LABORATORY OF MASS SPECTROMETRY

Sample preparation guidelines

A critical, and often overlooked, aspect of mass spectrometery is the sample preparation step prior to analysis. Very often it is sample preparation that determines whether or not a MS experiment will be successful or not. Samples can be submitted for mass spectrometric analysis in either solid form or as a solution in an appropriate solvent or buffer.

Solvents and Caveats for LC/MS

Solvents are typically chosen based on a compound of interest's solubility and compatibility with various ionization techniques used in LC/MS. Volatility and the solvent's ability to donate a proton are important in ESI and other atmospheric ionization techniques.

Protic primary solvents like methanol and mixtures with water, such as 1:1 methanol/water or 1:1 acetonitrile/H_2O, are used (although the water/methanol mixture increases viscosity well beyond either water or menthol as a neat solvent because of a resulting exothermic reaction). Water's relatively low vapor pressure can be detrimental to sensitivity when employed at 100%. Better sensitivity results when surface tension is decreased through addition of a volatile organic solvent. Surfactants with higher proton affinity, though they increase ion liberation from nebulized droplets, can also reduce sensitivity.

Aprotic co-solvents like 10% DMSO in water and isopropyl alcohol improve solubility for some compounds. Formic acid is often added at low levels (0.1%) to facilitate ionization by ensuring the analyte is more basic than the solvent. Even in small amounts, however, some acids, like TFA, though necessary for otherwise insoluble compounds, can limit sensitivity.

In the ESI ionization mode, buffers and salts (Na^+, K^+, and phosphate) cause a reduction in the vapor pressure and consequently a reduced signal. The increased surface tension of the droplets, and resultant reduction of volatility, can be remedied by using relatively more volatile buffers like ammonium acetate, formed by a weak acid-base pair.

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Solvent considerations

- Solvent in the gas phase limits ionization by ESI to molecules more basic than the solvent. The exception is photoionization (which is not acid/base ionization) but nonetheless mediated by solvent.
- Removing solvent and water vapor from the ionization region increases types of compounds that can be ionized at atmospheric pressure.
- Reducing liquid volume relative to the sample or analyte of interest contained in the liquid improves ESI performance (i.e., lower flow rates).

- **Useful Solvents**
  - **Water**
  - **Acetonitrile**
  - **Methanol**
  - **Ethanol**
  - **Propanol**
  - **Isopropanol**
- **Acceptable additives**
  - **Acetic acid**
  - **Formic acid**
  - **Ammonium hydroxide**
  - **Ammonium formate (salt concentration = 10 mM or less)**
  - **Ammonium acetate (salt concentration = 10 mM or less)**

- **Nonvolatile salts (phosphate, borate, citrate, Tris, CHAPS, HEPES, NaCl, KCl, NaOH, etc.)**
  - **Can deposit in source and plug capillaries thus requiring more cleaning and maintenance operations**
  - **Modern source designs can handle nonvolatiles better than older designs**

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- **Surface-active agents (surfactants/detergents) suppress electrospray ionization (DTT, SDS, Tween, Triton, glycerol, urea, etc.)**
- **Chaotropic agents like urea and guanidinium salts**
- **Inorganic acids are corrosive**
- **Trifluoroacetic acid (TFA)**
  - **To some extent suppresses positive-ion electrospray at levels exceeding 0.01%.**
  - **Greatly suppressed negative-ion electrospray.**
- **Triethylamine (TEA)**
  - **High PA (232 Kcal/mole) yields an intense [M+H]+ ion at *m/z* 102**
  - **Suppresses positive ion electrospray of less basic compounds.**
- **Tetrahydrofuran (THF)**
  - **100% THF is highly flammable, so APCI and most interface techniques use nitrogen as the nebulizer gas. (Using air creates an explosion hazard).**
  - **Reacts with PEEK® tubing.**

We recommend:

- Eppendorf brand tubes rinsed with MeOH and dried prior to use.

- The use of volatile buffers such as H2O, MeOH, Acetonitrile, Ammonium Bicarbonate, Acetic acid, Formic acid, Trifluoroacetic acid.

- All reagents should be of the highest quality available.

- No colored eppendorf tubes! These tubes often contain residual quantities of dyes which have

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been found to produce peaks in spectra. Since organic solvents are used in sample preparation of mass spec, the dyes can leach off the tubes into your samples. Use only clear plastic tubes.