trip circuit. Then remove the four screws attaching the corona needle assembly to the perspex chamber cover. Bend the needle as appropriate, then reassemble.

2. If an electrospray beam has already been found, then use the same tuning parameters in the first instance. The optima for the corona needle and sampling cone voltages will probably be different, but most or all of the others (ring electrode, focus & beam centre, and instrument lenses) are likely to remain the same.

3. Turn on the high voltage, and reposition the corona needle so that there are no, or only occasional, sparks jumping to the sampling cone.
4. Examine the spectrum for any water cluster ions, for instance at m/z 55, 73 or 91, and optimise the signal. Alternatively, carry out a survey scan to search for any ions to tune on.

5. If a spark occurs, it may be noticed that the ion being monitored does not recover to the original intensity afterwards, or only recovers slowly. This is normal behaviour.

6. A water cluster ion may be very stable with a cold APCI source, but it is common that the signal becomes very unstable, and may disappear completely, when the nebuliser is being pre-heated. This too is normal behaviour.

7. Various tricks can be tried to generate water ions. One method involves running the nebuliser with gas, heat and solvent for a short time (see section 9-5), then switching off the gas and solvent flows. As it cools, the nebuliser can facilitate the formation of much more intense water ions.

9-5. Operating The APCI Nebuliser

WARNING. The APCI nebuliser incorporates a 200 W heater which is controllable up to 1000 °C, and which can cause burns if touched. Furthermore, in the event of certain fault conditions, some of the external parts of the nebuliser and APCI chamber can also become extremely hot (or melt). never touch the nebuliser handle or the surface of the chamber without first checking that it is safe to do so, and always take care when carrying out any maintenance operations shortly after use of the interface.

9-5-1. Pre-Heating The Nebuliser

1. Start the APCI exhaust fan (section 9-2-3).

2. Adjust the position of the nebuliser so that it is roughly on axis in relation to the sampling cone orifice, and about 4 cm away.

3. Turn on the nebuliser gas, to give a value of around 12 on the flow meter, so that it can be heard hissing through the nebuliser.

4. Set the heater on/off switch to ‘on’, and increase the temperature slider in 100° steps to 500° or 600° C, over a period of a few minutes. Check that the temperature is regulating correctly; the readback should be reasonably stable.

(Gradual heating at this stage protects the nebuliser against damage if solvents have slowly soaked into the assembly).

5. Leave the nebuliser running with the heater and gas on (but no solvent flow) for 10 to 15 minutes. This heats up the nebuliser, sampling cone and chamber.
6. Connect up the sample line to the nebuliser, Figure 9-2-B. It is usually most convenient to use flexible (e.g. PEEK) tubing for the transfer line, although metal can be used if required.

Grip the flats on the in-line filter union with a spanner to prevent it rotating when the 1/16” nut is screwed in, and also be careful not to push the union into, or pull it out from, the nebuliser handle, since the in/out position determines the relative position of the nebuliser needles.

7. Switch on the solvent flow at 0.5 - 1 ml/minute, and observe the sampling cone for condensation. If liquid is seen, turn off the solvent flow and continue with the pre-heating for a few more minutes. (At this stage the condensation will do no harm, and may be beneficial in rinsing contamination away from the orifice).

8. Note that the nebuliser handle and the black plastic housing will become quite hot, perhaps too hot to touch for more than a short time, during normal operation. It is worthwhile becoming familiar with the approximate temperatures that these components reach, so that in the event of a fault developing it is easy to judge that the housing is overheating, and to take appropriate action.

9-5-2. Obtaining A Beam Using The Nebuliser

1. Set up the corona needle as described in section 9-4 for obtaining water clusters, if this has not already been done. Withdraw the needle tip so that it is ~10 - 15 mm from the sampling cone (when the nebuliser is operating, the corona needle has a different optimum position).

2. If an electrospray beam has already been found, then use the same tuning parameters in the first instance.

3. The easiest way to find and optimise an APCI beam is to continuously infuse a tuning compound. Otherwise, use as large a loop as possible (2 - 5 ml) and run at 0.5 ml/minute so that there is sufficient time to tune up. Some samples that are suitable for continuous infusion include:

   **Positive ions:**
   - 3-Picoline, 0.005% (v/v) in 1:1 water : acetonitrile; strong \([M + H]^+\) and / or \([M + acetonitrile]^+\) at m/z 94 and 135. Since this compound is volatile, it can be infused for long periods at much higher concentrations than this without causing any source contamination.
   - Pyridine, at the same concentration in the same solvent. This is more hazardous to handle than picoline, but is otherwise similar, with \([M + H]^+\) at 80.
   - Caffeine, at 1 ng/µl in 1:1 water : acetonitrile; strong \([M + H]^+\) and / or \([M + acetonitrile]^+\) at m/z 195 and 236. This does not seem to cause source contamination at this level, but it is best not to use this solution if the caffeine sensitivity specification is to be carried out soon afterwards.
   - PEG-200, 0.001% (v/v) in 1:1 water : acetonitrile.
Negative Ions:

Most organic acids should give a strong signal; 1% acetic acid in 1:1 water : methanol or water : acetonitrile is good, and will not cause any source contamination.

Anthraquinone at 1ng/µl should give a strong signal at m/z 207.

P-Nitrophenol will give a strong signal at m/z 138.

4. When a signal has been found, optimise the corona needle position and voltage, the nebuliser position, and the nebuliser gas and sheath gas flow rates. The sheath gas may decrease the signal intensity, but improve the stability and signal to noise ratio. Each of the above parameters is likely to affect the solvent declustering, and thus the signal to chemical noise ratio.

5. Also optimise the sampling cone and skimmer lens voltages - these may well have different optima compared to the values obtained with atmospheric water cluster ions.

6. Proceed to reoptimise all of the other relevant parameters, for instance the position of the APCI chamber in relation to the skimmer, the ring electrode voltage, the focus and beam centre voltages, and the other instrument lenses as described in section 4-8 for ESI.

7. The nebuliser temperature should also be optimised for the samples and solvent systems being used. This is best done by trial and error. As a guide, a higher temperature may help to prevent excessive cluster ion formation, and thus improve the sample ion formation or the signal to chemical noise ratio. However, if the sample is thermally labile it may also cause decomposition.

8. Save the various APCI source and instrument lens tuning parameters, by completing a Checklist, and/or by saving the data system values, for future reference.

9-5-3. Leaving The Nebuliser In Standby Mode

Rather than shutting down the nebuliser completely (see next section) in order to leave it just for a short period, for instance a couple of hours, consider doing the following:

1. Turn off the solvent flow, but do not disconnect the line.

2. Decrease the temperature to 350 - 400 °C, and leave the nebuliser gas flowing (perhaps at a reduced rate to conserve the gas).

It should then be possible to resume work quickly, without waiting for the interface to heat up from cold, and no harm should occur if the gas supply runs out unexpectedly.
9-5-4. Shutting Down The Nebuliser Completely

1. Turn off the solvent flow, and preferably place the injection valve in the closed position to prevent solvent slowly leaking through into the nebuliser when it is not in use (or disconnect the sample line at a convenient place). This prevents solvent soaking in to the heater assembly while not in use.

2. Turn the temperature slider down to zero, and set the heater on/off button in the APCI tuning menu to ‘off’. (The on/off button has no function if the stand-alone power supply is in use). It is good practice to save these settings in the current Parameters files, so that in the event of a data system crash the nebuliser will remain off by default.

3. Leave The Gas Flows On until the nebuliser temperature has fallen to 100°C or less.

4. It is good practice to unplug cable BB36 from the nebuliser at this stage, so that there is no possibility of it accidentally overheating.

5. Turn off the APCI exhaust fan if the APCI will not be used for some time.

6. If the system is being shut down in order to carry out maintenance, or in order to switch to another ionisation technique, be aware that even though the nebuliser heater has cooled, other parts (the nebuliser handle, and the chamber) will still be very hot!

9-6. A Guide To Running APCI Samples

This section briefly describes some of the special considerations required when running APCI samples. Some of the information listed in chapter 5 is also relevant.

9-6-1. Mobile Phases And Gradients

In general, many samples can be analysed successfully by loop injection using water/methanol or water/acetonitrile mixtures without any other additives. 1:1 water : acetonitrile tends to result in somewhat reduced solvent adduct formation compared to 1:1 water : methanol.

The optimum APCI nebuliser temperature depends on the solvent composition and flow rate, as well as the nature of the sample. If gradient LC-MS is to be performed, then several approaches can be taken to minimise the effect of the changing solvent composition, as follows.

• If possible, confine the gradient to a narrow composition range and optimise the nebuliser for a sample that elutes near the middle of the range. Up to around a 25% composition change, for instance 10 to 35% acetonitrile in water, can normally be tolerated without problems.
• If a medium range gradient is required, for instance 0 to 50% acetonitrile in water, and the flow rate can be in the region of 0.5 to 0.8 ml/minute, then the post-column addition of a make-up flow of organic solvent should be considered. If the make-up flow rate is the same as the LC flow, the overall change in composition would be brought into a manageable range in this example. This approach also has the advantage that a lockmass compound can be continuously introduced for high resolution SIR operation (see Figure 8-3-A).

• If a full range gradient, for example from 0 to 100% acetonitrile in water, must be run at 1 ml/minute then it is fairly straightforward to write a simple OPAL program that ramps the nebuliser temperature with the gradient. The high aqueous end of the gradient will require the higher temperature. One disadvantage of such OPAL programs is that while they are running it is not possible to carry out any other data system operations (for instance, new spectra or chromatograms cannot be called up).

9-6-2. Gas Phase Bacicity

As a general guide, basic compounds can be expected to run well in positive ion APCI, whilst acidic compounds should give good negative ion spectra.

The possibility exists to manipulate the gas phase reactions in order to improve ionisation of, or to selectively ionise, specific target compounds. This is usually achieved by careful choice of the mobile phase solvents, and/or by the addition of low levels of appropriate modifiers.

9-6-3. Calibration Compounds

Many of the mass calibration compounds listed in section 5-3-7 for electrospray will also run by APCI, with the exception of the peptides and proteins. Another difference is that the PPG’s may not produce multiply charged ion series by APCI.

Other options for APCI calibration include the use of the water cluster ions produced by the corona discharge alone (section 9-4), or the low mass solvent ion series that can be generated by the nebuliser under appropriate conditions.

It is important to inject only the minimum concentrations of calibrants necessary to obtain a good calibration, so as to avoid contamination of the APCI source. Small volume injections of 0.1 to 0.01% (v/v) PEG, for instance, could be employed, and continuous infusion of concentrations of around 0.001% (v/v) PEG can be used for LC-MS accurate mass experiments without significant contamination occurring.
Section 10

Routine Cleaning And Maintenance Of The APCI Interface
Routine Cleaning And Maintenance Of The APCI Interface

This chapter describes the routine cleaning procedures for the APCI part of the API interface. Refer to chapter 6 for the relevant procedures for cleaning of the skimmer/skimmer lens assembly, hexapole lens, transfer lenses, and cradle. (The procedures for cleaning the APCI and electrospray sampling cones are different).

The trouble shooting guide at the end of this chapter can be used to help identify faults and to determine which parts may need cleaning.

**Warning.** Be aware that the APCI nebuliser operates at high temperatures during normal operation, and can cause burns. Furthermore, in the event of certain fault conditions, some of the external parts of the nebuliser and APCI chamber can also become extremely hot (or melt). **Never** touch the nebuliser handle or the surface of the chamber without first checking that it is safe to do so. **Always** take care when carrying out any maintenance operations, since some components will retain the heat much longer than others.

Warning. If there is any possibility that the samples or solvents that have been sprayed are in any way hazardous, appropriate protective clothing, such as gloves and eye protection, must be worn.
Section 10
Routine Cleaning And Maintenance Of The APCI Interface
10-1. Maintainance Of The APCI Nebuliser

The APCI nebuliser can be worked on without venting the interface.

The cleaning intervals are determined primarily by the way in which the nebuliser has been used, but as a rough guide cleaning at 3 to 6 month intervals is likely to be required.

The components in the nebuliser are detailed in Figure 10-1-A.

10-1-1. Changing The In-Line Sample Filter

The main indications that the frit in the filter should be cleaned or changed are a significant increase in solvent back pressure (as indicated on the solvent delivery system pressure display), and/or leaking of solvent from any of the fittings between the pump and the nebuliser, possibly also accompanied by a loss of ion stability or sensitivity.

If the filter blocks frequently, then this indicates that there is a problem with the solvent delivery system, for instance worn piston seals or a damaged injection valve rotor, or possibly particulate contamination from the samples or from an HPLC column.

Other causes of high back pressure include blockage of the transfer line to the nebuliser (small-bore PEEK tubing can seal if fittings are overtightened). It is less likely that the sample needle will block, since this has a relatively large I.D.
To clean or replace the frit, do the following:

1. Undo this nut while gripping the flats on the filter union.
   - Do not disturb this union
   - Screwdriver or similar tool

2. If the filter does not come free, compress the ring on the Legris union with a suitable tool, and lift away the union and filter and slider block.

3. Separate the two halves of the filter.

FIGURE 10-1-B. REMOVAL OF THE FILTER FROM THE NEBULISER HANDLE, AND REPLACEMENT OF THE FRIT.
1. Turn off the heater and the solvent flow, allow the nebuliser to cool, then turn off the gas flows. Disconnect the sample line from the filter, and the nebuliser gas line.

2. Move the nebuliser outwards until the whole of the slotted cover on the nebuliser handle is exposed, then undo the five M1.6 x 3 ch. hd. screws and remove the cover.

3. While supporting the filter with a spanner to stop it twisting, loosen the 1/16" nut at the inner end of the filter. Note: do not disturb the position of the sample needle in the nebuliser assembly, otherwise it will be necessary to remove this assembly and readjust it.

4. The filter will now be free to slide, and may come free of the nebuliser handle.

5. If the filter cannot be extracted, then use a suitable tool such as a medium screwdriver to compress the ring on the inner end of the metal Legris fitting while pulling on the outer end, see Figure 10-1-B. This action should release the Legris fitting from the nebuliser pipe, so that the sliding block and filter are freed.

6. Undo the two halves of the filter union and tap out the frit and retaining ring, Figure 10-1-B. Inspect the frit for visible contamination, as this may provide some indication of its source.

7. The frit may possibly be cleaned by backflushing followed by ultrasonication (according to the standard procedures for cleaning HPLC frits), but it is simpler to replace it. The original frit is stainless steel, 0.5 μm pore, 0.062” thick x 0.062” diameter, trapped within a 0.25” diameter black PEEK ring (Upchurch Scientific order code A-102). It is better to replace it with a frit having 2 μm pore (Upchurch order code A-101, having a natural, tan PEEK ring).

8. Reassemble the filter, and refit it on the 1/16” end of the sample needle such that the arrow points in the direction of flow. A 1/16” teflon ferrule should be used; tighten the nut so that the filter is held securely. Be careful not to move the sample needle in or out during this procedure.

9. Reassemble the metal Legris union and sliding block on the nebuliser pipe, so that the filter is retained.

10. As a guide, the slider block should be flush with the end of the nebuliser handle, and the end of the filter should protrude by about 6 mm if the components are correctly positioned.
Measure this distance when needles are positioned correctly, and note it for future reference, so that the needle position can be subsequently estimated without disassembly (around 6mm is typical).
11. Connect up the nebuliser gas line and the sample line, and briefly turn on the solvent and gas flows to check for any leaks at the union between the filter and the sample needle (since the nebuliser is cold, the solvent flow must be turned off within a minute or so).

12. Refit the slotted cover to the nebuliser handle, and reposition the nebuliser in the APCI source chamber. Since the position is usually not very critical, it should suffice to approximate the earlier position. If this is not known, adjust it such that ~10 mm of the cover goes into the rectangular hole in the positioning mechanism.

13. The APCI interface should now be ready to use, following pre-heating. When the solvent is flowing again, note the new backpressure.

14. Check for any small leaks at the connections to the filter, and the filter body (it is possible to look through the slots in the cover). Warning. If a leak here goes undetected, then it is possible that a pool of solvent can collect in the nebuliser handle, and this can run out suddenly when the nebuliser is moved.

10-1-2. Checking The Sample Nebulisation

The nebulisation must be satisfactory if the overall performance of the interface is to be good. There are two main causes of deterioration, firstly accidental overheating or damage by corrosive solvents, and secondly accidental repositioning of the sample needle.

The following procedure should be used for checking the quality of the nebulisation:

1. Turn off the heater and the solvent flow, allow the nebuliser to cool, then turn off the gas flows.

2. Move the nebuliser outwards until the whole of the slotted cover on the nebuliser handle is exposed, then undo the M1.6 x 3 ch. hd. screws and remove the cover.

3. Loosen the 1/8” Swagelok nut on at the 1/8” to 1/4” union (Figure 10-1-A), while holding the sheath gas pipe to prevent the assembly twisting.

4. It should now be possible to withdraw the nebuliser pipe and sample needle assembly complete with the in-line filter and sliding block. If it jams during withdrawal then try to gently twist it free, without exerting undue force on the ceramic heater assembly.

5. The sprayer tip will probably be dirty and discoloured - this is normal, and it does not need to be rigorously cleaned (just remove any flaky particles with abrasive paper). The sample needle should protrude by ~1.3 mm through the outer tube, Figure 10-1-C. This distance is set by loosening the 1/16” nut at the nebuliser tee, and gently sliding the sample needle to the correct position. (Grip the 1/16” tube near the nebuliser tee with pliers, rather than pulling on the filter union).
Step 1: Inspect the spray (using safe solvents and appropriate protection)

SAFE solvent (pure water), 1 ml/minute

Nebulising gas flowing

20 mm spray

Steady and even spray of fine droplets, on axis or nearly on axis; gas jet can be felt 40 cm away

Step 2: Point the nebuliser into a beaker of water and observe how deeply the gas stream pokes down into the water.

40 to 60 mm typical; nebulisation satisfactory

Beaker of water

Nebuliser tip held vertically, at liquid surface
6. If the sample needle cannot be moved, it is possible that the tip has been damaged by overheating, and both the sample needle and the nebuliser needle assembly will have to be replaced.

7. Select a solvent system that is safe to spray into the laboratory atmosphere (pure water is a good choice). Wearing eye protection, point the needles in a safe direction, then turn on the solvent and nebuliser gas flows to observe the pneumatic nebulisation process. The spray plume should be reasonably on - axis, and should consist of fine droplets without sputtering, Figure 10-1- D.

If the spray is noticeably off - axis, then shut down the solvent and nebuliser gas flows and examine the tips of the needles using a good eyeglass. Polish the needles to produce clean, square - cut ends that are free of burrs and that are perfectly round.

If the spray is sputtering, then check that the gas supply pressure is 5 - 7 bar, and that the flow registering on the nebuliser flow meter is at or above 12 l/hour. Check the strength of the gas flow through the needle tip by pointing it into a beaker of water, Figure 10-1-D. If the gas flow is unsatisfactory then clean the outside of the sample needle (by gentle use of fine abrasive paper) and the inside of the nebuliser needle tip (using a fine wire) to ensure that the annulus is clear of obstructions.

8. When a good quality pneumatically nebulised spray is obtained, refit the nebuliser assembly in the 1/8” to 1/4” union such that the slider block is flush with the nebuliser handle, then refit the slotted cover.

9. The APCI nebuliser is now ready to use, following pre - heating.

10-1-3. Replacing The Heater Assembly

The ceramic heater assembly should have a long lifetime if handled carefully, but it may crack if knocked, or if its temperature rises rapidly after solvent has soaked into the ceramic putty surrounding the heating element. It does not normally need any cleaning, since any samples tend to be vapourised during the normal pre - heating procedure.

It is very unlikely that the heating element will burn out, but it is possible that the connections to the ceramic terminal block may corrode and fail. The element should have a resistance of close to 2.4 ohms when cold.
The procedure to replace a damaged heater assembly is given below:

1. Turn off the solvent flow and the heater. Turn off the gas flows when the nebuliser is cold. Turn off the instrument high voltage supplies.

2. Disconnect the external solvent, gas and electrical connections to the nebuliser.

3. Withdraw the nebuliser from the APCI source chamber so that the slotted cover can be removed.

4. Remove the four M3 x 5 mm screws that attach the nebuliser handle to the position adjusting mechanism, and lift away the nebuliser and handle.

5. Extract the brown and blue thermocouple wires from the electrical socket using a quickmate pin extractor, and remove the two heater wires from the ceramic connector block.

6. Loosen the 1/4” nut attaching the small (6 mm O.D.) ceramic tube to the 1/8” to 1/4” union.

7. Remove the four M1.6 x 5 mm csk. screws from the rectangular plate through which the large ceramic tube passes, and gently lift away the damaged heater assembly, Figure 10-1-A.

8. Follow the reverse of this procedure to install a new heater assembly, taking these points into account:

9. The ferrule used to attach the small ceramic tube to the 1/8” to 1/4” union should be able to be reused; otherwise replace it with a soft graphite ferrule.

10. Bend the two heater wires so that they do not touch each other or any other metal components. The glass fibre insulation on these wires loses its flexibility and tends to crumble, and so it is better to fit ceramic fish - spine beads if available.

11. Connect the thermocouple with the brown wire (+) as pin G and the blue wire as pin F in the electrical socket. Coil the metal part of the thermocouple wire so that it is not likely to touch either of the heater wires (it does not matter if it touches the metal pipework in the nebuliser handle).

12. When the heater is first used, it is possible the ceramics may outgas, producing dense fumes initially. Increment the temperature gradually in steps of 100° C when pre - heating the nebuliser.
Layers of yellow heatshrink insulation
(cut away any that is discoloured:
clean away exposed metal with
fine abrasive paper).

Ensure that this point is sharp

Straight needle, 18mm long. Clean it
with fine abrasive paper, and then
bend it so that it is precisely at the
same height as the sampling cone
orifice.

M1.6 x 3
grub screw

Sideways positioning
mechanism
(part of)

Figure 10-2-A. The Corona Needle
10-2. Cleaning The Corona Needle

The corona needle can be cleaned and sharpened without venting the interface. The high voltages must be turned off, but it is not necessary to shut down an operating nebuliser.

The interval between cleaning depends on the types of sample that have been run, and is related to the requirement to clean the sampling cone; in addition the corona needle may require sharpening more or less often depending in the amount of arcing that has occurred. The main indications for cleaning are a loss of stability and/or an increase in the background noise. As a general guide, clean the needle whenever very heavy contamination of the sampling cone has occurred (for instance if much too much PEG has accidentally been injected), or every third or fourth time that the sampling cone is cleaned during normal use.

1. Disconnect the high voltage cable, ES01, from the floating connector - this ensures that the HT is off by breaking the trip circuit.

2. Remove the four M3 x 8 mm screws that attach the corona needle assembly to the perspex plate, and lift the assembly away.

3. Allow the assembly to cool, if it was removed from an operating interface.

4. Wipe the yellow heat - shrink with an appropriate solvent to clean it, or cut it away and dispose of it if the contamination is heavy (its presence is not necessary).

5. Use fine abrasive paper to clean the exposed metal support rod and the needle itself so as to achieve a bright finish, then rinse in an appropriate solvent (e.g. water or methanol), Figure 10-2-A.

6. Inspect the sharpness of the corona needle using a good eyeglass. Excessive arcing tends to degrade the point. Sharpen it if necessary, using fine abrasive paper. If the corona needle is badly damaged, it can be replaced by any standard dress - maker's pin, cut to ~18 mm long. An Allen key suitable for the 1.6 mm grub screw at the end of the support rod is supplied (0.7 mm AF, Micromass code 5871024).

7. Refit the assembly in the housing using a couple of the M3 screws, and move the corona needle close to the sampling cone (while the HT remains off). Estimate any adjustment of the vertical position of the needle that may be necessary so that it is the same height as the sampling cone orifice. Remove the assembly, and bend the needle as appropriate until the alignment is good.

8. Fit the needle assembly permanently, connect the high voltage cable ES01, move the corona needle to its approximate previous position or slightly further away, and proceed to find the ion beam.
Areas requiring cleaning:

- Clean and clear; circular, with sharp edges.
- Hair or particle trapped within bore.
- Trapped particle or burr from one of the faces.

Hair across the hole: characteristic beam instability.
Bore partially occluded: characteristic decrease in turbo backing pressure.

Appearances of the bore on close inspection:

**Step 1:**
Inspect the bore, using a good eyeglass or low power microscope.

**Step 2:**
Swab off any loose deposits from the front and back faces, then clean and polish the front face using fine (e.g. 600 grade) abrasive paper. Rinse off any residue.

**Step 3:**
Squirt solvent through the bore, using a 1 ml disposable plastic syringe, or a glass syringe with a Rheodyne needle port cleaner pressed firmly over the hole (NOTE: WEAR EYE PROTECTION!). Alternatively, clean using a fine wire.

**Step 4:**
Rinse and dry, then re-inspect the bore prior to reassembly.

Clean this part with abrasive paper; remove any pitting.

Avoid damage to the vacuum sealing surface.

FIGURE 10-3-A. CLEANING THE APCI SAMPLING CONE (DO NOT USE THIS PROCEDURE TO CLEAN THE ELECTROSPRAY SAMPLING CONE).
10-3. Cleaning The APCI Sampling Cone

Indicators that the sampling cone requires cleaning are:
(a) heavy deposits can be seen on the sampling cone,
(b) a 2- or 3-fold decrease in sensitivity,
(c) a high level of background noise, or unexpected ions in the spectra,
(d) a marked decrease in stability that cannot be attributed to a nebulisation or ionisation problem, or
(e) a sudden and dramatic loss of sensitivity and/or stability that might be due to a hair or particle having lodged on the orifice.

A slow buildup of contamination may be accompanied by a gradual decrease in vacuum pressures. A complete blockage by for instance a particle will often result in a sudden large pressure decrease, possibly accompanied by high voltage discharges. This can occur because the pressure in the first pumping stage has fallen to the discharge range. In contrast, a hair lodged across the orifice rarely causes any noticeable change in the vacuum pressures.

Because this procedure involves venting the vacuum system, it is best to complete it as quickly as possible.

1. Completely shut down the nebuliser, and allow it to cool. Disconnect all of the sample, gas, and electrical connections to the APCI part of the interface, including the exhaust line.

2. Check that the three thumbscrews are tight, so that the APCI source chamber will not slip from its optimal position when the housing is vented.

3. Vent the APCI interface and source housing.

4. Loosen the top one of the three thumb screws, and lift out the APCI source chamber complete with the corona needle and nebuliser from the API housing. **Warning. Although the nebuliser cools quite quickly because of the gas flowing through it, the sampling cone and metal source chamber may still be very hot - handle these with due care.**

5. The first time the sampling cone is removed from the APCI source chamber, make a mark on it (for instance, scratch an ‘X’ on the cone and on the chamber) so that it can be put back in the same orientation.

6. The sampling cone can now be cleaned. **Warning. If there is any possibility that the samples or solvents that have been sprayed are in any way hazardous, appropriate protective clothing, such as gloves and eye protection, must be worn.**

7. Examine the sampling cone for contamination using a good eyeglass or a low-power microscope, refer to Figure 10-3-A. There are three areas requiring cleaning, namely the front, the back and the bore, and it is critical that they are each cleaned thoroughly. The deposits on the front face are best removed by polishing with fine grade abrasive paper until the surface is bright. Rinse off any residue with solvent.
Note - because of the surface damage to the APCI sampling cone resulting from occasional arcing and the harsh cleaning procedure required, the APCI and electrospray sampling cones should not be swapped over even though the items are originally identical.

8. The bore can usually be cleaned by squirting about 1 ml of an appropriate solvent through it using a disposable plastic syringe. Alternatively, the teflon needle port cleaner supplied with various Rheodyne HPLC injectors can be used to form a seal between a metal or glass syringe and the sampling cone.

9. Blow out any liquid remaining in the bore, and examine the bore very carefully using a good eyeglass or a low-magnification microscope. All deposits, burrs, or small obstructions must be removed for optimum performance to be obtained. Stubborn deposits may be removed using the fine cleaning wires supplied with Hamilton syringes, or by judicious use of a length of 150 um OD fused silica, for instance.

10. Inspect the inside of the metal part of the APCI source chamber for contamination, and clean it either with a solvent-soaked tissue, or with fine abrasive paper, as appropriate, to produce a bright finish. (Contamination here appears to be less important than that on the sampling cone, perhaps because it is further away from the region in which ions are formed and sampled).

11. Make sure that the sampling cone is free of any lint which might get sucked across the orifice, then reassemble it on the APCI source chamber.

12. Offer up the APCI chamber to the API housing, tighten the top thumbscrew that was loosened to remove it, and begin pumping down. Turbo pump controllers having the 'soft start' feature may take 45 minutes to bring the pump up to full speed, so that if the cleaning can be scheduled, it is useful to do it before lunch or at the end of the day.

13. (The corona needle can be removed and cleaned at this point, if required).

14. Make the gas and electrical connections to the APCI interface, and begin pre-heating the nebuliser. The interface will be ready to use when the turbo pump has reached at least 95% speed.
10-4. APCI Trouble Shooting Guide

This guide covers only the problems associated specifically with APCI; see also section 6-7 for other information.

**Ignition of solvents in the APCI chamber:**

Solvents having a high organic content can ignite due to the corona discharge, or sparks from the needle to the sampling cone. This is recognisable as dim flashes or popping noises, and is not normally likely to cause a major problem. It can be quickly quenched by turning off the solvent flow for a short time. Ignition can normally be prevented by withdrawing the corona needle slightly, and/or decreasing the voltage on it, or by slightly increasing the nebuliser or sheath gas flow rates. If the exhaust system is working properly then the likelihood of ignition is reduced. As a last resort, consider changing to a non-flammable solvent such as methylene chloride.

**Loss of the heater microprocessor from the optic loop:**

Discharges between the corona needle and the nebuliser assembly can cause unexpected faults with the instrument heater PCB, particularly on ‘V’ series instruments. One of the symptoms is that the heater supplies are lost from the optic loop. If the problem is persistent, contact factory personnel for advice.

**High background chemical noise, or unexpected ions in spectra:**

This is often the result of source contamination, and the only remedy is to clean the sampling cone (see chapter 10). If large quantities of calibration compounds such as polyethylene glycols or alkyl ethoxy sulphates have been injected, then these may stick to the chamber. As a general guide, use the lowest quantities of mass calibrants or samples commensurate with obtaining a satisfactory spectrum.

Other common causes of unexpected ions in the spectra include the use of low purity solvents, and possibly compounds present in the laboratory air. A spill of ammonia in an adjacent laboratory, for instance, may result in significant levels of ammonium adducts in the spectra.

**Regular, pulsing noise from the nebuliser:**

It is common for the nebuliser to produce a certain amount of audible noise. This will change in character from time to time, for instance in response to the flow disturbance when an injector is actuated. If the noise pulses regularly, then check for unstable flow from the solvent delivery system.
Unstable signal, and condensation on the sampling cone:

This can occur when the system has not been pre-heated sufficiently, when using solvents having a high aqueous content, or when the requested temperature is too low. Increase the temperature (or change the solvent flow rate or composition) until the condensation is no longer observed. It may also help to increase the gas flows slightly, to improve thermal transfer to the sampling cone.

Unstable signal, and fluctuating vacuum pressures:

If sputtering or condensation results in liquid being drawn in through the sampling cone orifice, then the rapid evaporation that subsequently takes place may cause sudden variations in both the turbo backing and source ionisation gauge pressures, as well as momentarily causing loss of the ion signal.

Nebuliser produces very high solvent back pressure:

Inspect, clean, and/or replace the frit in the in-line filter mounted in the handle of the nebuliser. The frit supplied as standard in the filter is 0.5 µm, but it is better to replace it with a 2 µm frit.

If the frit clogs regularly, then the cause of the problem should be established. Worn pump or injector seals may need to be replaced.

Nebuliser fails to work after it has become overheated:

The nebuliser can be damaged by excessive heating; in particular the braze at the needle tip can melt, if any of the following events occur:

1. The data system was rebooted after a crash, and the APCI heater defaulted to ‘on’ without any solvent or gas flow;
2. The gas supply ran out during an unattended analysis;
3. The temperature request was set too high (e.g. 800°C) without sufficient solvent or gas flow to keep the needle tip cool.

If the braze has melted, then it is likely that the needle assembly will need to be replaced. (Later designs incorporate welded needles which can better withstand the high temperatures).

Nebuliser fails to work after phosphoric acid solvent systems have been used:

The acid will corrode the hot nebuliser needles, which will need to be either cleaned or replaced. The damage is similar to that caused by overheating. Do not use phosphoric acid solvent systems!
**Nebuliser fails to heat up:**

1. Systems with the stand-alone power supply: The circuit breaker on the power supply has tripped, or a mains fuse has blown. If the red part of the circuit breaker is visible, it has tripped. If this is all right, disconnect the 15-way D-type plug from J6 on the power supply. If the diodes go off, then there is a fault with the mains supply. Check the fuses in the 13A mains plug, and in the mains socket on the power supply.

2. Systems with integral electronics on the heater PCB: One of the fuses has blown, or there is an optic loop (control) fault.

3. There is a faulty contact at the ceramic block within the handle of the nebuliser (the terminals can corrode if the screws were not tightened fully).

4. It is very unlikely that the heating element itself has failed; check that the resistance is ~2.4 ohms when the heater is cold.

**Nebuliser assembly is very wobbly on its positioning mechanism:**

The tolerances in the X-Y-Z positioning mechanism are such that the nebuliser can easily wobble around, and this can be perceived as a problem. However, the position of the nebuliser in relation to the corona needle and sampling cone is actually not very critical, perhaps because the spray plume is relatively broad, hence the wobble is not a problem.
Section 11

Operation In Other Ionisation Modes With The API Interface Installed
11-1. Compatibility And Limitations

The API interface is designed such that a range of other ionisation techniques can be used whilst the interface is still attached to the instrument. This facilitates, for instance, easy LSIMS calibration for electrospray spectra, or the opportunity to carry out an ‘emergency’ GC-MS analysis in a busy laboratory. The ionisation techniques that are compatible are listed in Table 11-1.

One of the main difficulties is that if the inlets 1 pump is used to back the turbo, then the side vacuum lock cannot be pumped down in the normal way. This can easily be overcome by the purchase of an extra Edwards E2M8 (inlets 2) rotary pump if it is intended that the electrospray interface will be left in place much of the time.

A second difficulty is that no movement of the various LSIMS targets with respect to the beam axis is possible, and so the correct positioning of the Caesium gun is somewhat more critical. This can limit the Dynamic LSIMS performance, but will probably not make any noticeable difference to static LSIMS performance.

<table>
<thead>
<tr>
<th>Technique or Interface</th>
<th>Compatible ?</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Static LSIMS</td>
<td>Yes;</td>
<td>Use either the Dynamic LSIMS side reference probe (good for CsI, etc.), or the accurate mass LSIMS probe (better for samples)</td>
</tr>
<tr>
<td></td>
<td>pump out side lock with care</td>
<td></td>
</tr>
<tr>
<td>Dynamic LSIMS</td>
<td>Yes;</td>
<td>A special side entry Dynamic LSIMS probe is available</td>
</tr>
<tr>
<td></td>
<td>pump out side lock with care</td>
<td></td>
</tr>
<tr>
<td>TLC LSIMS</td>
<td>Yes;</td>
<td>No limitations</td>
</tr>
<tr>
<td></td>
<td>pump out side lock with care</td>
<td></td>
</tr>
<tr>
<td>Laser Desorption</td>
<td>Yes;</td>
<td>No limitations</td>
</tr>
<tr>
<td></td>
<td>pump out side lock with care</td>
<td></td>
</tr>
<tr>
<td>EI/CI, solids probe</td>
<td>Yes;</td>
<td>Inner sources must be mounted manually</td>
</tr>
<tr>
<td></td>
<td>pump out side lock with care</td>
<td></td>
</tr>
<tr>
<td>EI/CI, GC and septum inlet</td>
<td>Yes</td>
<td>No limitations</td>
</tr>
<tr>
<td>AGHIS</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td>Particle beam, TSP/PSP</td>
<td>(Yes)</td>
<td>Both are possible, but due to the bulk of the trolleys, and a second plug panel being required, complete removal of API is recommended</td>
</tr>
<tr>
<td>FD</td>
<td>No</td>
<td>Front lock required</td>
</tr>
</tbody>
</table>

Table 11-1. Ionisation Modes / Interfaces That Can Be Operated Whilst API Is Still Fitted.
Step 1:
Remove the sampling cone from the end of the electrospray atmospheric pressure chamber or the APCI source chamber, and store it in a safe place.

Step 2:
Fit the sampling cone blank, M805213BD1, to the ESI or APCI chamber, this way round.

Note: the recess must be on this side

(Part of the electrospray atmospheric pressure chamber shown)
11-2. Blanking Off The API Interface

1. Shut off any solvent flows. If APCI is fitted, cool the nebuliser prior to shutting off the gas flow.

2. Shut the analyser isolation valve. Turn off the source ionisation gauge and close the butterfly valve. Turn off the turbo pump.

3. Check that the three black plastic thumbscrews are holding the atmospheric pressure chamber in place - this will prevent it from moving when the interface is vented, thus enabling an API beam to be found relatively quickly when the interface is next in use.

4. Vent the electrospray or APCI interface, and remove the electrospray atmospheric pressure chamber or the APCI source chamber, as appropriate. Remove the sampling cone from the end of the chamber, and fit the blanking plate, Figure 11-2-A. Refit the chamber in the housing.

5. Remove the electrospray cradle from the source housing region, and install the new ion source and associated hardware as appropriate (see the following sections).

6. Pump down the API housing using the first-stage rotary pump, the second stage pump that backs the turbo, and the instrument housing via the side lock in the normal way.

7. Start the turbo pump. It can be run either at standby speed or at full speed in this mode.

8. **Close The KF-25 Speedivalve** on the first-stage pumping line. This step is important - it is done to prevent backstreaming of the first-stage rotary pump oil when there is no gas load on this line.

9. The source housing pressure should fall rapidly towards \( \sim 1 \times 10^{-6} \) mbar, since there is no gas load on the API interface. The new ionisation technique can be used immediately, without waiting for the turbo pump to reach full speed.

10. Park the electrospray probe either in the API housing, or in the spray tester, such that the probe microswitch is closed (otherwise the source HT will not come on). Similarly, if APCI is fitted, reconnect the high voltage plug ES01 to the corona needle socket.

11. Note - recall of other instrument menus. No harm will be done to the API interface if an ESI or APCI source is still selected when the instrument is switched to 'operate' - however, high voltage will be applied to the API interface. If instead an EI or LSIMS source is selected, then all of the API high voltages will remain off when the HT is turned on.

12. Note that EI or LSIMS can be operated at 8kV after the API interface has been blanked off.
Side probe compatibility
The current design (with a short insulator and plate) will fit on both the original Solids probe having a solid steel shaft, and the water cooled Solids probe having a hollow shaft and a copper end.
The early design will not fit in the vacuum lock if used with the water cooled probe.

Steel plate (Reversible), primarily for reference compounds,
early design: 49mm long
current design: 46mm long

Overall assembly code:
M802051AC1

Another version, suitable for ‘X’ series instruments having Mk1 optics,
is M802067AC1, not shown here.

FIGURE 11-3-A. THE SIDE ENTRY REFERENCE PROBE TIP FOR LSIMS

Alternative:
protect the lens stack
using a cover of aluminum foil

These insulators must be protected from contamination

API transfer lens stack

M805248BD1
lens cover for LSIMS operation

Slide cover over the transfer lens so that the two side pumping holes are orientated horizontally

FIGURE 11-3-B. PROTECTING THE API TRANSFER LENS AGAINST CONTAMINATION WHILST USING LSIMS.
11-3. Static LSIMS Operation

Static LSIMS analyses can be carried out using the side entry reference probe supplied with the Dynamic LSIMS accessory, two versions of which are shown in Figure 11-3-A. (These parts are supplied as standard with the Dynamic LSIMS accessory, and not with the electrospray interface, thus should be ordered separately). The side entry reference probe target is designed primarily for use with reference compounds, and may not prove ideal for certain types of sample analyses.

Another static LSIMS probe which is better suited to sample analysis is the accurate mass probe (code for the complete kit: M804112DC1). This probe is not provided as standard with either the static LSIMS or electrospray interfaces, and should be ordered separately if required.

The end of the electrospray lens stack which protrudes into the instrument source housing must be protected from the primary Caesium beam during LSIMS analyses, either by using the cap provided, or by using aluminium foil, Figure 11-3-B. This beam, which hits the glass lid and is reflected back down towards the lenses, can eventually contaminate the black plastic insulators, resulting in a loss of API sensitivity and/or stability. Contamination can usually be seen as a white, grey, brown or carbon - black bloom on these insulators (refer to section 6-6-2 for cleaning procedures).

Care must be exercised when pumping out the side vacuum lock if the turbo pump is backed by inlets 1. In this case, the speedivalve below the turbo pump should be closed temporarily (with the turbo still running) for just long enough to evacuate the vacuum lock.

11-4. Dynamic LSIMS Operation

The side entry Dynamic LSIMS probe can be useful if frequent switching between API and Dynamic LSIMS interfaces is necessary. This probe kit is only available as a special option. However, in general the performance of the axial (standard) Dynamic LSIMS probe is somewhat superior, since it is easier to correctly position this probe in relation to the Caesium beam and the instrument ion extraction area, and the tip assembly has a smaller dead volume. It is therefore preferable to completely remove API and to use the axial probe for Dynamic LSIMS wherever possible.

As with static LSIMS, the exposed end of the API transfer lens stack should be shielded from the primary Caesium beam to protect it from contamination; see Figure 11-3-B.
FIGURE 11-5-A. ADDITIONAL COMPONENTS REQUIRED TO CLAMP THE INNER SOURCE IN THE EI/CI CRADLE.
11-5. EI / CI Operation

Since the front probe lock is not available, the inner source cannot be exchanged in the normal way. Instead, the API housing and instrument source region are vented, the inner source volume is exchanged, and the instrument is pumped down according to the procedures detailed in the previous sections.

It is necessary to use a clamp plate and a spring to hold the inner source tightly in the cradle, as shown in Figure 11-5-A. lvl (These parts are supplied as standard with the electrospray interface).

It is probably not necessary to fit the LSIMS shield (Figure 11-3-B) to the exposed end of the API transfer lens stack whilst operating in EI/CI mode, since there is much less source contamination.

The GC and the septum inlet can be used in the normal way, but care must be exercised when pumping out the septum inlet or when using the side vacuum lock if the turbo pump is backed by inlets 1. In this case, the speedivalve below the turbo pump should be closed temporarily (with the turbo still running) for just long enough to evacuate the vacuum lock.

11-6. Changing Back To ESI or APCI Ionisation

1. Turn off or shut down the ionisation technique currently installed. Shut the analyser isolation valve. Turn off the source ionisation gauge and close the butterfly valve. Turn off the turbo pump and vent the source.

2. Remove the glass lid and exchange the current ion source and any associated hardware for the electrospray cradle assembly. Slide the steel ring across (use a screwdriver) and connect the two wires to the end of the electrospray transfer lens stack (refer to Figure 4-2-D).

3. Remove the electrospray atmospheric pressure chamber or the APCI source from the API housing, remove the blanking plate from the end, and refit the sampling cone (inspect the sampling cone for contamination, and clean it prior to reassembly if necessary).

4. Pump down the API interface in the normal way.

5. Recall the appropriate ESI or APCI Instrument parameters. Note: NEVER switch on the HT while other instrument parameters are on display! If the high voltages are turned on while the electrospray cradle is fitted and for instance an EI or LSIMS source is selected, then accelerating voltage will be applied to the skimmer whilst the surrounding components are grounded. This can cause damage.

6. Check that the accelerating voltage and Limits values (Figure 4-4-B) are returned to 4000.
7. Wait until the turbo pump has reached at least 95% speed before turning on the HT and attempting to find an ESI or APCI ion beam.

8. If the ESI atmospheric pressure chamber or APCI source has remained in the same position when the interface was vented, and if the appropriate set of Lens Tune parameters are recalled or the potentiometers on the pot box are returned to previously noted optimum positions, a beam should be obtained relatively easily.
Section 11  Operation In Other Ionisation Modes With The API Interface Installed
Section 12

Installation and Operation of the Nanoflow Electrospray Interface

micromass
12-1. Safety Information

The following contains important information concerning the safe use of the nanoflow electrospray interface and should be read before using the system.

- Always wear eye protection when handling fused silica.
- Always wear eye protection when handling borosilicate capillaries.

- Always turn the system to standby before unscrewing the acetal body from the ampling cone assembly. When the system is in operate the screws under the protective acetal caps are at high voltage.

- **WARNING**: Care should be taken handling borosilicate capillaries as they are extremely sharp.

- **WARNING**: Always remove any back pressure from the borosilicate sample probe before removing the borosilicate capillary, to avoid the capillary being forcibly ejected when the pressure on the conductive elastomer is released. The connection from the regulated supply should always be made via the back pressure inlet at the bottom of the acetal body, and the connection from the probe should be made to the stud directly above the back pressure inlet. The short length of clear tygon tube running from the probe acts as a tether preventing removal of the probe when the back pressure connection is made.

- Ensure that the solvent delivery system used with the fused silica sprayer is grounded to prevent charging.
- If a bath gas is used an exhaust line from the interface should be fitted.

All procedures must be performed by suitably trained personnel, aware of the inherent hazards and observing every precaution.

If the equipment is used in a manner not specified by the manufacturer the protection provided by the equipment may be impaired.
12-2. Introduction.

The Nanoflow technique allows sample to be introduced into the electrospray source at flow rates of between 5nL/min to 1μL/min. For a given concentration of sample, the absolute ion currents produced at these reduced flow rates are comparable to the ion currents produced at a flow rate of 10μL/min using the standard electrospray probe.

This great reduction in the flow rate leads to the high sensitivity gains possible in this technique. For instance for the same concentration of sample and the same experimental conditions, 1000 times less sample would have to be consumed at a flow rate of 10nL/min compared to a flow rate of 10 μL/min to produce data of comparable quality.

The reduced flow rate, and hence reduced sample consumption, results in ion signals persisting for long periods of time even when relatively small volumes are introduced. This allows the possibility of performing multiple experiments such as MS, MS/MS and high resolution accurate mass, on a single loading of 1 to 2 μL of solution.

To facilitate high resolution accurate mass measurements, without the need for mixing reference material with the sample, the nanoflow interface incorporates provision for the simultaneous introduction two probes. Using one probe for sample and the other for reference material accurate mass determinations may be made without contamination of the sample.
Section 12 Installation and Operation of the Nanoflow Electrospray Interface

Schematic showing front of the interface:

- Adjustable probe in stop
- Sprung loaded out position catch knob
- Bath gas exhaust
- Bath gas inlet
- Back pressure inlet
- Interconnection box
- High voltage BNC connection for probes
- (ES01) probe voltage and microswitch connector
- (ES02) sampling cone Voltage
- Screw retaining acetal to ionisation chamber NOTE: High voltage when in operate

Schematic of probe:

- Sprayer tip
- Leuer fitting
- Tygon tube
- Probe guide / out stop catch rod / microswitch actuator
12-3. **Description of the Nanoflow Interface.**

The nanoflow interface is designed to fit onto the standard electrospray source in place of the counter electrode assembly and consists of a black acetal body with two probe insertion ports attached to a metal ionisation chamber to which a sampling cone identical to that used for standard electrospray is fitted.

Attached to the body of the interface is a 4 mm Legris fitting allowing a bath gas to be directed into the ionisation chamber. A 6 mm Legris fitting allows an exhaust line to be fitted. A second 4 mm Legris fitting, directly under the acetal body, allows back pressure to be applied to the borosilicate sprayer probe.

The probe insertion ports each contain a microswitch which provide a high voltage safety interlock. High voltages can only be applied to the interface when both probes are inserted. The insertion locks also contain a catch mechanism which allow the probes to be withdrawn to a fixed “out” position without removing them completely from the interface. This feature provides a means of rapidly switching between the two probes without activating the safety interlock and turning the high voltage to the system off.

The distance from the tip of the sprayer to the sampling cone is set by an adjustable stop at the top of each probe insertion port.

Two probes are supplied with the standard system which are identical apart from modifications to the tip to allow configuration for different sprayer types. The probes and the tips are interchangeable and the system may be configured with only one probe if required. Attached under each probe handle is a metal rod which when the probe is inserted closes the safety interlock microswitch and locates into a catch mechanism.

Electrical connection to the probes is made via a high voltage BNC socket on an interconnection box on the right hand side of the interface. A flying lead terminating in a BNC plug is attached to each probe.

Two sprayer types are available.

### a. Metal Coated Borosilicate tip

These afford the lowest flow rates (5 - 50 nL/min) and the minimum sample loading volume (1 - 2 uL), and consist of a glass capillary tube which has been drawn to a small orifice size at one end and coated with metal to allow electrical contact to be made. Typically the diameter of the sprayer orifice is between 1 and 5 \( \mu \)m. Sample is loaded, using a suitable syringe, into the back of the capillary. The capillary is then positioned in close proximity to the sampling cone orifice and a potential difference of 1 to 2 KV is applied between the sampling cone and the sprayer tip. Liquid is drawn out of the capillary purely by electrostatic force and ions formed in the spray drift towards the sampling cone and enter the mass spectrometer.

To avoid cross contamination a separate capillary should be used for each new sample to be analysed.
b) Fused Silica Tip

This employs a short length of 20 μm i.d., square cut, fused silica as the sprayer orifice and is designed to be coupled to a continuous flow solvent delivery system such as a Harvard syringe pump or a HPLC pump fitted with an appropriate splitter. The fused silica sprayer is operated at a flow rate of between 100 nL/min and 1 μL/min and may be used in conjunction with a loop injector, coupled to a nano-LC column or used to continuously infuse analyte.

The fused silica is not metal coated and electrical contact is made through the liquid within a low dead volume union (dead volume approximately 5 nL). This union allows the 20 μm i.d. sprayer fused silica to be joined to a length of 25 μm i.d. fused silica acting as a transfer line to the tip.

12-4. Installation of the Interface

This procedure assumes that the standard electrospray interface is fitted to the mass spectrometer and that the source housing has been vented to atmosphere.

- Unplug the electrical connection to the standard electrospray probe ES01, and the electrical connection to sampling cone and counter electrode ES02.
- Remove the probe guide flange, and flange containing the feedthrough for ES02.
- Remove the counter electrode assembly.
- Replace the counter electrode/pepper pot assembly with the nanoflow interface making sure that the connection box is on the right hand side and the two probe insertion ports are in a roughly horizontal plane.
- The interface is initially held in place by the three positioning adjuster screws on the electrospray housing and will become secure when the source housing is evacuated.
- Evacuate the source housing.
- Connect the ES02 to the feedthrough on the front of the interface. This connector carries the sampling cone high voltage and also an interlock which enables the high voltage only when this connector is inserted.
- Connect ES01 to the feedthrough at the bottom of the interconnection box on the right hand side of the interface. This feedthrough carries the high voltage supply for the probe tips and also the probe safety interlock microswitch connections.
12-5. Assembly and Operation of the Fused Silica Sprayer

**WARNING:** Always wear eye protection when handling fused silica.

- Cut approximately 600 mm of the 25 µm i.d. fused silica.
- Cut approximately 40 mm of the 20 µm i.d. fused silica.
  
  *It is recommended a white background is used when handling the 20 µm i.d. fused silica.*

- Cut approximately 30 mm of orange stripe PEEK tubing
- Cut approximately 30 mm red stripe PEEK tubing
- Cut approximately 30 cm of 1.7 i.d. PTFE tubing.
- Locate the ultra low dead volume Valco union (ZU1XC) and the Valco plug cap.
- Remove the probe handle. Thread the tube PTFE tube through the hole in the back of the probe handle and push securely over the tube protruding from the probe shaft. This will act as a guide for the 25 µm i.d. fused silica transfer line.
- Replace the probe handle.
- Thread one end of the 25 µm i.d. fused silica through the orange PEEK sleeve.
- Set the correct pilot depth for the PEEK and a Valco ferrule using the plug cap provided.
  
  *Do not overtighten when setting the pilot depth.*

- Remove the PEEK from the plug cap and the nut from the PEEK.
- With a small amount of fused silica protruding, cut a small piece (approximately 2 mm) off the end, then ensure that the fused silica is flush with the sleeve using a scalpel blade to push it back.
  
  *This ensures that the fused silica has a clean end.*

- Using the plug cap again set the correct pilot depth for the red stripe PEEK sleeving and a Valco ferrule
- Remove the PEEK from the plug cap and the nut from the PEEK.
- Cut the PEEK so that approximately 5 mm is protruding from the back end of the ferrule, and hence none can be seen when the nut is replaced.
- Thread the 20 µm i.d. fused silica through the PEEK ensuring that the fused silica is flush with the sleeve using a scalpel blade
- Attach the red PEEK sleeved fused silica to one end of the low dead volume union using a normal Valco nut.
• Attach the orange PEEK sleeved fused silica to the other end of the Valco union.

• Ensure both pieces of fused silica are tightly held.

• Thread the 25µm fused silica through the probe shaft and through the PTFE sleeve.

• Screw the Valco adapter into the probe shaft (finger tight)

• Cut down the 20µm i.d. fused silica such that 4 - 6 mm protrudes from the nut.

• Set the probe in stop on the main body of the interface fully out by turning the knurled knob anticlockwise.

  When this probe is used to introduce a calibration reference material for accurate mass measurement the left hand side, angled, probe insertion port is normally used.

• Attach the 25µm fused silica to the solvent delivery system.

  A Harvard Syringe pump or HPLC pump with a suitable splitter. The pump should be grounded to prevent charging.

• Ensure that any air bubbles are removed from the liquid flow.

  This may be achieved simply by running the pump at 2-3 µL/min until all the lines are full of liquid.

• Reduce the flow to the required rate (approximately 150nL/min)

• Fully Insert the probe until the probe handle hits the probe in stop adjuster.

  To insert the probe, pull down on the black sprung loaded knob on the underside of the insertion port. Guide the metal rod on the probe handle through the hole under the probe shaft. Release the catch Knob.

• Attach the flying lead from the probe handle to one of the BNC sockets on the underside of the connector box on the right hand side of the interface.

• Connect the bath gas inlet tube to the smaller of the two push fit Legris fittings on the left hand side of the interface.

  The nebuliser supply from the standard interface may be used for this purpose. The necessity and flow rate for the bath gas should be determined experimentally.

• On the source tune menu set the needle voltage to zero.

• Turn the instrument into operate.

  Voltage will only be supplied if probes are inserted to both inlet ports activating both microswitches.

  A stable beam should be obtained with a needle voltage of ~2.5kV relative to the sampling cone voltage. However, there will be an upper and lower limit where the beam starts to go unstable. It has been found that the system operates efficiently when the needle voltage is set to the mid point.
With time, the needle voltage needed to obtain a stable beam will start to rise. This usually means that the sample cone requires cleaning with methanol. See the section on routine maintenance.

It is important that the end of the 20µm i.d. fused silica is cut cleanly, unstable signals can often be traced to this component.

12-6. Assembly and Operation of the Borosilicate Sprayer.

To remove the borosilicate capillary from its case lift vertically while pressing down on the foam with two fingers.

Caution: It is important to take great care when handling the borosilicate tips as they are extremely fragile. Always handle using square end of capillary.

• Remove the probe handle. Thread the Tygon tubing through the hole in the back of the probe handle and push securely over the tube protruding from the probe shaft. Replace the handle. Fit the Leuer connection into the exposed end of the tubing. This will allow back pressure to be applied to the capillary if it is required.

• Identify the tip to be used and remove carefully from the case.

• Locate the swagelok union in which the capillary is to be held and remove the knurled nut.

• If not already available, cut a 5mm length of blue elastomer.

• Pass, in turn, the knurled nut, the elastomer and the union, over the square end of the capillary.

• Secure the nut, finger tight, such that approximately 18mm of capillary protrudes from the nut

Loading the Sample.

There are five types of capillary currently available from Micromass, each with a slightly different tip profile:

• Types A, C, and E have been found to work optimally when using, for example, peptides.

• Types B and D work optimally for larger molecules or for fully aqueous solutions.

10 of the type A tips are supplied with each system. It is recommended that individual laboratories should produce their own tips if this sprayer is to be used routinely. Commercial capillary drawing equipment and metal sputtering devices are available. It is suspected that the tips have a finite shelf life probably due to the liquid flow characteristics of glass.

The capillary may be reused if the same sample is to be analysed, but to avoid cross contamination it is recommended that a new capillary is used for each sample.

If possible, filter the sample using a 0.2µm Anopore filter.
It has been found that the beam stability improves when 50% Methanol is used as opposed to 50% Acetonitrile with 50% water.

The sample can now be loaded into the capillary using either a fused silica syringe needle or disposable gel loader tips as follows.

**Fused silica syringe**

Push the needle to the base of the tip before depressing the syringe plunger.

*A fused silica needle syringe is commercially available (Hamilton 75RNFS) which allows accurate volumes to be injected.*

**Gel Loader tips**

Inject the sample about half of the way into the length of the borosilicate capillary.

Hold the capillary vertically until the sample runs to the tip.

*Gel loader tips allow accurate volumes to be injected without the possibility of cross contamination, however, there is some evidence that plasticisers in the tips can contribute to chemical background in spectra.*

**Operation**

Once the sample has been loaded into the capillary.

- Secure the Swagelock union onto the probe.
- Set the probe in stop on the main body of the interface fully in by turning the knurled knob clockwise.
- Insert the probe fully into the source.
  
  *To inset the probe pull down on the black sprung loaded Knob on the underside of the insertion port and guide the metal rod on the probe handle through the hole under the probe shaft. Release the catch Knob.*
- Attach the flying lead from the probe handle to one of the BNC sockets on the underside of the connector box on the right hand side of the interface.
- Connect the bath gas inlet tube to the smaller of the two push fit Legris fittings on the left hand side of the interface.
  
  *The nebuliser supply from the standard interface may be used for this purpose. The necessity and flow rate for the bath gas should be determined experimentally.*
- On the source tune menu set the **needle** voltage to zero.
- Turn the instrument into operate.
  
  *Voltage will only be supplied if probes are inserted to both inlet ports activating both microswitches.*
- Adjust the **needle** voltage to optimise beam stability and signal intensity.
  
  *The needle voltage should not need to be raised above 3KV relative to the sampling cone and may require reducing to obtain a stable beam. A high needle voltage often indicates the need for cleaning the sampling cone.*
Initiating Spray and Improving Stability.

If no signal is seen or the signal intensity or stability is poor one of, or a combination of, the following procedures may be employed. If spray is interrupted during an analysis the procedures described may be employed to reinitiate the spray.

A) Raise the needle voltage rapidly to maximum for one or two seconds then lower again to a standard operational voltage.

B) Turn the system to standby for one or two seconds then back to operate at a standard operating voltage.

If this procedure is employed to reinitiate spray during an accurate mass determination at high resolution, data from the calibrant and the sample should be acquired again to minimise errors.

Connect a nitrogen supply fitted with a 0-30psi regulator to the 4mm legris fitting directly under the acetal body. The Leuer fitting on the Tygon tubing exiting the rear of the probe handle should then be firmly pushed over the stud above the legris fitting on the front face of the acetal body.

Apply back pressure to the capillary.

The nitrogen back pressure should not exceed 1.4 bar (20 psi)

WARNING: Always remove back pressure before removing the borosilicate capillary to avoid the capillary being forcibly ejected when the pressure on the conductive elastomer is released. The Tygon tube must be of a length such that removal of the probe without first removing the pressure is impossible.

C) Remove any back pressure applied to the capillary and remove the probe. Using a fine tissue very lightly touch the tip. A drop of liquid may appear.

This requires a steady hand and experience to avoid completely snapping off the fine glass filament at the end of the tip.

D) Remove any back pressure applied to the capillary and remove the probe. Using a magnification device and a manipulator, touch the tip against a flat surface taking care not to completely snap off the fine glass filament at the end of the tip. A drop of liquid may appear.

NOTE: When using the borosilicate glass tips a back pressure should always be applied, ideally with a nitrogen cylinder, to ensure a reliable spray.

Calculation of flow rates

If an unknown amount of sample is placed in the capillary, the amount of sample consumed may be calculated as follows.

Measure the internal diameter of the capillary (approximately 0.58mm)

Measure the length (in mm) of liquid used during the run

By holding the capillary up to light it is possible to see the meniscus level of the liquid before and after analysis.

Calculate the amount of sample in mm$^3$ used during the run.

$mm^3 = \mu L$

Based on the duration of the run calculate the average flow rate.
Storage of Loaded Tips

Loaded tips may be stored in the refrigerator for use at a later date providing that the sample is stable.

Recovery of Unused Sample.

Once analysis is complete unused sample may be recovered from the tip of the capillary using a fused silica syringe. This sample may then be analysed by other methods if required.

Instructions for Use of the Micro-Manipulator

The micro-manipulator assembly allows the tip of the borosilicate sprayer to be touched against a surface to ‘distress’ the fine capillary which forms the sprayer. Distressing this tip can often re-start a blocked sprayer.

The kit supplied consist of:
- 1 50X pocket microscope
- 1 Micro manipulator table assembly
- 1 M3x 8mm dowel
- 1 adjuster screw with tapered tip

**Warning: The borosilicate tip is very sharp and care should be taken in its handling.**

- Unscrew and remove the tip of the nanoflow probe without disturbing the borosilicate sprayer capillary.
- Place the whole tip on the manipulator so that it is gently retained by the spring clip and the sprayer tip is roughly 1mm away from the flat plate.
- Whilst illuminating the flat plate from the rear with a suitable light source view the tip through the microscope. The guide on the microscope is designed to rest on the top of the flat plate and focusing is achieved by sliding the microscope through the guide. Once focused the microscope can be held by the guide sleeve.
- Using the adjuster screw touch the tip of the sprayer against the flat plate. It is much easier to see the flat plate through the microscope if a small dot is made on the front of the plate with a felt tip pen. Often a small fragment of the capillary will be snapped off.
- Remove the tip and attach to the probe.
12-7. Accurate Mass Measurements Using a Combination of Probes.

The standard system is configured with two probes, one with a tip suitable for use with the borosilicate sprayers, the other with a tip suitable for use with the fused silica sprayer. In normal operation the borosilicate sprayer is inserted into the front probe port so that the capillary is at 90 degrees the front of the sampling cone. The fused silica sprayer is inserted into the side probe port. Both probes must be inserted to allow the system to operate.

An accurate mass measurement on a given peak in the spectrum of the sample can be achieved as follows.

- Continuously infuse a suitable reference material using the fused silica sprayer (reference sprayer)

  The reference material should be chosen so that two peaks are present at m/z values which effectively bracket, in mass, the peak of interest. The mass difference between the two bracketing reference peaks should be arranged to be as small as possible to minimise errors.

  **In positive ion operation:** Poly ethylene glycol may be used (0.001%) which is commercially available with several different average mass distributions to accommodate the mass of the sample peak. It is possible to alter the ion species produced using suitable additives to favour the production of certain adduct ions. This can lead to a simplification of the spectra. For instance:

  - Addition of NaCl will produce predominantly sodiated PEG peaks. (Care should be taken to add only the minimum of NaCl as excessive amounts leads to rapid contamination of the sampling cone with a white deposit and degrades performance.)
  
  - Addition of an acid will produce predominantly protonated species.
  
  **Addition of ammonia solution** will produce predominantly NH₄ adducts.

  **In negative ion operation:** a 0.001% solution of PEG diacid is recommended.

- Obtain a beam and tune to an appropriate calibrant peak close to the m/z value of the sample to be measured. Determine, and record, the optimum needle voltage.

- Tune the mass spectrometer to the desired resolution using the reference material.

  The resolution required depends mainly on the nature of the sample and reference. It is necessary to obtain a resolution high enough to resolve out any interference’s on any of the peaks used. A resolution between 5000 and 10000 is sufficient for most applications. It is important at this stage to obtain the maximum transmission at the resolution required and to ensure that the peak is as symmetrical as possible.

- Set up a voltage scanning experiment, in continuum mode, over a m/z range which will incorporate two reference peaks, one on each side of the sample peak.

  A directory of calibration reference files can be viewed by typing the command: `dir [vg.ref]*.*` in a large window from the `$` prompt.
A list of the reference peaks in a given reference file can be printed by typing the command `Laser [vg.ref] XXXX.*;*` from the $ prompt where XXXX is the name of the reference file.

A scan rate of 5 to 10 seconds per scan is normally used, and the entry for resolution in the experiment page should be set to the resolution of the instrument.

- Withdraw the reference probe so that it clicks into the out position.
- Adjust the needle voltage to that determined as the optimum for the sample.
- Fully insert the borosilicate sprayer (sample probe) and obtain a signal from the sample peak to be analysed. Restart the spray if required.

  At this point it is advisable to check that the peak of interest is as symmetrical as the peak from the reference material and that any interference peaks have been resolved. An asymmetrical sample peak when the reference peak appears symmetrical may indicate an unresolved interference at this m/z value.

- Start the acquisition and acquire enough spectra so that when averaged the peak of interest has a clear centroid.
- Very statistical data may cause errors in the mass measurement.
- Withdraw the sample probe until it clicks into the out position.
- Fully insert the reference probe and adjust the **needle** voltage to that determined as optimum for the reference material.

- **In the same acquisition** acquire enough spectra so that when averaged the two reference peaks have clear centroids.

  By alternating between the sample and the reference probe several consecutive accurate mass measurements, on the same peak, may be possible during the same acquisition or the above procedure may be followed for several accurate mass measurements using separate acquisitions. Statistical computations on several measurements can, therefore, be made.

**Processing the Data for Accurate Mass.**

Once sufficient data has been acquired, an accurate mass measurement may be determined as follows:

- Average the scans from the part of the mass chromatogram containing the reference peaks only.
- Smooth, peak detect, and centroid the data.

  *Peak detection parameters are not described in this document and it is often advisable to experiment with a standard sample with a known empirical formula to determine the best peak detection conditions.*

- Select **calibrate** from the spectrum and automatically calibrate the spectrum with the correct reference file nominated in the calibration menu.

  The calibration file produced will automatically appear in the mass measurement window and will be used to mass measure any subsequent data displayed.
• Average the scans from the part of the mass chromatogram containing the sample peaks only.

• Smooth, peak detect and centroid the data using exactly the same parameters used to process the reference peaks.

• The resulting centroided data should be accurately mass measured to within $\pm 5$ ppm.

The number of decimal places displayed can be increased by selecting annotate then modify from the spectrum header banner.

12-8. Background noise.

A common problem which can occur, particularly when using the borosilicate sprayer, is that of background noise appearing in the spectrum. This is often produced by an APCI type ionisation of atmospheric water in the high potential field strengths at the tip of the capillary. This background noise can appear as an obvious series of water cluster ions with equal mass spacing or as general noise along the mass axis with peaks at every mass. The use of a bath gas can reduce this effect.

Normally a flow of nitrogen at approximately 40L/hour is sufficient and can be supplied and regulated using the nebuliser supply on the standard electrospray interface.

Excessive background with no peaks from the sample present can indicate an interruption in the flow from the capillary.

In some instances the introduction of a flow of bath gas can also increase the signal intensity of the analyte.

12-9. Routine Maintenance

A. Sampling Cone

Because of the very low flow rate which the technique employs, the stability of the system is very susceptible to contamination on the surface of the sampling cone. During routine operation it is advisable to wipe the sampling cone each day. This can be achieved without venting the source housing as follows:

• Turn the system to standby.

<table>
<thead>
<tr>
<th>WARNING: The screws which hold the black acetal body onto the ionisation chamber are at high voltage when the system is in operate.</th>
</tr>
</thead>
</table>

• Remove both probes.

• Remove ES01 and ES02 from the acetal body.

• Remove the protective plugs over the three retaining screws which pass through the front of the acetal body.

• Unscrew the three retaining screws and remove the acetal body.

• Using a lint free cloth soaked in methanol, wipe the sampling cone thoroughly.

• Replace the acetal body, electrical connections and probes.

• Replace the protective plugs which cover the three retaining screws.
If the sampling cone is very heavily contaminated it may be necessary to remove it and ultrasonic in a suitable solvent.

To remove the sampling cone:

- Turn the system to standby.
- Remove the probes and the electrical connections from the acetal body.
- Vent the source housing to air.
- Loosen the three knurled positioning screws on the electrospray housing and remove the interface.
- Remove the four retaining screws holding the sampling cone onto the metal ionisation chamber and clean.

B. Fused Silica Sprayer

The fused silica sprayer should ideally be flushed out with pure solvent at the end of each day to minimise the risk of blocking. If the probe becomes blocked the blockage is usually at the tip of the 20μm i.d. fused silica. A small amount of this fused silica can be cut off to allow the probe to spray again alternatively a new length of fused silica can easily be installed.

Borosilicate Sprayer

The condition of the blue conductive elastomer which holds the capillary into the tip of the probe, should be checked for ware, and replaced periodically. Poor electrical contact at this point can cause beam instability.
## 12-10. Part list

<table>
<thead>
<tr>
<th>Description</th>
<th>Code no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>TOP LEVEL CODE</td>
<td>M805463DC1</td>
</tr>
<tr>
<td>PROBE HANDLE</td>
<td>M805451CD1</td>
</tr>
<tr>
<td>RETAINING PLATE</td>
<td>M805453AD1</td>
</tr>
<tr>
<td>PROBE GUIDE SHAFT</td>
<td>M805452BD1</td>
</tr>
<tr>
<td>LOCATING ROD</td>
<td>M805454AD1</td>
</tr>
<tr>
<td>PROBE SHAFT SUPPORT ASSY</td>
<td>M805470AD1</td>
</tr>
<tr>
<td>PLUG BNC HV R317005</td>
<td>0880109</td>
</tr>
<tr>
<td>MODIFIED SWAGELOCK CONNECTOR FEMALE</td>
<td>M805470AD1</td>
</tr>
<tr>
<td>CONDUCTIVE ELASTOMER</td>
<td>6028626</td>
</tr>
<tr>
<td>VITON O RING 012</td>
<td>5711032</td>
</tr>
<tr>
<td>ADAPTER</td>
<td>M805469BD1</td>
</tr>
<tr>
<td>VALCO ZU1XC LOW DEAD VOLUME UNION</td>
<td>6070232</td>
</tr>
<tr>
<td>O RING VITON Φ 4.1x1.6 (375-039)</td>
<td>5711215</td>
</tr>
<tr>
<td>FUSED SILICA 20X90X30</td>
<td>6028621</td>
</tr>
<tr>
<td>RED PEEK TUBE 1/16&quot;X0.005</td>
<td>6436005</td>
</tr>
<tr>
<td>FUSED SILICA 25X375</td>
<td>6028622</td>
</tr>
<tr>
<td>ORANGE PEEK 1/16&quot; X 0.02</td>
<td>6436002</td>
</tr>
<tr>
<td>VALCO PLUG CAP ZC1</td>
<td>6070233</td>
</tr>
<tr>
<td>VALCO FERRULE</td>
<td>6070129</td>
</tr>
<tr>
<td>VALCO NUT</td>
<td>6060806</td>
</tr>
<tr>
<td>PTFE TUBE Φ 1.7 X 0.3WALL</td>
<td>6430202</td>
</tr>
<tr>
<td>O RING VITON 011</td>
<td>5711018</td>
</tr>
<tr>
<td>CUTTER FUSED SILICA</td>
<td>6028630</td>
</tr>
<tr>
<td>BOROSILICATE TIP TYPE A</td>
<td>6028623</td>
</tr>
<tr>
<td>BOROSILICATE TIP TYPE B</td>
<td>6028624</td>
</tr>
<tr>
<td>BOROSILICATE TIP TYPE C</td>
<td>6028625</td>
</tr>
<tr>
<td>BOROSILICATE TIP TYPE D</td>
<td>6028627</td>
</tr>
<tr>
<td>BOROSILICATE TIP TYPE E</td>
<td>6028628</td>
</tr>
<tr>
<td>TYGON TUBE 0.8I.D. X 0.8 WALL</td>
<td>6490332</td>
</tr>
<tr>
<td>LEUER FITTING CUT DOWN SYRINGE</td>
<td>5810104</td>
</tr>
</tbody>
</table>
Appendix A
Appendix A

4-6. High Voltage Setup

**WARNING.** Consider all high voltages as hazardous. Do not attempt to measure them unless you are competent to do so, and unless appropriate test equipment is available! Do not proceed if in any doubt whatsoever; instead contact your local service representative.

There are six separate high voltage supplies in the electrospray trolley, and in addition the instrument accelerating voltage supply energises the skimmer.

4-6-1. High Voltage Supplies - ‘X’, ‘S’ and ‘V’ Series Instruments

These instruments all utilise MA3268-200 (column 1) high voltage PCB's for both the instrument accelerating voltage and the electrospray trolley HT supplies, as shown schematically in Figure 4-6-A.
* NOTE: THE COVER OF THIS UNIT SHOULD ONLY BE REMOVED BY QUALIFIED PERSONNEL. THE VOLTAGES INSIDE ARE DANGEROUS.

FIGURE 4-6-B HIGH VOLTAGE SUPPLIES ON 'M' SERIES INSTRUMENTS
When the large panel at the top back of the electrospray trolley is removed, and when one of the instrument panels is removed, the operation of these units can be observed. The green diodes marked ‘Output’ and ‘-ve O/P’ indicate when the high voltage is on, in positive and negative modes respectively. The green diodes marked ‘+Vs’, ‘15V’ and ‘-15V’ should be illuminated continuously - if not, a fault condition exists.

In all of these instruments, an 8V reference voltage produces a corresponding 8000V output from the supplies. The electrospray control PCB, MA3606-200, has links that must be set to position ‘A’ to be compatible.

4-6-2. High Voltage Supplies - ‘M’ Series Instruments

These instruments utilise MA3268 high voltage PCB’s for the electrospray HT supplies, and an Applied Kilovolts supply for the accelerating voltage as shown schematically in Figure 4-6-B.

The operation of the instrument accelerating voltage supply is most easily monitored using the SIOSLOAD diagnostics.

When the large panel at the top back of the electrospray trolley is removed, the operation of the electrospray HT supplies can be observed. The green diodes marked ‘Output’ and ‘-ve O/P’ indicate when the high voltage is on, in positive and negative modes respectively. The green diodes marked ‘+Vs’, ‘15V’ and ‘-15V’ should be illuminated continuously - if not, a fault condition exists.

In the ‘M’ instruments, a 10V reference voltage produces a corresponding 8000V output from the supplies. The electrospray control PCB, MA3606-200, has links that must be set to position ‘B’ to be compatible.
4-6-3. Adjustment Procedure

It is essential that the electrospray trolley high voltage outputs are carefully matched with the instrument accelerating voltage supply, using the following procedure. This must be repeated for both positive and negative ionisation modes.

1. **Important!** All six electrospray high voltage leads and also the accelerating voltage supply should be unplugged, due to the complexity of the interconnections (and close proximity of the individual components) in the interface. It is usually easiest to remove the white plugs from both the electrospray and the accelerating voltage supplies on the ‘X’, ‘S’ and ‘V’ series instruments. The accelerating voltage can alternatively be disconnected from the left-hand source feedthrough, BB08. On ‘M’ series instruments the easiest and safest way is to disconnect BB08 (it is not wise to open up the source control unit unless absolutely necessary on these instruments).

2. In the electrospray positive tuning menu (Figure 4-4-C), set the following sliders to zero:
   - Needle Voltage
   - Sampling Cone
   - Skimmer Lens
   - Ring Electrode

   (The sliders will already be at zero when the menus are first called up; however, to make sure that the values are correctly updated by the SIOS it is important to move one of these sliders up, then return it to zero to avoid problems later).

3. Set the Ion Energy pot or slider to its middle position, or to a known optimum for 4 kV operation. Check that the voltage is 4000 in the Limits menu (Figure 4-4-B).

4. Turn off the Span control (or set a value of 1 in the Peak display window).

5. Put the electrospray probe into the housing, or the spray tester, so that all of the microswitches are closed.

6. Switch the instrument into Operate. The readbacks should now register the high voltages.

7. Using an HT probe and a digital multimeter with at least 3 digits, measure the instrument accelerating voltage (**Warning! Do not attempt this unless you are competent to do so!**)

8. Adjust the electrospray voltages, using the ‘+ve o/p adjust’ pots, to give the outputs shown in Figure 4-6-C with respect to the accelerating voltage.

<table>
<thead>
<tr>
<th>Needle Voltage</th>
<th>Sampling Cone</th>
<th>Hexapole HT</th>
</tr>
</thead>
<tbody>
<tr>
<td>$V_{acc} + 3 \text{ kV}$</td>
<td>$V_{acc}$ exactly</td>
<td>$V_{acc} - 10 \text{ V}$</td>
</tr>
<tr>
<td>Counter Electrode</td>
<td>Ring Electrode</td>
<td>Skimmer Lens</td>
</tr>
<tr>
<td>$V_{acc} + 1 \text{ kV}$</td>
<td>$V_{acc}$ exactly</td>
<td>$V_{acc}$ exactly</td>
</tr>
</tbody>
</table>

**FIGURE 4-6-C ELECTROSPRAY MA3606 CONTROL UNIT - POSITIVE ION SETUP**
Select positive ion mode. Use an HT probe to measure the sampling cone voltage. Move the sampling cone slider until the voltage is 50V above the accelerating voltage. This should correspond to ~20% on the slider.

Use an HT probe to measure the skimmer lens voltage. 

**a)** For systems with independent control of the skimmer lens voltage, move the skimmer lens slider until the voltage is 60% above the accelerating voltage. This should correspond to ~25% on the slider.

**b)** For systems with the skimmer lens voltage linked to the sampling cone voltage, move the skimmer lens slider to 100%. This should correspond to a skimmer lens voltage of ~18V above the sampling cone voltage, by changing the sampling cone voltage slider check that the skimmer lens voltage tracks with the sampling cone voltage to maintain this voltage difference.

Use an HT probe to measure the ring electrode voltage. Move the ring electrode slider until the voltage is 20 V below the accelerating voltage. This should correspond to ~20% on the slider.

The hexapole HT may need slight optimisation, but this can only be done once a beam is obtained, and an HT probe is not required. The method involves fine tuning of the adjustment pots in both positive and negative modes so as to maximise the signals.

9. For negative ion operation the following voltages should be set, using the ‘-ve o/p adjust’ pots, in a similar manner to that described above for positive ionisation, but with the opposite polarity:-

<table>
<thead>
<tr>
<th>Supply</th>
<th>Connector</th>
<th>Voltage (-ve mode)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Needle</td>
<td>EB02J3</td>
<td>2.5 kV below Vacc (i.e. - 6.5 kV)</td>
</tr>
<tr>
<td>Counter Electrode</td>
<td>EB02J6</td>
<td>1 kV below Vacc</td>
</tr>
<tr>
<td>Sampling Cone</td>
<td>EB17J3</td>
<td>Vacc</td>
</tr>
<tr>
<td>Skimmer Lens</td>
<td>EB33J6</td>
<td>Vacc</td>
</tr>
<tr>
<td>Hexapole HT</td>
<td>EB33J3</td>
<td>10 V above Vacc</td>
</tr>
<tr>
<td>Ring Electrode</td>
<td>EB17J6</td>
<td>Vacc</td>
</tr>
</tbody>
</table>
FIGURE 4-7-A GRAPH OF RF AMPLITUDE VERSUS M/Z (NOT TO SCALE)
4-7. Hexapole RF Setup

The hexapole RF is scanned with mass to give the optimum transmission of ions. The scan control signal is produced by a diode function generator circuit on the electrospray control PCB (MA3606-200) in the trolley, using a magnet reference signal. The boards mounted on top of the API housing (MA3599-200 and MA3599-201) produce the RF, which is fed to the rods directly beneath in the housing. The hexapole RF will be set up by the engineer during installation, and it is unlikely that it will require anything other than slight and occasional adjustments. Refer to the Quick Guides below just to check the setup and operation, or instead to the detailed instructions if the complete procedure is required.

4-7-1. Quick guide - Hexapole RF lens

1. The RF frequency is around 1.1 MHz. If the frequency is much less than this, then low mass ions may be transmitted at reduced sensitivity. The waveform should be sinusoidal, but ions can still be transmitted at high sensitivity if the waveform has quite odd shapes.

2. During normal magnet scans, the RF amplitude of the lens is scanned linearly with the m/z of the ions to be transmitted; the higher the m/z the less critical the actual RF required.

3. The RF PCB produces some residual RF with zero input signal (this occurs at m/z values below ~50). The maximum RF it can produce is ~1600V peak-to-peak (equivalent to 800V measured on one side of the output stage). This corresponds to an m/z value of 3000 to 4000.

4. During B/E linked scans (and similar scan modes) the RF is held constant at the function m/z, even though the magnet is being scanned.

5. The RF can be monitored directly by observing the neon indicator on the RF PCB (it is visible through the holes in the cover). It will glow brightly at maximum RF; and will dim steadily as the RF decreases; it will extinguish completely at RF amplitudes corresponding to m/z values of ~600 to ~800. If it remains brightly lit at lower m/z values, and significant ripple is present on any ion signals, the circuit may be oscillating.

6. If the RF is monitored and tuned using an oscilloscope, then a X100 scope probe MUST be used. A X10 probe will significantly distort the operation of the circuit, thus it may only be used to monitor, but not to tune, the RF.

7. The RF is scanned using a magnet reference signal; the magnet reference is converted into the correct form by the diode function generator on the electrospray control PCB. The diode function generator utilises four break points and four slopes to cause the RF to track correctly with mass. Once it has been set up it is unlikely to require further adjustment. However, the setup is valid only for one accelerating voltage and one type of magnet (and one set of coils).
Figure 4-7-B. Graph of RF input voltage versus magnet reference signal, showing setup of break points (not to scale).

NOTE: Incorrect set up of any of the 'Slope' pots, RV6, RV7, RV8 or RV10, can cause the RF input signal to drop to zero and go back up again, as illustrated for RV6 above.

FIGURE 4-7-B GRAPH OF RF INPUT VOLTAGE VERSUS MAGNET REFERENCE SIGNAL, SHOWING SETUP OF BREAK POINTS (NOT TO SCALE)
8. A schematic of the RF signal vs. mass to charge is given in Figure 4-7-A.

[RF graph currently in text above - will be Fig 4-7-A]

4-7-2. Quick Guide - Diode Function Generator Setup

If the DFG needs quick checking or optimisation, refer to Figure 4-7-B, and try the following.

1. Measure the magnet reference voltages (TP23 wrt. TP13 on MA3606-200 with an extender card, or pin 8 wrt. pin 9 on BR01 at the HPLC panel) to determine the break point voltages M1 to M5, by selecting masses of 4000 (M1), 560 (M2), 100 (M3), 70 (M4) and 50 (M5).

2. Check and/or set up M1 to M4 using the pairs (RV2 + TP16 for M1), (RV3 + TP17 for M2), (RV4 + TP21 for M3), and (RV5 + TP22 for M4), respectively (the 4 test points on the front of the pcb below the optic unions).

3. Use a 10-turn pot to feed RF input signals into pin 10 of ES03 manually, & determine the optima for ions at 571, ~102, ~70 and ~55. Use the 0 V and +15 V test points to provide the power. Measure the optimum input voltages (while the HT is still on) on pin 10 wrt. pin 4.

4. Select mass 571 (#M2, ie a little above the break-point M2), and display the ion. Monitor the ion signal and the RF input voltage on pin 10 wrt. pin 4, and adjust RV6 to optimise the signal and reach the optimum input voltage as measured previously.

5. Select an ion at mass ~102 (#M3), and adjust RV7 to optimise the signal & match the optimum input voltage, as above.

6. Select an ion at mass ~75 (#M4), and adjust RV8 to optimise the signal & match the optimum input voltage, as above.

7. Select an ion at mass ~55 (#M5), and adjust RV10 to optimise the signal & match the optimum input voltage, as above.

8. The DFG should now be set up correctly. To test it, jump through the masses 571, 102, 75 and 55 check that the RF input voltages & ion signals are as expected.
4-7-3. Full Setup Procedure

The setup procedure is very similar to the Y-focus setup on the early AutoSpec instruments. Tools required to complete the procedure are as follows:

1. An extender card, #3244-210, is essential. One is usually present in one of the free slots in the old ES trolley, but do not assume one will be available on site! None are supplied with ‘M’ instruments!

2. An oscilloscope is optional; a X100 scope probe must be used with it. If no scope is available, the RF can be coarsely monitored by the neon indicator on the RF PCB (it will glow with increasing brightness as the RF input voltage is increased from approx. 3V to the maximum of 10 to 13V).

3. An electrospray beam is essential, since the final iteration of the set-up requires fine tuning based on ions at 571 (gramicidin S 2+) and on low mass solvent ions at about 102, 75 and 54. A solution of 10 ng/µl gramicidin S in 1:1 water : methanol + 1% acetic acid should be used. If gramicidin S is unavailable, then 10 ng/µl leucine enkephalin (m/z 556) is a good substitute.

4-7-3-1. Finding A Beam Initially

1. Set an RF input voltage of 1 to 2V to the 3599-200 RF PCB by requesting a magnet mass of 571 at 4kV accelerating voltage. This will be suitable for finding a beam using gramicidin S at 571 or leucine enkephalin at 556. The input signal can be measured on J2 pin 10 wrt. pin 4 on this board, and on TP24 wrt. TP13 on the 3606-200 control PCB.

2. If the 3606 PCB has been correctly pre-set, the RF input voltage will be satisfactory; if not, adjust RV1 and/or RV11. If this fails, then carry out a ‘blind’ set-up procedure prior to finding a beam.

3. If this is not possible, then use a breakout connector in J2 of the 3599-200 RF PCB to disable the signal from the 3606-200 PCB by switching out pin 10. Tap off a 1 to 2V signal from the +15V and ground test points (TP4 & TP0) using resistors, and inject this on to J2 pin 10. (A 10 kohm 10-turn pot could be used as a manual control).
1. Set the output of RV11 so that it gives +12 to +13V as measured on the left-hand side of R154 (the resistor sited immediately below TP29). (In some cases the setup seems to be easier if +6 to +8V is set instead).

2. Measure the magnet reference signals on TP23 at 4kV accelerating voltage at the following masses: 4000, 560, 100, 70 & 50. (Call these values M1 to M5 respectively; they will all be negative).

3. Adjust RV2 to give the positive value of M1 on TP16. This is the first break point (BP1).

4. Adjust RV3 to give the positive value of M2 on TP17. This is the second break point (BP2).

5. Adjust RV4 to give the positive value of M3 on TP21. This is the third break point (BP3).

6. Adjust RV5 to give the positive value of M4 on TP22. This is the fourth break point (BP4).

7. Select mass 4000 on the Instrument menu (BP1). Adjust RV1 until the RF reference signal measured on TP24 is just saturating; i.e. just reaches its maximum value. This will be around 14V. (The RF output will now be maximum at m/z values above 4000).

8. Select mass 560 on the instrument (BP2). Now adjust RV6 so that 1.5V is measured on TP24. This sets the slope of the line between BP1 and BP2. (1.5V is a rough value for transmission of the 2+ ion of gramicidin S at m/z 571; note that the break point is a little lower than the m/z value).

9. Select mass 100 on the instrument (BP3). Adjust RV7 so that 200mV is measured on TP24. (A very rough value for transmission of the solvent ion at approx. 102).

10. Select mass 70 on the instrument (BP4). Adjust RV8 so that 150mV is measured on TP24. (A very rough value for transmission of the solvent ion at approx. 74).

11. Select mass 50 on the instrument. Adjust RV10 so that a signal of around 100mV is measured on TP24. (A very rough value for transmission of the solvent ion at approx. 54).

12. Check that the input signal to the squarer, IC28, as measured on TP26, always remains in the range 1 to approx. 10V (the polarity does not matter). If the input drops into the range -1V to +1V at low mass values then the circuit may fail to work properly. See below for details.

13. The ‘blind’ set-up procedure is now complete.
14. Table 4-7-A lists the set-up values for a high-field AutoSpec ‘M’ series magnet (5000 MR @ 8kV; values for ‘X’, ‘S’ and ‘V’ HF 4500 MR magnets can be taken as the same for the purpose of the ‘blind’ set-up).

<table>
<thead>
<tr>
<th>Magnet Mass</th>
<th>Magnet Ref (TP23)</th>
<th>Break Point #</th>
<th>Adjust...... Sitting At mass:</th>
<th>In……….. Pot:</th>
<th>Sequence To Give RF Ref (On TP24)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4000</td>
<td>-5.65 (m1)</td>
<td>BP1</td>
<td>4000</td>
<td>RV1</td>
<td>Max (13-14V)</td>
</tr>
<tr>
<td>560</td>
<td>-2.11 (M2)</td>
<td>BP2</td>
<td>560</td>
<td>RV6</td>
<td>1.5V</td>
</tr>
<tr>
<td>100</td>
<td>-0.88 (M3)</td>
<td>BP3</td>
<td>100</td>
<td>RV7</td>
<td>0.20V</td>
</tr>
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<td>70</td>
<td>-0.76 (M4)</td>
<td>BP4</td>
<td>70</td>
<td>RV8</td>
<td>0.15V</td>
</tr>
<tr>
<td>50</td>
<td>-0.65 (M5)</td>
<td>——</td>
<td>50</td>
<td>RV10</td>
<td>0.10V</td>
</tr>
</tbody>
</table>

4-7-3-3. Accurate Setup Procedure For 3606 PCB

1. Essentially the same procedure is followed, except that the precise optima for the RF voltages are determined experimentally.

2. The RF optima for gramicidin-S 1+ and 2+ ions and higher m/z ions are quite broad, and can best be estimated from the middle value of an 80% or 90% transmission window. Optima for ions of m/z less than 200 are much narrower; as a consequence they may be hard to find if the RF is incorrect.

3. Note that if the input signal to the squarer, TP26, is close to the range -1V to +1V then the circuit may fail to work properly. When the Instrument mass is changed from say 102 to 54 by typing ‘54’ in the instrument picture, the magnet current is switched to zero then back up again. The swing to zero coupled with the relatively low new request, which may be just below -1V, may cause a fault condition such that the squarer output (TP25) remains at zero. The ion beam will vanish. If instead the mass is changed by typing ‘-48’ in the instrument picture, the magnet will not switch to zero and the squarer will continue to work correctly. Similarly, if the magnet mass is scanned or dragged downwards, then the squarer will work correctly.

4. It is important to check the operation of the squarer. Ideally it should be set up (by means of a suitable adjustment to RV11) so that the magnet can be jumped from 75 to 0 to 75 without losing the ion beam.

5. Follow the steps detailed in points 1. to 7. of the ‘blind’ procedure: (a) check the setting of RV11, (b) determine the magnet reference voltages at mass 4000, 560, 100, 70 and 50, and (c) check the set-up of the break points.

6. Find the 2+ ion of gramicidin-S (571, just above BP2). Determine the optimum RF required. This can be done in two ways. If RV1 (the overall gain pot) is tweaked, then the set-up of the DFG is not altered, and if RV1 is returned to the same setting (using TP24) the previous characteristics will be retained. This is useful if the intention is to check rather than change the set-up. However, if RV6 is adjusted then the pots further down the chain, RV7, 8 and 10, will also have to be re optimised.
7. Make a note of the signal intensity of the 571 gramicidin ion.

8. Find the solvent ion at around m/z 102 (just above BP3). Check the optimum RF required using RV1, and/or re optimise RV7 if necessary (if RV7 is adjusted then RV8 and 10 will also need to be adjusted). Note the signal intensity.

9. Find the solvent ion at around 75 (just above BP4). Check with RV1 and/or re optimise with RV8, and note the intensity.

10. Find the solvent ion at around 54. Check with RV1 and/or re optimise with RV10, and note the intensity.

11. Check that the RF reference signal (TP24) remains positive at a lower mass value, for instance 23 (corresponding to Na\(^+\)). It may be possible to find the Na\(^+\) ion; however, do not confuse it with N\(_2\)\(^+\) at mass 28, since this ion is always present independent of the applied RF voltage.

12. Check the operation of the squarer at low mass values as described above. If it is necessary to readjust RV11 then this set-up procedure should be repeated.

13. The set-up should now be complete. Check it by acquiring magnet scan data over the range 1500 to 20, and compare the intensities of the ions at 54, 75, 102, 571 (and 1142) with the recorded values: a good match confirms that the set-up procedure has been successful.

14. IMPORTANT. As a final check, monitor the RF input voltage whilst dragging the magnet accross a wide mass range, say from 20 to 5000, to ensure that the RF input does not drop to zero between the break points (refer to Figure 4-7-B)

4-7-3-4. Related comments, 3606 PCB

1. The squarer has an inherent ripple, of perhaps 10mV. This is insignificant at masses above 100, but may cause some nuisance at lower masses.

2. When a standard multimeter is connected to TP26 (the squarer input), it can load the circuit such that the output is altered: again this can be a nuisance at low masses. In one case a 40mV change occurred when tuning on the ion at mass 54, which resulted in a large intensity change.

3. The cables running between the 3606 control PCB and the 3599 RF PCB have significant impedance, such that an RF output signal of say 100mV measured on TP24 will be around 90mV if measured on J2 pin 10 wrt. pin 4 on the RF board.

4. (The magnet reference signal can be found on: (a) BS02J3 pin 5 wrt. pin 6 on the source control unit of an AutoSpec ‘S’, (b) J16 pin 3 wrt. pin 16 in the beam control unit of an AutoSpec ‘M’; and goes into EB65J03 pin 3 wrt. pin 4 on the electrospray trolley).
4-7-3-5. Setup Of RF Boards, 3599 PCB

1. The capacitance across the hexapole rods should have been measured after assembly; around 35 to 40 pF is expected between feedthrough pins 3 and 4. When the interface is under vacuum, 15 to 20 pF is expected. A much lower value indicates that (a) a wire is disconnected inside, or (b) the wire goes to the wrong pins. The hexapole wires are copper, while the Ring Electrode link wire to feedthrough pin 2 is nichrome.

2. The feedthrough should be mounted such that pins 3 + 4 are nearest to the instrument housing lid.

3. The lower board, 3599-201, should be mounted on the feedthrough flange using cap head screws. **Note that this board carries high voltage, and that RF may be present when the instrument is in standby.**

4. The three flying leads should be connected to their nearest feedthrough pins; FL4 to 4, FL6 to 3, and FL5 to 2 (FL5 is the Ring Electrode connection). Ensure that these leads do not touch the upper board, 3599-200.

5. Mount the upper board, 3599-200. **Note that this board carries up to 1600V peak-to-peak RF.** The RF is at the back of the board (near the instrument housing lid); the rest of the board is at low voltage. **Early Designs: the RF remains on even when the instrument is in standby.** Later designs (boards with a neon indicator) include an HT Enable circuit.

6. Plug the remaining two flying leads FL3 and FL9 from the lower board in to J1 and J3 on the upper board. Ensure that these leads will remain clear of the metal cover.

7. The two cables, ES03 and ES04, both carry the trip switch circuit, so that the source voltages will not come on if either are disconnected.

8. Check for the presence of +15V, -15V and +24V on the test points (TP0 is at ground). If this is a retrofit kit, an intermittent +15V supply probably indicates that the transformer and/or the rectifier in the trolley power supply have not been upgraded.

9. Check the RF reference signal reaching the board; between 1 and 2V is expected on J2 pin 10 wrt. pin 4 or 11 or TP0 for optimal transmission of m/z 571 at 4kV accelerating voltage (refer to the notes on the 3606 PCB for more information).

10. Measure the voltage on TP1 wrt. TP0, and adjust the trimpot RV1 to give a minimum value - this procedure matches the RF frequency of the board to the system. For guidance, typical voltages are ~2.5, 3.5, 4.5 and 18V for reference signals of 1.0, 1.5, 2.0 and 14V respectively. Note that this adjustment should be iterative, beginning with low and ending with high reference signals. It must be done while the interface is under vacuum since the capacitance is different in air.
11. Optionally, using an oscilloscope with a X100 probe, iteratively adjust the trimpot RV1 to give the maximum actual RF output on J1 with a 6V, then 10V, then 12V, then 14V reference signal. Check the voltages on TP1 - they should remain close to the values noted above. If they differ significantly the board may run hot. This procedure should be done with care.

12. Optionally, check the RF frequency using an oscilloscope or a frequency meter and a X100 scope probe on TP2 or TP3; it should be about 1.1 Mhz.

13. Optionally, check the RF amplitude using an oscilloscope with a X100 probe, either on pins J1 or J3, or on the test point nearby. Typical voltages are ~100, 140, 180 and 800V for reference signals of 1.0, 1.5, 2.0 and 14V respectively. (Since this measures only one side of the circuit, the peak-to-peak voltage is double, e.g. 2x800V). (Alternatively, the neon indicator will glow with varying intensity at RF reference signals between ~3 and 10V to indicate the approximate levels of RF voltage).

4-7-3-6. Comments & Fault Conditions Of 3599 PCB

1. If RF is not present, or is present at a very low level, check the following: (a) the fuse FS1, (b) the condition of the resistor R4, (c) the connections of the flying leads between the top and bottom boards, and between the bottom board and the feedthrough pins, and (d) the presence of the driver waveform on TP2 and TP3, using an oscilloscope. If one of the leads to the hexapole rods is not connected, then the fuse may blow or R4 may overheat or burn out. If IC2 is working, a ~14V semi-square waveform on TP2 and a similar square waveform on TP3 will be observable. If IC2 (HEF4049BP, Micromass part 1984049) has failed, there will be no signal on TP3, and R4 will be running hot.

2. The HT Enable circuit (fitted on boards having a neon indicator) requires a minimum of +15V on pin 2 of J2 on the 3599 PCB - if this is not present the RF will remain off. If nearly 15V is present, then the circuit may switch the RF on and off at variable frequencies. This can be identified using an oscilloscope, and may appear as a thicker line on the peaks and troughs than near the zero position on a triggered waveform (the instrument Span must be off, because the scanning also affects the RF level).

3. If RV1 is not set to the optimum (to give a minimum voltage on TP1), then the highest RF output will be somewhat below the maximum, of around 1600V peak-to-peak. However, within the controllable range the relationship between reference voltage and output voltage will be identical, and so any slight adjustments will not cause any problems.

4. If RV1 is not set to the optimum then certain components, in particular R4 and IC2, may overheat if the RF reference signal remains at maximum for a long period.
TABLE 4-7-B TYPICAL VOLTAGES FOR ‘M’ SERIES 5000 M.R. MAGNET

In which:  
TP23 = inverted magnet ref.;  
TP29 = DFG output;  
TP26 = squarer input;  
TP25 = squarer input;  
TP24 = RF ref.;  
TP1 = ~RF power;  
and RF OUT = RF output measured on J1 with a X100 probe;  
and RV11 was set to give a +11.48V (measured at R154).

<table>
<thead>
<tr>
<th>M/Z (4kV)</th>
<th>3606 TP23 (volts)</th>
<th>3606 TP29 (volts)</th>
<th>3606 TP26 (volts)</th>
<th>3606 TP25 (volts)</th>
<th>3606 TP24 (volts)</th>
<th>3599 TP1 (volts)</th>
<th>RF OUT (volts)</th>
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<tr>
<td>8665</td>
<td>-10.0</td>
<td>0.048</td>
<td>-11.51</td>
<td>12.28</td>
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<td>780</td>
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</tbody>
</table>
5. If the transmission of very low mass ions (e.g. solvent ions at ~54, 75 and 102) is well below the expected level, then check the RF frequency. These ions require around 1 MHz for maximal transmission, while ions of higher m/z such as those of gramicidin S are satisfactorily transmitted at frequencies above 500 KHz. If an oscilloscope or frequency meter are not available, turn RV1 anti clockwise to increase the frequency and see if the ion signal increases. If it does, then the frequency will have to be measured. (Return RV1 to the optimum afterwards).

6. If instability is observed on the gramicidin S ion cluster, or if the peak is breaking up badly, check that the +15V supply to the RF board is steady. Intermittent problems may occur if the regulator in the trolley power supply has not been upgraded (in the case of retrofit systems), or is running hot. (Also check the -15V and +24V and the reference signal for instability).

7. Instability may also be apparent when the voltage differences between the Skimmer (i.e. accelerating voltage) and the hexapoles, or alternatively between the hexapoles and the Ring Electrode, are too great. Typically only about 10V is necessary between these components. If the instability changes when the Ion Energy or Ring Electrode controls are altered, then this is the likely cause. The output of the hexapole high voltage supply may require manual adjustment.

8. Table 4-7-B lists all of the relevant voltages obtained during a typical setup procedure:

i. These values are meant as an example only; the exact values will depend on the precise set-up, and on the magnet type.

ii. The break points were at m/z 4000, 560, 100 and 75, as detailed in the set-up procedure.

iii. The RF reaches maximum output at above m/z 2800; this is to be expected since the maximum input voltage that the RF PCB will accept is in the region of 10 to 13 V (in this case ~10.5V). The maximum depends to some extent on the tuning of the RF PCB.