BIOE 507 Quiz 3: Mass Spectroscopy April 1st 2021

Please provide brief answers to the following:

1. State the basic principles governing chromatographic separations.

Chromatographic separation is driven by equilibrium of different components in the mobile phase and stationary phase. Different analyte components have distinct affinities towards the mobile phase and stationary phase, causing different solubilities in the two phases. The higher affinity to the stationary phase, the lower the analyte moves through the column and vice versa. The time difference to pass through the column results in the separation of the components.

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

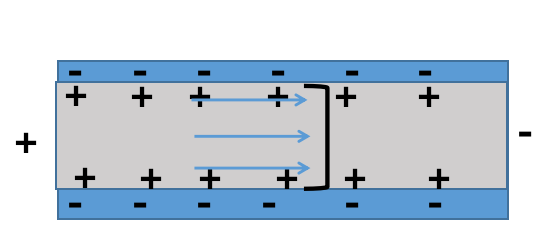
It contains resolution and separation efficiency.

Resolution measures how much two components in a chromatogram are separated from each other. The equation defining the property is R=2(t1-t2)/(w1+w2), where t1 and t2 are the retention time of the two components and w1 and w2 refer to the width of each peak.

Separation efficiency is the number of theoretical plates, which is a concept from fractional distillation, as a distinct region where a single equilibrium is maintained. Its value is related by the length of the column as N=L/H=L^2/σ^2=t^2/σ^2, where L is the length of the column, H is the height equivalent to a theoretical plate, σ is the standard deviation of an elution peak.

1. Briefly define electroosmotic flow and approximately draw a flow profile

Pressure-driven flow is a flow that the liquid flows faster in the center regions compared to peripheral regions, forming a parabolic profile flow. 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.



The blue arrows indicate the flow direction. The inner face is charged with positive ions, forming the electric double layer.

1. Briefly describe mass spectra characterization based on mass 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 called 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. Mass resolving power measures the ability of the analyzer to distinguish how close of two peaks could be.

1. Define the terms ESI, MALDI, QIT, TOF in 2-3 sentences each.

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.

MALDI: matrix assisted laser desorption ionization. It’s a technique that uses a laser energy absorbing matrix to generate ions form large molecules.

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 to control the process.

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.

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

Multi-Stage MS (MSn) 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. What are the factors contributing to resolution of separation in Mass Spectrometry and why?

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. For example, in ESI, Sample & gas flowrates, employed solvent, ionization temperature and voltage may affect the

ionization efficiency. Matrix material is the key factor affecting its performance in MALDI.