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Section A

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Assignment of Course # 606 Analytical Techniques II

**Question number 1:**

Discuss the function of sonicator and Filteration in HPLC.

**Answer:**

Bubble formation on mixing of solvents can lead to a number of problems in HPLC analysis which can be prevented by degassing of mobile phase.

Why degassing is required?

One of the biggest reason is Unstable and noisy baselines.

Lower flow rate precision of pump due to cavitation in piston chamber or effect on check valve performance. Excessive pressure can develop which can lead to eventual pump failure.

Air bubbles passing through detectors lead to spurious peaks.

Air bubbles can contribute to flow transfer of mobile phase through the HPLC column due to creation of dead volumes.

Before a discussion on the features of degassing techniques let us understand how the problem arises. Solvents equilibrate with atmospheric gases on exposure to laboratory environment. On mixing the solvents the solubility of air is less than it is in same proportion of pure solvents and excess air will tend to bubble out.

Outgassing can occur on mixing or anywhere in the LC system where rough surfaces produce nucleation sites for bubble formation. Practically it is not necessary to remove the entire dissolved air but only a fraction can be removed to bring it below the super saturation level in the mobile phase. Low pressure mixing systems are more prone to bubble formation. In low-pressure mixing the solvents are mixed at atmospheric pressure and outgassing can take place anywhere down the flow path.

On the other hand, in high-pressure mixing solvent are blended after passing through the pumps and mixing takes place under high pressure in the mixing chamber. The mixture may be saturated but under elevated pressure outgassing is prevented. Bubble formation, if any, can take place when the mobile phase exits the column and returns to atmospheric pressure.

Degassing techniques:

Commonly used degassing practices for HPLC mobile phase are:

* Helium purging
* Vacuum degassing
* Sonication (Main Focus)

**Sonication or Function of Sonicator**

Boiling is the most effective technique to get rid of dissolved air completely but it is never advised because of loss of volatile components along with the gases and also it takes a long time to equilibrate the mobile phase to the required ambient temperature conditions. Sonication using ultrasonic baths is common in most laboratories but as a stand-alone technique it removes only up to 30% dissolved air so sonication in combination with any other technique is recommended.

For practical purposes it is advisable to degas mobile phase in both low and high pressure mixing systems and combination of different techniques can eliminate most of the problems associated with bubble formation.

As on-line vacuum degassing is offered on most commercially available systems sonication in combination with on-line degassing gives satisfactory results.

It is recommended that sonication is used in combination with one of the other techniques. For practical purposes, it is advisable to degas the mobile phase in both low and high pressure mixing systems.

**Filtration in HPLC**

It is essential to filter the mobile phase components even if you have sourced solvents from reputed suppliers and taken precautions to ensure that buffer salts have been fully dissolved. Suspended particulate matter can result in build up inside columns and result in development of back pressure thereby affecting flow rate and damage to pump components. In the worst scenario there can be mobile phase leakages at joint fittings accompanied with stoppage of the analytical run. Now the question arises on how to minimize such occurrences. Some steps are suggested which will help overcome such situations

After preparing mobile phase mixture filter through a 0.45 µ size filter using a vacuum

Prepare mobile phase fresh before use. In case this is not possible every time at least look for any visible suspended impurities or bacterial growth. In such cases it is suggested that the mobile phase should be discarded and another lot freshly prepared

Use online filters in HPLC solvent bottles to remove any sub- 10 µ size suspended particles. Clean the filters by sonication from time to time

Filter sample and standard solutions before collection in vials before injection

Make use of guard columns

The suggested precautions will contribute to the useful life of your HPLC system and at same time improve the reproducibility of your chromatographic separations.

**Question number 2:**

Write the name of detectors used in HPLC and GC, what are difference between them?

**Answer:**

**Detectors for HPLC are:**

1. UV-Vis Detectors:

The SPD-20A and SPD-20AV are general-purpose UV-Vis detectors offering an exceptional level of sensitivity and stability. With improved light-source compensation and stray light correction, high sensitivity is achieved across an extremely broad linear range (2.5AU). A temperature controlled flow cell assists in reducing noise and allows for baseline stability.

1. Photodiode Array Detectors (M30A, M20A, 30AM):

The SPD-M20A is a photodiode array detector (PDA) that achieves high sensitivity and superior linearity (2.0AU) due to improved light-source compensation and stray light correction. Two slit widths are available, 1.2nm for high-resolution work and 8nm for quantitative runs. Temperature controlled flow cells are standard to reduce noise and assist in baseline stability.

The SPD-M30A is a photodiode array detector (PDA) that uses a newly designed capillary cell to achieve ultra-low dispersion in UHPLC separations. This detector also supports high-sensitivity analysis due to the improved signal level and reduced noise level. Stray light compensation technology and temperature control functions allow for reliable analysis.

The SPD-30AM is a UHPLC multi-wavelength photodiode detector for high-speed multi-wavelength (up to 4 discrete channels) sampling. This detector was designed to satisfy demands for greater accuracy and sensitivity than a typical UV-Vis detector but with a flexible multi-wavelength design.

1. Refractive Index Detector:

The RID-20A is a high-performance, easy-to-use refractive index detector that offers excellent stability. A dual temperature control structure and an improved thermal design is adopted for the optical system to provide better baseline stability and a shorter initial stabilization time.

1. Fluorescence Detectors:

The RF-20A and RF-20Axs are fluorescence detectors for UHPLC and HPLC separations with industry-leading sensitivity and fast sampling. These detectors offer superb ease-of-maintenance, thanks to cell and lamp replacements from the front panel with no additional position adjustment. The RF-20Axs also offers a temperature controlled flow cell with cooling functions allowing excellent peak area reproducibility with respect to room temperature fluctuations.

1. Evaporative Light Scattering Detector:

The ELSD-LT II is an evaporative light scattering detector using a unique nebulizer and evaporation tube to allow low-temperature operation. This universal detector is a powerful tool for the gradient analysis of compounds that cannot be analyzed using an absorbance detector.

1. Conductivity Detector:

Conductivity Detector for Shimadzu

The CDD-10AVP is a conductivity detector applicable for ion chromatography or organic acid analyses. This detector offers low noise and low drift assurance with a wide dynamic range. High sensitivity is achieved even as a non-suppressed system which also allows for a high degree of versatility.

**Detectors for GC are:**

1. FLAME IONIZATION DETECTOR (FID):

Mechanism: Compounds are burned in a hydrogen-air flame. Carbon containing compounds produce ions that are attracted to the collector. The number of ions hitting the collector is measured and a signal is generated.

Selectivity: Compounds with C-H bonds. A poor response for some non-hydrogen containing organics (e.g., hexa-chlorobenzene).

Sensitivity: 0.1-10 ng

Linear range: 105-107

Gases: Combustion - hydrogen and air; Makeup - helium or nitrogen

Temperature: 250-300°C, and 400-450°C for high temperature analyses.

1. NITROGEN PHOSPHORUS DETECTOR (NPD):

Mechanism: Compounds are burned in a plasma surrounding a rubidium bead supplied with hydrogen and air. Nitrogen and phosphorous containing compounds produce ions that are attracted to the collector. The number of ions hitting the collector is measured and a signal is generated.

Selectivity: Nitrogen and phosphorous containing compounds

Sensitivity: 1-10 pg

Linear range: 104-10-6

Gases: Combustion - hydrogen and air; Makeup – helium

Temperature: 250-300°C

1. ELECTRON CAPTURE DETECTOR (ECD):

Mechanism: Electrons are supplied from a 63Ni foil lining the detector cell. A current is generated in the cell. Electronegative compounds capture electrons resulting in a reduction in the current. The amount of current loss is indirectly measured and a signal is generated.

Selectivity: Halogens, nitrates and conjugated carbonyls

Sensitivity: 0.1-10 pg (halogenated compounds); 1-100 pg

(nitrates); 0.1-1 ng (carbonyls)

Linear range: 103-104

Gases: Nitrogen or argon/methane

Temperature: 300-400°C

1. THERMAL CONDUCTIVITY DETECTOR (TCD):

Mechanism: A detector cell contains a heated filament with an applied current. As carrier gas containing solutes passes through the cell, a change in the filament current occurs. The current change is compared against the current in a reference cell. The difference is measured and a signal is generated.

Selectivity: All compounds except for the carrier gas

Sensitivity: 5-20 ng

Linear range: 105-106

Gases: Makeup - same as the carrier gas

Temperature: 150-250°C

1. FLAME PHOTOMETRIC DETECTOR (FPD):

Mechanism: Compounds are burned in a hydrogen-air flame. Sulfur and phosphorous containing compounds produce light emitting species (sulfur at 394 nm and phosphorous at 526 nm). A monochromatic filter allows only one of the wavelengths to pass. A photomultiplier tube is used to measure the amount of light and a signal is generated. A different filter is required for each detection mode.

Selectivity: Sulfur or phosphorous containing compounds. Only one at a time.

Sensitivity: 10-100 pg (sulfur); 1-10 pg (phosphorous)

Linear range: Non-linear (sulfur); 103-105 (phosphorous)

Gases: Combustion - hydrogen and air; Makeup – nitrogen

Temperature: 250-300°C

1. PHOTOIONIZATION DETECTOR (PID):

Mechanism: Compounds eluting into a cell are bombarded with high energy photons emitted from a lamp. Compounds with ionization potentials below the photon energy are ionized. The resulting ions are attracted to an electrode, measured, and a signal is generated.

Selectivity: Depends on lamp energy. Usually used for aromatics and olefins (10 eV lamp).

Sensitivity: 25-50 pg (aromatics); 50-200 pg (olefins)

Linear range: 105-106

Gases: Makeup - same as the carrier gas

Temperature: 200°C

1. ELECTROLYTIC CONDUCTIVITY DETECTOR (ELCD):

Mechanism: Compounds are mixed with a reaction gas and passed through a high temperature reaction tube. Specific reaction products are created which mix with a solvent and pass through an electrolytic conductivity cell. The change in the electrolytic conductivity of the solvent is measured and a signal is generated. Reaction tube temperature and solvent determine which types of compounds are detected.

Selectivity: Halogens, sulfur or nitrogen containing compounds. Only one at a time.

Sensitivity: 5-10 pg (halogens); 10-20 pg (S); 10-20 pg (N)

Linear range: 105-106 (halogens); 104-105 (N); 103.5-104(S)

Gases: Hydrogen (halogens and nitrogen); air (sulfur)

Temperature: 800-1000°C (halogens), 850-925°C (N), 750-825°C (S)

1. MASS SPECTROMETER (MS):

Mechanism: The detector is maintained under vacuum. Compounds are bombarded with electrons (EI) or gas molecules (CI). Compounds fragment into characteristic charged ions or fragments. The resulting ions are focused and accelerated into a mass filter. The mass filter selectively allows all ions of a specific mass to pass through to the electron multiplier. All of the ions of the specific mass are detected. The mass filter then allows the next mass to pass through while excluding all others. The mass filter scans stepwise through the designated range of masses several times per second. The total number of ions are counted for each scan. The abundance or number of ions per scan is plotted versus time to obtain the chromatogram (called the TIC). A mass spectrum is obtained for each scan which plots the various ion masses versus their abundance or number.

Selectivity: Any compound that produces fragments within the selected mass range. May be an inclusive range of masses (full scan) or only select ions (SIM).

Sensitivity: 1-10 ng (full scan); 1-10 pg (SIM)

Linear range: 105-106

Gases: None

Temperature: 250-300°C (transfer line), 150-250°C (source)

Difference:

HPLC detection is commonly based on nondestructive detection such as UV, RI, photodiode array detectors, conductivity and laser detection. On the other hand, Gas Chromatography detection is based largely on destructive principles such as a FID, NPD and FPD.

**Question number 3**:

Discuss properties of ion exchange resin and explain its working.

**Answer:**

Properties of ion exchange resins

1. Swelling:

Ion exchange resins are hygroscopic. The amount of moisture hydrated by a resin is determined by the cross-linking and the type of functional group. Low cross-linking gel resins with functional groups of sulfonic acid or quaternary ammonium contain large amounts of water resulting in swelling. Frequent swelling and contraction reduce the resin life.

1. Capacity:

Capacity is a number of chemical equivalents of ions that can be taken up by a unit amount of the resin (dry weight/wet weight/wet volume). Cross-linking decreases the capacity measured on the dry basis (fewer functional groups may be attached to highly cross-linked polymer molecules). However, cross-linking also decreases hydration of the resin therefore the capacity measured on the wet basis increases with an increase of the cross-linking level.

1. Particle size:

Ion exchange resins are available in different particle (bed) size. Common ion exchange resins are manufactured in form of polydispersed spherical beds with the size distributed within the range 0.01-0.05” (0.25-1.25mm) or in form of uniform particle size (UPS). Smaller particles improve the kinetics of the ion exchanging reaction but cause increase of the water pressure drop and decrease of the flow rate.

1. Stability:

Mechanical (physical) stability of ion exchange resins is determined mainly by the toughness of the polymer structure (cross-linking) and by the frequency of swelling-contraction cycles. Chemical degradation of ion exchange resins may be caused by fouling the resin pores by precipitates (e.g., iron hydroxide), breaking polymer structure, loss of ion exchange capacity due to a modification of the functional groups.

***Strong Acid Cation resins***

Strong Acid Cation (SAC) resins behave similar to strong acids.

Strong Acid Cation resins are available in two forms: hydrogen (R-SO3H) or sodium (R-SO3Na).

The typical strong acid cation exchange reaction:

2(R-SO3Na) + CaCl2 = (R-SO3)2Ca + 2NaCl

Cross-linking level of the Strong Acid Cation resins is 8-10%.

The ion exchange capacity of Strong Acid Cation resins does not depend on the solution PH.

Strong Acid Cation resins are used for water softening and demineralization.

The exhausted Strong Acid Cation resins may be regenerated.

Regeneration in hydrogen (acid) form is performed by a strong acid (e.g., HCL). Regeneration in sodium (salt) form is performed by sodium chloride solution (NaCl).

***Weak Acid Cation resins***

Weak Acid Cation (WAC) resins behave similar to weak acids.

Weak Acid Cation resins are available in hydrogen form (R-COOH).

Weak Acid Cation resins have high affinity for hydrogen ions therefore they are easily regenerated by stoichiometric amount of acid.

The ion exchange capacity of Weak Acid Cation resins increases with an increase of the solution PH. WAC resins are not used for treatment acidic (PH<6) solutions.

Weak Acid Cation resins are used for demineralization and de-alkalization of water.

***Strong Base Anion resins***

Strong Base Anion (SBA) resins behave similar to strong bases.

Strong Base Anion resins are available in hydroxide form: (R-NH3OH).

The typical strong base anion exchange reaction:

R-NH3OH + HNO3 = R-NH3NO3 + H2O

Strong Base Anion resins are used for demineralization and dealkalization of water.

The exhausted Strong Base Anion resins may be regenerated by concentrated sodium hydroxide (NaOH).

***Weak Base Anion resins***

Weak Base Anion (WBA)resins behave similar to weak bases.

The typical weak base anion exchange reaction:

R-NH2 + HNO3 = R-NH3NO3

The ion exchange capacity of Weak Base Anion resins increases with a decrease of the solution PH. WBA resins are not used for treatment basic (PH>6) solutions.

Weak Base Anion resins sorb only anions of strong acids (chlorides, nitrates, sulfates).

Weak Base Anion are easily regenerated by small amounts of weak bases (such as ammonia or sodium carbonate), which neutralize the acid taken up by the resin.