BIOE 507 Questions (Mass Spectroscopy lectures – Week of March 15th)

Please answer/explain the questions below in a paragraph of less (5-10 lines).

1. What are the basic principles governing chromatographic separations.

Chromatographic separation is driven by equilibrium of different components in the mobile phase and stationary phase. Different components of the analyte have different affinities towards the mobile phase and stationary phase, which means they have different solubilities in the two phases. The higher affinity to the stationary phase, the slower the molecule will move through the column and vice versa. The time differences to pass through the column result in the separation of the components.

1. What are the “Figures of Merit Characterization” of separations.

It includes resolution and separation efficiency.

Resolution measures how much two compounds in a chromatogram are separated from each other. Its equation is defined as follows. R=2(t2-t1)/(w1+w2) where t1 and t2 are the retention times of the two compounds and w1 and w2 refer to the width of each peak.

Separation efficiency is the number of theoretical plates, a concept arising from fractional distillation, as a distinct region where a single equilibrium is maintained. Its values is related by the length of the column as N=L/H=L^2/σ^2=t^2/σ^2=(2.35t)^2/w1/2^2, where L is the length of column, H is the height equivalent to a theoretical plate, σ is standard deviation of an elution peak and w1/2 refers to the full width at half max of the peak.

1. Describe the flow profile during an electroosmotic flow.

Unlike a parabolic profile flow generated in pressure-driven flow where the liquid lows faster in the center regions compared to peripheral regions, the electroosmotic flow’s flow profile is almost planar. Electroosmotic flow is caused by the Coulomb force induced by the electric field on the net mobile electric charge in the solution. The electric field is formed by the equilibrium between the solid surface and the solution leading the interface to acquire a layer of mobile ions known as an electrical double layer. The velocities of electroosmotic flow are independent of the conduit size, as long as the electrical double layer is much smaller than the length scale of the channel.

1. How is mass spectra characterized – explain this based on mass accuracy and resolving power.

Mass spectrometers separate the ions according to their mass-to-charge ratio (m/z). The results are represented on a plot of intensity as a function of the m/z which is known as mass spectrum. There are 2 important analyzer characteristics: mass resolving power and mass accuracy. Mass accuracy is the ratio of the m/z measurement error to the true m/z. It determines how precise the analyzer in measuring an ion. Its definition is △m/z=(real m/z - measured m/z)/ real m/z \* 10^6. Mass resolving power measures the ability of the analyzer to distinguish how close of two peaks could be. It is defined as R=(m/z)/(△m/z).

1. Explain the terms ESI, MALDI, QIT, TOF. State the key factors affecting these.

ESI: electrospray ionization. It’s a technique to produce ions using an electrospray where a high voltage is applied to a liquid to generate aerosol. Sample & gas flowrates, employed solvent, ionization temperature and voltage may affect the ionization efficiency.

MALDI: matrix assisted laser desorption ionization. It’s a technique that uses a laser energy absorbing matrix to generate ions form large molecules. Matrix material and layer type are the key factors affecting its performance.

QIT: quadrapole ion trap. It’s a type of ion trap that uses dynamic electric field to trap ions. Operating voltage and frequency are the key factors.

TOF: time of flight. It’s a methods of mass spectrometer which measures m/z based on the velocity over at fixed distance which equates to time. Kinetic energy which is determined by the distance and voltage of acceleration region is the major factor.

1. What is multi-Stage MS. Why and where is this useful.

Multi-Stage MS (MS^n) is a technique in mass spectrum analysis where two or more mass spectrometers are coupled together using additional reaction steps to increase their abilities to analyze ion samples.

After the ions by specific m/z are separated by the first MS analyzer, these ions could be selected and split into smaller ions by utilizing ion fragmentation methods, such as collision induced dissociation and electron transfer dissociation. Then these fragmented ions could by detected via the following MS analyzer. In this way, ions with similar m/z ratio could be distinguished and identified. The resolution of the system is improved.

This technique is very useful in the analysis of biomolecules including proteins, metabolites and peptides, which are large and have complex spectrum with similar m/z ratios.

1. In Mass Spectrometry what are the factors contributing to its resolution of separation.

The major factors affecting the resolution are the mass selection methods. For example, when detecting ions with m/z=1000, time of flight method which is to measure the flight time of ions could reach resolution 10^3 to 10^4; magnetic sector which measures momentum/charge has resolution 10^5; Fourier transform ion cyclotron resonance which detect ions based on cyclotron frequency has resolution 10^6; Ion trap which measure the frequency has resolution 10^4. Particularly, orbitrap, a kind of ion trap, has resolution up to 5\*10^6.

Other factors including ionization methods, sample preparation systems, signal processing procedures including noise reduction, SNR improvement would also have impact on the resolution of the analyzer.