

The Dalhousie Chemistry Mass Spectrometry Laboratory

The Dalhousie Chemistry Mass Spectrometry Laboratory (DalChem MS Lab) is equipped with four mass spectrometers, namely a **CEC 21-110B sector instrument** [regular electron ionization mass spectrometry {EIMS}, accurate mass measurements], a **VG/Micromass Quattro triple quadrupole spectrometer** [EIMS, chemical ionization mass spectro metry {CIMS}, electrospray ionization or atmospheric pressure chemical ionization mass spectro metry {ESI/APC IMS}], a **Thermo Finnigan LCQ Duo ion trap** [routine ESIMS, accessible to trained users] and a **Bruker microTOF Focus orthogonal ESI-TOF MS** [routine ESIMS, including high resolution/accurate mass, service]. Service MALDI mass spectra are available, courtesy of the Finnigan vMALDI LTQ linear ion trap instrument in the Doucette lab (Alan.Doucette@dal.ca). Each spectrometer has certain features that allow it to be used to acquire certain types of mass spectra. It is important that the user have some familiarity with these features so that productive choices can be made. In addition, the Quattro, the LCQ, the MALDI and the ESI-TOF instruments can be coupled to a liquid chromatography apparatus, while the Quattro can also be coupled to a gas chromatograph. The right choices of experiments will improve the chances of getting useful spectral data. Choices can be discussed with Mr. Xiao Feng, the Mass Spectrometry Technologist (the MSTech), Room 124, telephone 902-494-1663. Some options other than those listed above are also possible – please discuss with the MSTech.

1. Submission of samples

Samples, accompanied by a completed MS REQUEST FORM (on the DalChem Mass Spectrometry website under “Centres and Facilities”), may be submitted to Room 124 or Room 507 whenever the MSTech is present. Solid or liquid samples for EI/CIMS must be fully pumped down on a rotary oil pump to remove any residual solvent. All samples must be supplied in clearly labelled closed sample vials. To comply with WHMIS regulations labels must include the user's name AND the sample number or other identifier as listed on the MS Request Form. Samples cannot be accepted in miscellaneous laboratory flasks, although in some cases they may be preloaded into sealed capillary tubes of the correct size; discuss with the MSTech.

2. Timing

Spectra will be run as soon as possible in order of receipt, subject of course to spectrometer malfunctions. Emergency requests for spectra should be discussed with the MSTech. Users are responsible for collecting their sample vials from the MSTech; charges for spectra will be billed semi-annually. Status reports are accessible on-line (please see the DalChem Mass Spectrometry website under “Centres and Facilities”).

2. Bruker microTOF Focus orthogonal ESI-TOF MS

The Bruker microTof Focus orthogonal ESI-TOF MS can be used to acquire ESI spectra, with both low resolution and high resolution (i.e. accurate mass analysis) available in positive and negative mode. Accurate mass analysis is perhaps the most rapid route to assist in the determination of molecular formulae. A software package is used to automatically provide molecular formula assignment. Deconvolution software is also available to provide the most accurate assignment of molecular weight for extremely large compounds (biopolymers). This instrument can be coupled to HPLC instrumentation, for LC-MS. This instrument is operated by the MSTech, or by trained users following an instrument training course. Resolution – 3-5 ppm. 15,000 resolution.

3. CEC 21-110B mass spectrometer

4.1 This mass spectrometer is used for routine electron-ionization mass spectra (EIMS) and especially for accurate mass measurements. The spectrometer can be scanned over a mass range of 10 to 800 Daltons in a low-resolution ($M/\Delta M = 2500$) mode, or over a narrow mass range in a high-resolution ($M/\Delta M = 10\,000$) mode. Present limitations in the operation of the spectrometer are that routine mass measurements below m/z 60 are not reliable and relative ion intensities are not very accurate. Good relative intensity measurements can be made on adjacent ions in isotope clusters although the process is slow and is only viable for a limited number of measurements.

4.2 Thermally stable solids or higher boiling oils are introduced into the spectrometer using CLEAN glass capillary tubes. Samples MUST be freed of volatile solvents by pumping on a rotary oil pump at 0.1 torr for 30 min. Normally the capillaries are loaded by the MSTech but special requests are possible.

4.3 The capillaries are placed into a heatable/coolable glass or quartz probe, which passes directly into the spectrometer source.

4.4 Volatile samples vaporize as the probe is heated by an electronic temperature programmer. Successive scans record the vaporization of the compound and differences between scans can indicate inhomogeneity or thermal instability of the sample.

4.5 Volatile liquids or gases can be injected into a capillary inlet. Discuss your needs with the MSTech.

4.6 Details of the conditions under which each spectrum was obtained are given on each plotted spectrum and on the data table containing peak intensities. The number of counts read from the detector for the base peak are listed on the side of the spectrum. This number is related to the number of ions detected which can often be related to the sample size.

4.7 The plotted spectra, relative intensity vs m/z values, provide a picture of centroid data, obtained by digital smoothing of the actual instrument output. Doubly charged ions are shown, but meta stable ions are not.

4.8 The mass scale is calibrated at least daily, but errors may occur and users should check their spectra to see that they make sense. Users who wish to plot

their own spectra may submit a 3.5" diskette on to which a file of ASCII data will be copied; these data can be imported into any spreadsheet program. Original data are kept for at least one month and are then purged. Users are responsible for keeping their own backups.

4.9 Accurate mass measurements are done by peak matching an ion to the mass of a suitable perfluorokerosene (PFK) reference ion. This is computer controlled and can produce very good results even on ions of low intensity. Users should be aware of significant P-1 ions, which will cause peak distortion and an unreliable result. All accurate mass measurements will be done only after a low-resolution spectrum has been provided or run.

4.10 Results from accurate mass measurements can be reported as follows:

Accurate mass measurements were made on a CEC 21-110B mass spectrometer operated at a mass resolution of 8000 (10% valley) by computer-controlled peak matching to appropriate PFK reference ions. Spectra were obtained using electron ionization at 70 volts and a source temperature of ... °C, with samples being introduced by means of a heated quartz probe. Mass measurements were routinely within 3 mmu (... ppm) of the expected value.

4.11 Intensity measurements of adjacent peaks for isotope enrichment experiments can be made to $\pm 1\%$ using a special computer-controlled arrangement, similar to that for peak matching. This specialized work should be discussed with the MSTech.

4.11 This instrument is only operated by the MSTech.

4. VG/Quattro mass spectrometer:

5.1 EI spectra can be run in a similar way to the CEC 21-110B MS. Sections 4.2 – 4.4 above apply; the mass range is 2 to 4000 Th at unit mass resolution. The regular EI/CI probe on the Quattro MS has a more sophisticated temperature controller than that on the CEC 21-110B MS which means that thermal desorption experiments can be done. The Quattro MS also can be fitted with a very fast-heating, high temperature probe which can be used for compounds having higher molecular masses.

5.2 The Quattro MS can be used for acquiring CI spectra. Reagent gases available are methane, isobutane and ammonia. CI operations inherently lead to quite rapid contamination of the source and to reduced filament life which makes them relatively more expensive to run.

5.3 Results from CI mass spectra can be reported as follows: Chemical ionization mass spectra (CIMS) were acquired on a VG/Micromass Quattro triple quadrupole mass spectrometer equipped with an EI/CI source using methane as the ionizing reagent gas to give protonated molecules, $[M+H]^+$, source temperature = ... °C, sample temperature = ... °C.

5.4 A gas chromatograph (H.P./Agilent) can be connected. Users should discuss their needs with the MSTech. The Quattro in either EI or CI modes is used as a detector for positive or negative ions. Users will need to arrange for the provision of a GC, as well as to provide the column and operating conditions.

5.5 The Quattro MS can be adapted for sample introduction using either ESI or APCI MS using either flow injection, infusion or the eluent from an HPLC. The spectrometer is ideally suited for the acquisition of MS and MS/MS spectra at low collision energies. In some cases, pseudo-MS/MS/MS spectra can be acquired. MS spectra can be acquired in full-scan or single-ion monitoring modes, while MS/MS spectra can be acquired in fragment ion, precursor ion, or constant neutral loss modes. The spectrometer is thus a powerful analytical instrument and it can be used for relatively sophisticated ion fragmentation mechanistic studies.

5.6 For ESI MS, possible solvents include water, methanol, 2-propanol or acetonitrile. Non-volatile buffers containing anions such as **phosphate or sulfate cannot** be used. Positive or negative ions already present in the solution may be observed or, in the positive-ion mode, neutral species may become complexed with protons and/or with ammonium, sodium or other cations. Neutral molecules may also cluster with ions if the chemistry is correct. All ions may give solvent clusters. Electrochemical redox processes may also be observed.

5.7 The following are general conditions, written in a format suitable for publication, under which ESI mass spectra could be obtained using the Quattro mass spectrometer. Select the correct conditions, solvents, or voltages that apply to your experiments. Electrospray ionization (ESI) mass spectra were obtained using a Micromass (VG) Quattro triple quadrupole spectrometer operating in the positive/negative {select one} ion mode under the following conditions: electrospray voltage = 3.75 kV, source cone voltage = 15 V {a variable }, source temperature = 100 °C, bath and nebulizer gases were nitrogen at optimized flow rates. Analytes were dissolved in methanol ({select solvent} ca 2 mM) and introduced into the mass spectrometer using a flow injection valve and a stream of methanol solvent {select solvent} flowing at 20 μ L/min. Collision-induced dissociation (CID) mass spectra (MS/MS) were acquired by using argon as a collision gas at a collision energy of 20 eV {a variable } (laboratory frame) and at such a pressure so as to reduce the intensity of the main beam by ca 50%.

5.8 APCI spectra can be acquired in the same solvents, as well as hexanes or toluene, as used for the ESI spectra. Molecular ions, M⁺ or protonated or deprotonated molecules ([M+H]⁺ or [MSH]⁻) may be produced.

5.9 Results from APCI mass spectra could be possibly reported as follows: Atmospheric pressure chemical ionization mass spectra (APCIMS) were obtained using a Micromass (VG) Quattro triple quadrupole spectrometer operated in the negative ion mode. Solutions in distilled acetonitrile (ca. 2 mM) were introduced into the spectrometer using a flow injection valve into a stream of acetonitrile from a syringe pump (30 μ L min⁻¹), leading to the formation of deprotonated molecules, [M-H]⁻. Instrument conditions: bath, nebulizer and sheath gases were nitrogen at optimized flow rates; corona needle = 4000 V; source temperature = ... °C; probe temperature = 350 °C.

5.9 This instrument is usually operated by the MSTech.

6. Thermo Finnigan LCQ Ion Trap MS

The Finnigan LCQ Ion Trap MS can be used to acquire ESI or APCI MS from compounds at essentially any molecular weight (> 100,000 Da). Both positive and negative mode is available. The ion trap can acquire tandem mass spectra (MS^2), if fragmentation data for structural elucidation is desired. ESI (electrospray ionization) is a relatively mild ionization technique, and involves spraying a solution of the compound/material (either through direct infusion from a syringe, or by coupling the ion trap to LC). Ionization is typically through proton transfer or salt adduct formation, and thus almost any compound that can be solubilized in solvents including water/alcohols/ acetonitrile is potentially suited for ESI ionization. APCI (atmospheric pressure chemical ionization) is available for those compounds that do not ionize well with ESI (such as those with no ionisable groups). This instrument is operated by the MSTech, as well as routinely by trained users.

7. Finnigan vMALDI LTQ linear ion trap MS

The Finnigan vMALDI LTQ MS can be used to acquire service MALDI spectra. MALDI (matrix-assisted laser desorption ionization) is a relatively mild ionization technique, often used for biomolecules and molecules with large molecular weight that may be susceptible to fragmentation under other ionization conditions. Consequently, this technique is useful for obtaining structural information, via both low resolution and MS^n experiments. It should be noted that the matrices used are generally light-absorbing carboxylic acids and so compounds/materials that are particularly acid sensitive may not be compatible with MALDI. Via consultation with the Doucette research group (alan.doucette@dal.ca) a variety of more specialized services are available including LC-MALDI (separation, drying, re-dissolution and spotting), MS/MS, protein ID, ingel digestion and sample clean-up/ziptip. This instrument is operated by research personnel in the Doucette group as well as by the MSTech.