MICELLAR CHROMATOGRAPHY

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ASBSTRACT:

Micelles are composed of surfactant, or detergent, monomers with a hydrophobic moiety, or tail, on one end, and a hydrophilic moiety, or head group, on the other. The polar head group may be anionic, cationic, zwitterionic, or non-ionic. When the concentration of a surfactant in solution reaches its critical micelle concentration (CMC), it forms micelles which are aggregates of the monomers. The CMC is different for each surfactant, as is the number of monomers which make up the micelle, termed the aggregation number many of the characteristics of micelles differ from those of bulk solvents. For example, the micelles are, by nature, spatially heterogeneous with a hydrocarbon, nearly anhydrous core and a highly solvated, polar head group. They have a high surface-to-volume ratio due to their small size and generally spherical shape. Their surrounding environment (pH, ionic strength, buffer ion, presence of a co-solvent, and temperature) has an influence on their size, shape, critical micelle concentration, aggregation number and other properties.

Key Works: *Micellar Chromatography, HPLC, Micelles, Krafft Point*

INTRODUCTION

Analytical Chemistry is the study of the separation, identification, and quantification of the chemical components of natural and artificial materials. Qualitative analysis gives an indication of the identity of the chemical species in the sample and quantitative analysis determines the amount of one or more of these components. The separation of components is often performed prior to analysis.

Analytical methods can be separated into classical and instrumental. Classical methods (also known as wet chemistry methods) use separations such as precipitation, extraction, and distillation and qualitative analysis by color, odor, or melting point. Quantitative analysis is achieved by measurement of weight or volume. Instrumental methods use an apparatus to measure physical quantities of the analytes such as light absorption, fluorescence, or conductivity. The separation of materials is accomplished using chromatography or electrophoresis methods.

Analytical chemistry is also focused on improvements in experimental design, chemometrics, and the creation of new measurement tools to provide better chemical information. Analytical chemistry has applications in forensics, bioanalysis, clinical analysis, environmental analysis, and materials analysis.

APPLICATIONS

- Genomics - DNA sequencing and its related research. Genetic fingerprinting and DNA microarray are very popular tools and research fields.

- Proteomics - the analysis of protein concentrations and modifications, especially in response to various stressors, at various developmental stages, or in various parts of the body.
- Metabolomics - similar to proteomics, but dealing with metabolites.
- Transcriptomics - mRNA and its associated field
- Lipidomics - lipids and its associated field
- Peptidomics - peptides and its associated field
- Metalomics - similar to proteomics and metabolomics, but dealing with metal concentrations and especially with their binding to proteins and other molecules.

CHROMATOGRAPHY

Chromatography (from Greek chroma "color" and graphein "to write") is the collective term for a set of laboratory techniques for the separation of mixtures. The mixture is dissolved in a fluid called the mobile phase, which carries it through a structure holding another material called the stationary phase. The various constituents of the mixture travel at different speeds, causing them to separate. The separation is based on differential partitioning between the mobile and stationary phases. Subtle differences in a compound's partition coefficient result in differential retention on the stationary phase and thus changing the separation. Chromatography may be preparative or analytical. The purpose of preparative chromatography is to separate the components of a mixture for more advanced use (and is thus a form of purification). Analytical chromatography is done normally with smaller amounts of material and is for measuring the relative proportions of analytes in a mixture. The two are not mutually exclusive. Chromatography is a technique used in analytical chemistry to separate and identify components of mixtures. The name comes from the Greek term for "color writing" because this method was originally used to separate colored samples. Today a variety of chromatography methods work by separating the individual components of mixtures so that each one can be analyzed and identified. Instruments used to perform chromatography are known as chromatographs, which have become essential pieces of equipment in chemical laboratories. With these devices, scientists can tell what chemical compounds are present in complex mixtures such as smog, cigarette smoke, petroleum products, or even coffee aroma.

MICELLES

Micelles are composed of surfactant, or detergent, monomers with a hydrophobic moiety, or tail, on one end, and a hydrophilic moiety, or head group, on the other. The polar head group may be anionic, cationic, zwitterionic, or non-ionic. When the concentration of a surfactant in solution reaches its critical micelle concentration (CMC), it forms micelles which are aggregates of the monomers. The CMC is different for each surfactant, as is the number of monomers which make up the micelle, termed the aggregation number Many of the characteristics of micelles differ from those of bulk solvents. For example, the micelles are, by nature, spatially heterogeneous with a hydrocarbon, nearly anhydrous core and a highly solvated, polar head group. They have a high surface-to-volume ratio due to their small size and generally spherical shape. Their surrounding environment (pH, ionic strength, buffer ion, presence of a co-solvent, and temperature) has an influence on their size, shape, critical micelle concentration, aggregation number and other properties.

Micellar liquid chromatography

Micellar liquid chromatography (MLC) is a form of reversed phase liquid chromatography that uses an aqueous micellar solution as the mobile phase.

Theory

The use of micelles in high performance liquid chromatography was first introduced by Armstrong and Henry in 1980. The technique is used mainly to enhance retention and selectivity of various solutes that would otherwise be inseparable or poorly resolved. Micellar liquid chromatography (MLC) has been used in a variety of applications including separation of mixtures of charged and neutral solutes, direct injection of serum and other physiological fluids, analysis of pharmaceutical compounds, separation of enantiomers, analysis of inorganic organometallics, and a host of others.

One of the main drawbacks of the technique is the reduced efficiency that is caused by the micelles. Despite the sometimes poor efficiency, MLC is a better choice than ion-exchange LC or ion-pairing LC for separation of charged molecules and mixtures of charged and neutral species. Some of the aspects which will be discussed are the theoretical aspects of MLC, the use of models in predicting retentive characteristics of MLC, the effect of micelles on efficiency and selectivity, and general applications of MLC.

Basic Principles of MLC

Micellar liquid chromatography (MLC) is an efficient alternative to conventional reversed-phase liquid chromatography with hydro-organic mobile phases. Almost three decades of experience have resulted in an increasing production of analytical applications. Current concern about the environment also reveals MLC as an interesting technique for ―green‖ chemistry because it uses mobile phases containing 90% or more water. These micellar mobile phases have a low toxicity and are not producing hazardous wastes. The stationary phase is modified with an approximately constant amount of surfactant monomers, and the solubilising capability of the mobile phase is altered by the presence of micelles, giving rise to a great variety of interactions (hydrophobic, ionic, and steric) with major implications in retention and selectivity. From its beginnings in 1980, the technique has evolved up to becoming in a real alternative in some instances (and a complement in others) to classical RPLC with aqueous-organic mixtures, owing to its peculiar features and unique advantages. The addition of an organic solvent to the mobile phase was, however, soon suggested in order to enhance the low efficiencies and weak elution strength associated with the mobile phases that contained only micelles.

Particularities of the Micellar Mobile Phase

Micelles provide hydrophobic and electrostatic (for ionic surfactants) sites of interaction. In the micelles, three sites of solubilisation can be identified: the core (hydrophobic), the surface (hydrophilic), and the palisade layer (the region between the surfactant head groups and the core). Solutes associated to micelles experience a microenvironment that is different from that of bulk solvent.

Krafft Point

The Krafft point is defined for ionic surfactants as the temperature at which the solubility of a surfactant monomer becomes equal to the CMC. Below the Krafft point temperature, the solubility is quite low and the solution appears to contain no micelles. Chromatographic work in MLC should be conducted above this temperature to avoid surfactant precipitation. This means that the Krafft point should be well below room temperature. The Krafft point for SDS and CTAB is around 15°C and 20–25°C, respectively.

pH of the Mobile Phase

MLC employs the same packing materials as classical RPLC, which for conventional columns have a limited working pH range of 2.5–7.5. Appropriate pH values depend on the nature of the analytes and the surfactant selected. The pH of the micellar mobile phase is commonly fixed with phosphoric or citric acid buffers. For mobile phases

containing SDS, potassium salts are not recommended as potassium dodecyl sulphate presents a high Krafft point and precipitates from aqueous solutions at room temperature.

Micellar electrokinetic chromatography

Micellar electrokinetic chromatography (MEKC), is a chromatography technique, used in analytical chemistry. It is a modification of capillary electrophoresis (CE), where the samples are separated by differential partitioning between micelles (pseudo-stationary phase) and a surrounding aqueous buffer solution (mobile phase).

The basic set-up and detection methods used for MEKC are the same as those used in CE. The difference is that the solution contains a surfactant at a concentration that is greater than the critical micelle concentration (CMC). Above this concentration, surfactant monomers are in equilibrium with micelles.

In most applications, MEKC is performed in open capillaries under alkaline conditions to generate a strong electroosmotic flow. Sodium dodecyl sulfate (SDS) is the most commonly used surfactant in MEKC applications. The anionic character of the sulfate groups of SDS cause the surfactant and micelles to have electrophoretic mobility that is counter to the direction of the strong electroosmotic flow. As a result, the surfactant monomers and micelles migrate quite slowly, though their net movement is still toward the cathode.

Analytes that are insoluble in the interior of micelles should migrate at the electroosmotic flow velocity, u_o and be detected at the retention time of the buffer,t_m Analytes that solubilize completely within the micelles (analytes that are highly hydrophobic) should migrate at the micelle velocity,u_c , and elute at the final elution time,t_c

Applications of MEKC

The simplicity and efficiency of MEKC have made it an attractive technique for a variety of applications. Further improvements can be made to the selectivity of MEKC by adding chiral selectors or chiral surfactants to the system. Unfortunately, this technique is not suitable for protein analysis because proteins are generally too large to partition into a surfactant micelle and tend to bind to surfactant monomers to form SDS-protein complexes.

Recent applications of MEKC include the analysis of uncharged pesticides, essential and branched-chain amino acids in nutraceutical products, hydrocarbon and alcohol contents of the marjoram herb.

INSTRUMENTATION

Introduction to HPLC.

High Performance Liquid Chromatography (HPLC) is one mode of chromatography, one of the most used analytical techniques. Chromatographic process can be defined as separation technique involving mass-transfer between stationary and mobile phase. HPLC utilizes a liquid mobile phase to separate the components of a mixture. The stationary phase can be a liquid or a solid phase. These components are first dissolved in a solvent, and then forced to flow through a chromatographic column under a high pressure. In the column, the mixture separates into its components. The amount of resolution is important, and is dependent upon the extent of interaction between the solute components and the stationary phase. The stationary phase is defined as the immobile packing material in the column. The interaction of the solute with mobile and stationary phases can be manipulated through different choices of both solvents and stationary phases. As a result, HPLC acquires a high degree of versatility not found in other chromatographic systems and it has the ability to easily separate a wide variety of chemical mixtures.

Types of HPLC: There are many ways to classify liquid column chromatography. If this classification is based on the nature of the stationary phase and the separation process, three modes can be specified.

a) Adsorption chromatography: the stationary phase is an adsorbent (like silica gel or any other silica based packing) and the separation is based on repeated adsorption-desorption steps.

b) Ion-exchange chromatography: the stationary bed has an ionically charged surface of opposite charge to the sample ions. This technique is used almost exclusively with ionic or ionizable samples. The stronger the charge on the sample, the stronger it will be attracted to the ionic surface and thus, the longer it will take to elute. The mobile phase is an aqueous buffer, where both pH and ionic strength are used to control elution time

c) Size exclusion chromatography: The column is filled with material having precisely controlled pore sizes, and the sample is simply screened or filtered according to its solvated molecular size. Larger molecules are rapidly washed through the column; smaller molecules penetrate inside the porous of the packing particles and elute later. This technique is also called gel filtration or gel permeation chromatography.

Stationary Phases (Adsorbents)

HPLC separations are based on the surface interactions, and depend on the types of the adsorption sites. Modern HPLC adsorbents are the small rigid porous particles with high surface area. Main adsorbent parameters are: Particle size: 3 to 10 ìm Particle size distribution: as narrow as possible, usually within 10% of the mean. The last parameter in the list represents an adsorbent surface chemistry. Depending on the type of the ligand attached to the surface, the adsorbent could be normal phase (-OH, -NH2), or reversed-phase (C5, C8, C 18 CN, NH2), and even anion (CH2NR3 +OH-), or cation (R-SO3 -H+) exchangers.

Mobile phase

In HPLC, the type and composition of the eluent is one of the variables influencing the separation. Despite the large variety of solvents used in HPLC, there are several common properties: Purity Detector compatibility Solubility of the sample Low viscosity Chemical inertness For normal phase mode, solvents are mainly nonpolar; for reversed-phase, eluents are usually a mixture of water with some polar organic solvent such as acetonitrile or methanol. Size-exclusion HPLC has special requirements. SEC eluents have to dissolve polymers, but the most important is that SEC eluent has to suppress possible interactions of the sample molecule with the surface of the packing material.

Functional description of the instrument

a) Mobile phase reservoir, filtering
b) Pump
c) Injector
d) Column
e) Detector
f) Data system

Mobile phase reservoir, filtering

The most common type of solvent reservoir is a glass bottle. Most of the manufacturers supply these bottles with special caps, Teflon tubing and filters to connect to the pump inlet and to the purge gas (helium) used to remove dissolved air. Helium purging and storage of the solvent under helium is not sufficient for degassing aqueous solvents. It is useful to apply a vacuum for 5-10 min. and then keep the solvent under a helium atmosphere.

Pump

High pressure pumps are needed to force solvents through packed stationary phase beds. Smaller bed particles require higher pressures. There are many advantages to using smaller particles, but they may not be essential for all separations. The most important advantages are: higher resolution, faster analyses, and increased sample load capacity. However, only the most demanding separations require these advances in significant amounts. Many separation problems can be resolved with larger particle packings that require less pressure. Flow rate stability is another important pump feature that distinguishes pumps. Very stable flow rates are usually not essential for

analytical chromatography. However, if the user plans to use a system in size exclusion mode, then there must be a pump which provides an extremely stable flow rate.

Injector

Sample introduction can be accomplished in various ways. The simplest method is to use an injection valve. In more sophisticated LC systems, automatic sampling devices incorporated where the sample is introduced with the help of autosamplers and microprocessors. In liquid chromatography, liquid samples may be injected directly and solid samples need only be dissolved in an appropriate solvent. The solvent need not be the mobile phase, but frequently it is judiciously chosen to avoid detector interference, column/component interference, loss in efficiency or all of these. It is always best to remove particles from the sample by filtering over a 5 ìm filter, or centrifuging, since continuous injections of particulate material will eventually cause blockages in injection devices or columns. Sample sizes may vary widely.

Colums

The availability of highly sensitive detectors frequently allows use of the small samples which yield the highest column performance. Typical sample mass with 4.6 mm ID columns range from the nanogram level up to about 2 mg diluted in 20 ml of solvent. In general, it will be noted that much less sample preparation is required in LC than in GC since unwanted or interfering compounds, or both, may often be extracted, or eliminated, by selective detection.

Detector

Today, optical detectors are used most frequently in liquid chromatographic systems. These detectors pass a beam of light through the flowing column effluent as it passes through a low volume flow cell. The variations in light intensity caused by UV absorption, fluorescence emission or change in refractive index, from the sample components passing through the cell, are monitored as changes in the output voltage. These voltage changes are recorded on a strip chart recorder and frequently are fed into a computer to provide retention time and peak area data. The most commonly used detector in LC is the ultraviolet absorption detector. A variable wavelength detector of this type, capable of monitoring from 190 to 400 nm, will be found suitable for the detection of the majority samples. Other detectors in common use include: Photo Diode Array UV detector (PAD), refractive index (RI), fluorescence (FLU), electrochemical (EC). The RI detector is universal but also the less sensitive one. FLU and EC detectors are quite sensitive (up to 10-15 pmole) but also quite selective.

Data system

Since the detector signal is electronic, using modern data collection techniques can aid the signal analysis. In addition, some systems can store data in a retrievable form for highly sophisticated computer analysis at a later time. The main goal in using electronic data systems is to increase analysis accuracy and precision, while reducing operator attention. There are several types of data systems, each differing in terms of available features.

Method

A reservoir holds the solvent [called the mobile phase, because it moves]. A high-pressure pump [solvent delivery system or solvent manager] is used to generate and meter a specified flow rate of mobile phase, typically milliliters per minute. An injector [sample manager or autosampler] is able to introduce [inject] the sample into the continuously flowing mobile phase stream that carries the sample into the HPL column. The column contains the chromatographic packing material needed to effect the separation. This packing material is called the stationary phase because it is held in place by the column hardware. A detector is needed to see the separated compound bands as they elute from the HPLC column [most compounds have no color, so we cannot see them with our eyes]. The mobile phase exits the detector and can be sent to waste, or collected, as desired. When the mobile phase contains a separated

compound band, HPLC provides the ability to collect this fraction of the eluate containing that purified compound for further study. This is called preparative chromatography. Note that high-pressure tubing and fittings are used to interconnect the pump, injector, column, and detector components to form the conduit for the mobile phase, sample, and The detector is wired to the computer data station, the HPLC system component that records the electrical signal needed to generate the chromatogram on its display and to identify and quantitate the concentration of the sample constituents. Since sample compound characteristics can be very different, several types of detectors have been developed. For example, if a compound can absorb ultraviolet light, a UV-absorbance detector is used. If the compound fluoresces, a fluorescence detector is used. If the compound does not have either of these characteristics, a more universal type of detector is used, such as an evaporative-light-scattering detector [ELSD]. The most powerful approach is the use multiple detectors in series. For example, a UV and/or ELSD detector may be used in combination with a mass spectrometer [MS] to analyze the results of the chromatographic separation. This provides, from a single injection, more comprehensive information about an analyte. The practice of coupling a mass spectrometer to an HPLC system is called LC/MS.

Detector

As the separated dye bands leave the column, they pass immediately into the detector. The detector contains a flow cell that *sees* [detects] each separated compound band against a background of mobile phase [see Figure161]. [In reality, solutions of many compounds at typical HPLC analytical concentrations are colorless.] An appropriate detector has the ability to sense the presence of a compound and send its corresponding electrical signal to a computer data station. A choice is made among many different types of detectors, depending upon the characteristics and concentrations of the compounds that need to be separated and analyzed, as discussed earlier.

Normal-Phase HPLC

In his separations of plant extracts, Tswett was successful using a polar stationary phase [chalk in a glass column; see Figure A] with a much less polar [non-polar] mobile phase. This classical mode of chromatography became known as normal phase.

represents a normal-phase chromatographic separation of our three-dye test mixture. The stationary phase is polar and retains the polar yellow dye most strongly. The relatively non-polar blue dye is won in the retention competition by the mobile phase, a non-polar solvent, and elutes quickly. Since the blue dye is most like the mobile phase [both are non-polar], it moves faster. It is typical for normal-phase chromatography on silica that the mobile phase is 100% organic; no water is used.

Reversed-Phase HPLC

The term reversed-phase describes the chromatography mode that is just the opposite of normal phase, namely the use of a polar mobile phase and a non-polar [hydrophobic] stationary phase. Illustrates the black three-dye mixture being separated using such a protocol.

Now the most strongly retained compound is the more non-polar blue dye, as its attraction to the non-polar stationary phase is greatest. The polar yellow dye, being weakly retained, is won in competition by the polar, aqueous mobile phase, moves the fastest through the bed, and elutes earliest like attracts like. Today, because it is more reproducible and has broad applicability, reversed-phase chromatography is used for approximately 75% of all HPLC methods. Most of these protocols use as the mobile phase an aqueous blend of water with a miscible, polar organic solvent, such as acetonitrile or methanol. This typically ensures the proper interaction of analytes with the non-polar, hydrophobic particle surface. A C18–bonded silica [sometimes called ODS] is the most popular type of reversed-phase HPLC packing.

Micellar Liquid Chromatographic Determination of Carbaryl and 1-Naphthol in Water, Soil, and Vegetables

Chemicals and Reagents

Carbaryl was supplied by Union Carbide and 1-naphthol was purchased from Sigma-Aldrich Stock standard solutions containing 100 μg mL−1 of carbaryl and 1-naphthol were prepared. Pesticides were dissolved in few millilitres of methanol in an ultrasonic bath and were made up to the mark in the volumetric flask with SDS 0.15 M-6% (v/v) pentanol buffered at pH 3. Distilled-deionized water was used throughout, and solutions were stored at 4°C. Finally, they were conveniently diluted before the analysis. Micellar mobile phases were prepared by using sodium dodecyl sulphate of 99% purity purchased from Merck, Brij-35, or N-cetylpyridinium chloride monohydrate (NCPC) obtained from Across Organics. The buffer salt was sodium dihydrogen phosphate, and 1-propanol, 1- butanol, or 1-pentanol, all of which were of HPLC grade, came from Scharlab and were used as organic modifiers. All the solutions were filtered through 0.45 μm nylon membranes and stored at 4°C.

Apparatus

The chromatographic system consisted in an Agilent 1100 series high-performance liquid chromatography equipped with a quaternary pump, an online degasser, an autosampler, a thermostated column compartment, and a fluorescence detector. Chromatographic signals were acquired and processed with the Agilent ChemStation software package which was used to obtain the chromatographic peak parameters (dead time, retention time, efficiencies, and asymmetry factors). These data were later processed with SPSS 17.0. All the pH measurements were made with a GLP 22 Crison pH-meter, provided with a combined Ag/AgCl/glass electrode. The analytical balance used was a Mettler-Toledo AX105 Delta-Range. The vortex shaker and sonification units were made by Selecta.

Chromatographic Conditions

Chromatographic separation of the pesticides was performed in a Kromasil C18 column (250 mm × 4.6 mm i.d., 5 μm particle size) obtained from Scharlab. The selected mobile phase was 0.15 M SDS-6% (v/v) pentanol-0.01 M NaH2PO4 buffered at pH 3. The flow rate was 1 mL min−1, the injection volume was 20 μL, and the column temperature was maintained at 25°C. The excitation and emission wavelengths selected for the detection of the studied compounds were 225 nm and 333 nm, respectively.2.4. Sample PreparationCarbaryl and 1-naphthol were added in water, soil, and vegetables. Water was directly added with carbaryl and 1-naphthol at the desired concentrations. Then 1g of the soil or vegetable samples was weighed, finely ground using a mincer, centrifuged at 5000rpm for 5min, mixed with 10mL of 0.15M SDS-6% (v/v) pentanol-pH 3, spiked with the pesticides, stirred for 10min, and finally filtered directly into the autosampler vials through nylon membranes.

CONCLUSIONS

The micellar liquid chromatography procedure developed herein is rapid and allows the simultaneous determination of carbaryl and 1-naphthol in water, soil, and vegetables matrices with high sensitivity. The designed method easily achieves a high sample throughput with a short preparation time, and proves useful to determine the content of both pesticides. The proposed chromatographic procedure provides good results to determine the pesticides in the matrices studied in terms of linearity, accuracy, precision, recoveries, robustness, and sensitivity at the ng mL−1 level. Compared to other methods developed for the determination of pesticides, micellar mobile phases are less flammable, less expensive, less toxic, and biodegradable and can cosolubilize hydrophobic and hydrophilic analytes in this kind of matrixes. The elution of hydrophobic and hydrophilic analytes in the same MLC run is possible without a gradient elution. The use of an interpretative optimization strategy in MLC also makes more efficient and reliable mobile phase selection.

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