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Review Article

Micellar Liquid Chromatography

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Abstract: 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.

Keywords: Micellar Liquid Chromatography (MLC), Surfactant Monomers, Micellar Mobile Phases.

Introduction

Micellar liquid chromatography (MLC), which uses mobile phases containing a surfactant above its critical micellar concentration (CMC), is an alternative to conventional reversed phase liquid chromatography and provides a solution to the direct injection of physiological or food samples by solubilising proteins (that are eluted together or shortly after the solvent front)[1]. The possibility of directly injecting samples into the chromatograph simplifies and expedites treatment, which confers analytical procedures greater accuracy and a lower cost.

The versatility of MLC is due to the wide variety of interactions that are established among the eluted solutes, the stationary phase, the aqueous phase and micelles. Their eluent characteristics allow the analysis of compounds with a wide range of polarities. The presence of a surfactant not only modifies the interactions established inside the column but also reduces the necessary amount of organic solvent in the mobile phase, which can be recycled due to low evaporation. MLC shares the basic components of reversed-phase liquid chromatographic (RPLC) systems, that is, a non-polar stationary phase and a polar aqueous mobile phase. However, hydro-organic mobile phases in conventional RPLC are homogeneous, whereas micellar solutions are microscopically heterogeneous, being composed of two distinct media: the amphiphilic micellar aggregates (micellar pseudo phase) and the surrounding bulk water or aqueous-organic solvent that contains surfactant monomers in a concentration approximately equal to the CMC.

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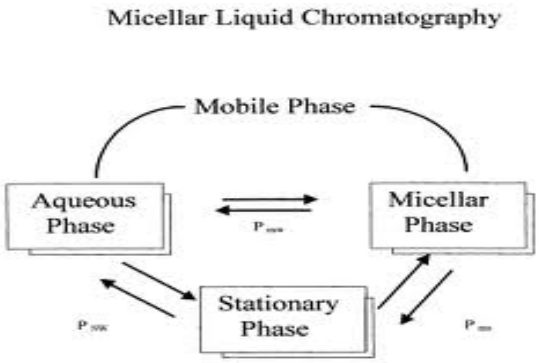

Fig. 1: Outline of micellar liquid chromatography

Theory:

The use of micelles in high performance liquid chromatography was first introduced by Armstrong and Henry in 1980[2]. 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[3]. 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.

Reverse phase high-performance liquid chromatography (RP-HPLC) involves a non-polar stationary phase, often a hydrocarbon chain, and a polar mobile or liquid phase. The mobile phase generally consists of an aqueous portion with an organic addition, such as methanol or acetonitrile. When a solution of analytes is injected into the system, the components begin to partition out of the mobile phase and interact with the stationary phase. Each component interacts with the stationary phase in a different manner depending upon its polarity and hydrophobicity. In reverse phase HPLC, the solute with the greatest polarity will interact less with the stationary phase and spend more time in the mobile phase. As the polarity of the components decreases, the time spent in the column increases. Thus, a separation of components is achieved based on polarity[4]. The addition of micelles to the mobile phase introduces a third phase into which the solutes may partition.

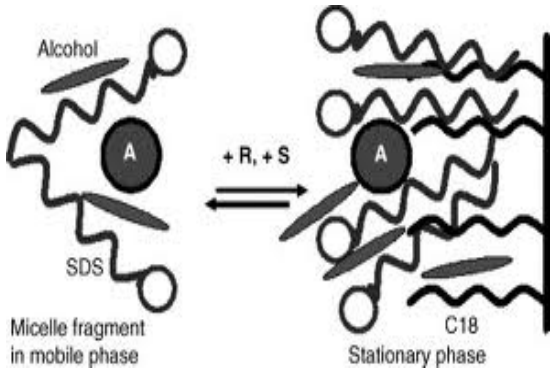

Fig.2: Micelle in mobile phase

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, zwitter ionic, 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 (AN).

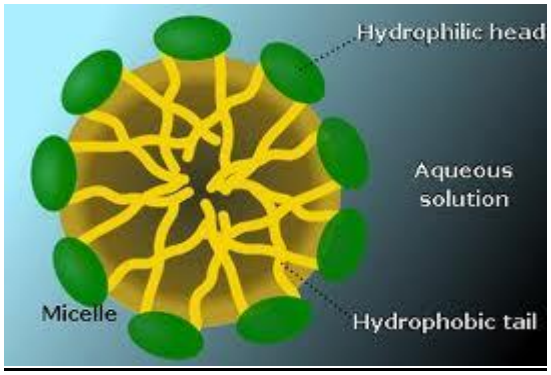

Fig. 3: Micelle diagram

Table.1: lists some common detergents used to form micelles along with their CMC and AN where available.

TYPE	NAME	CMC(mM)	AN
Anionic	Glycocholic acid, sodium salt	13	2-4
	Sodium dodecyl sulfate (SDS)	8.27	2
	Taurocholic acid, sodium salt	10-15	62
	Sodium tetradecyl sulfate	2.1	4
	Cetyltrimethylammonium chloride	1	
	Cetyltrimethylammonium bromide (CTAB)	1.3	78
Cationic	Dodecyltrimethlyammonium bromide (DTAB)	14	50
	Hexadecyltrimethylammonium bromide	0.026	169
	3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS)	8	10
	3-[(3-cholamidopropyl)dimethylammonio]-2-hydroxy-1-propanesulfonate (CHAPSO)	8	11
Zwitterionic	N-Dodecyl-N,N-dimethylammonio-3-propane sulfonate	3.3	
	n-Decyl-b-D-glucopyranoside	2.2	
	Triton X-100	0.24	140
Nonionic	Polyoxyethylene (23) dodecanol (BRIJ 35)	0.1	
	Polyoxyethylene [20]-sorbitane monooleate (Tween 80)	0.01	
	Polyoxyethylene [20]-sorbitane monolaurate (Tween 20)	0.059	

Particularities of the Micellar Mobile Phase:

- **Critical Micellar Concentration 1**: A suitable surfactant for MLC should have a low CMC. A high CMC would imply operating at high surfactant concentration, which would result in viscous solutions, giving undesirable high system pressure and background noise in UV detectors. The selection is often limited to the following surfactants: the anionic sodium dodecyl sulphate (SDS), the cationic cetyltrimethylammonium bromide (CTAB), and the nonionic Brij-35. The CMC values of these surfactants in pure water are low enough for MLC. It should also be taken into account that the CMC is strongly affected by the presence of an organic solvent. The changes are related to the modification of the structure of the micelle, which also induces, at least partially, the reduced retention in MLC. Recently, some novel ionic liquid-based surfactants like 1-

hexadecyl-3-butylimidazolium bromide have been used in MLC[5].

- **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[6]. 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[7].

  Non-ionic surfactants also have a specific temperature, that if exceeded, phase separation occurs, which is called the cloud point[8]. Chromatographic work with these surfactants should be conducted below this temperature (e.g., Brij-35, is ca. 100◦C for aqueous 1–6% solutions, whereas for Triton X–100 this value is 64◦C).

- **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[5]. 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.

- **Organic Solvents: Types and Concentration:** The selection of the appropriate organic solvent modifier in MLC should consider the polarities of the analytes. For polar compounds, sufficiently short retention times (below 20 min) are obtained with 1-propanol, 2-propanol, or acetonitrile. For nonpolar compounds or compounds with high affinity for the surfactant adsorbed on the stationary phase, stronger solvents as 1-butanol or 1-pentanol are needed[9]. However, it should be noted that the two latter alcohols give rise to micro emulsion formation at sufficiently high concentration. In practice, the amount of organic solvent that can be added is limited by its solubility. It should be noted that at high organic solvent concentration, the micelles disaggregate and the mobile phase contains only free surfactant molecules. The organic solvent contents that preserve the integrity of micelles are below 15% for propanol and acetonitriles, 10% for butanol, and 6% for pentanol[10].

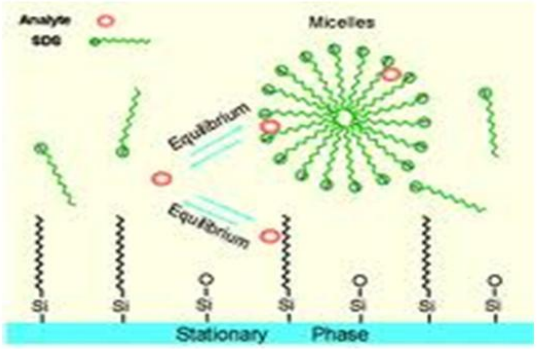

Fig.4: Distribution of analyte between stationary phase and mobile phase

Modified Stationary Phase

- **Column:** The alkyl-bonded C18 is the stationary phase most widely used in MLC, but other columns can be selected (e.g., C8 and cyanopropyls).

- **Surfactant Adsorption:** Alkyl-bonded phase columns are strongly modified when SDS, CTAB, or Brij-35 is incorporated into the mobile phase. Surfactant adsorption on the porous RPLC packing affects drastically the chromatographic retention, owing to the change of diverse surface properties of the stationary phase (e.g., polarity, structure, pore volume, and surface area). Surfactant molecules coat the stationary phase pores, reducing appreciably their volume.

  Ionic compounds are frequently added to micellar mobile phases for pH buffering and, eventually, ionic strength adjustment. Salt addition may change the amount of adsorbed ionic surfactant due to the reduction of both electrostatic repulsion and surfactant CMC, and the enhancement of hydrophobic interactions[11].

- **Presence of an Organic Solvent in the Mobile Phase:** Organic solvents are added to micellar mobile phases to improve peak efficiencies and reduce retention times, giving rise to the so-called hybrid micellar mobile phases. Competition between alcohols and surfactant molecules for adsorption sites on the stationary phase explains the linear reduction in the amount of adsorbed surfactant with increasing concentration of alcohol in the mobile phase. Mobile phases rich in organic solvent can sweep completely the adsorbed surfactant molecules from the bonded phase.

- **Use of large-pore stationary phases:** The apparent lack of strength of pure micellar mobile phases (i.e. without organic solvent) has been attributed to the exclusion of micelles from the pores, within which >99% of the stationary phase resides. Since the excluded micelles do not have direct access to the solutes associated to the stationary phase (except when these have diffused out of the pores)[12], even high concentrations of micelles are not sufficient to elute moderately to highly hydrophobic compounds.

  It should be noted that as the pore size of a porous material is increased, the specific surface area is reduced. As a consequence, the volume of bonded stationary phase is also decreased. Therefore, under equal mobile phase conditions, the retention of a solute in a large-pore column will necessarily be smaller than on an otherwise identical small-pore column. For this reason, in order to get a valid conclusion, the authors compared the behaviour of solutes of diverse nature with hydro-organic and micellar mobile phases, and found that the large-pore columns really allow better penetration of the micelles into the pores, such that they are able to reach the solutes at the internal surface of the stationary phase better, and elute them in less time.

- **Suppression of silanol activity:** The surface of porous silica is covered with silanol groups (Si–OH), which are ionised in slightly acidic and basic media, being responsible for the polarity of silica. Less polar pickings' are obtained by derivatisation of the silanol groups, although some remain underivatised. The ionic interaction of positively charged species with free silanols is the main reason of the reduced efficiency and peak tailing in the separation of basic compounds with conventional C8 and C18 columns. This effect can be prevented by reducing the pH of the mobile phase to suppress silanol ionisation.

Solute micelle and solute–stationary phase interactions:

The unique capabilities of micellar mobile phases are attributed to the ability of micelles to selectively compartmentalize and organize solutes at the molecular level. Solutes are separated on the basis of their differential partitioning between bulk solvent and micelles in the mobile phase or surfactant-coated stationary phase. For water-insoluble species, partitioning can also occur via direct transfer of solutes between the micellar pseudophase and the modified stationary phase. The partitioning equilibria in MLC can be described by three coefficients: PWS (partition between aqueous solvent and stationary phase), PWM (between aqueous solvent and micelles), and PMS (between micelles and stationary phase). The coefficients PWS and PWM account for the solute affinity to the stationary phase and micelles, respectively, and have opposite effects on solute retention: as PWS increases the retention increases, whereas as PWM increases the retention is reduced due to the stronger association to micelles.

- **Nature of the interactions:** The retention behaviour depends on the interactions established by the solute with the surfactant-modified stationary phase and micelles. Neutral solutes eluted with non-ionic and ionic surfactants, and charged solutes eluted with non-ionic surfactants will only be affected by non-polar, dipole–dipole and proton donor–acceptor interactions[13]. Besides these interactions, charged solutes will interact electrostatically with ionic surfactants (i.e. with the charged surfactant layer on the stationary phase and the charged outer layer of micelles). With ionic surfactants, two situations are possible according to the charges of solute and surfactant: repulsion or attraction (by both surfactant-modified stationary phase and micelles). In the case of electrostatic repulsion, charged solutes cannot be retained by the stationary phase and elute at the dead volume, unless significant hydrophobic interaction with the modified bonded layer exists. In contrast, combined electrostatic attraction and hydrophobic interactions with the modified stationary phase may give rise to strong retention in MLC. Mixtures of polar and non-polar solutes can be resolved, provided an appropriate surfactant is chosen.

- **Binding, non-binding and anti-binding solutes:** The function of the micellar pseudophase in MLC has been compared to that of the organic modifier in conventional RPLC, since for most solutes an increasing surfactant concentration in the mobile phase results in decreasing retention. This behaviour contrasts to that in IPC, where the addition of an ionic surfactant increases the retention of solutes owing to electrostatic attraction to the modified stationary phase. However, it should be noted that in MLC, the elution strength increases with surfactant concentration only if the solute interacts with micelles.

  According to their elution behaviour with a micellar mobile phase, solutes were classified into three categories: solutes binding to micelles, and non-binding and anti-binding solutes14. Solutes that associate or bind to micelles show decreased retention when the concentration of micelles in the mobile phase is increased. For solutes that do not associate

with micelles, the retention may remain unaltered by changing the micelle contents (non-binding), or increase with increasing micelle concentration (anti-binding).The most frequent behaviour is binding to micelles, while antibinding is quite uncommon.

Description of the retention behavior in pure micellar mobile phases:

Several theoretical approaches have been proposed to describe the retention of binding solutes as a function of the "micellar" concentration ([M], which should be understood as the concentration of monomers of surfactant forming micelles, calculated as the total concentration of surfactant minus the CMC):

1. The partitioning model of Armstrong and Nome

2. The equilibrium approaches of Arunyanart and Cline-Love

3. Foley.
   The proposed models allow measuring the strength of solute–micelle and solute–stationary phase interactions.

Efficiency:

The main limitation in the use of MLC is the reduction in efficiency (peak broadening) that is observed when purely aqueous micellar mobile phases are used[14.] Several explanations for the poor efficiency have been theorized. Poor wetting of the stationary phase by the micellar aqueous mobile phase, slow mass transfer between the micelles and the stationary phase, and poor mass transfer within the stationary phase have all been postulated as possible causes. To enhance efficiency, the most common approaches have been the addition of small amounts of isopropyl alcohol and increase in temperature. A review by Berthod studied the combined theories presented above and applied the Knox equation to independently determine the cause of the reduced efficiency. The Knox equation is commonly used in HPLC to describe the different contributions to overall band broadening of a solute. The Knox equation is expressed as16:

$$h = An^{1/3} + B/n + Cn$$

Where:

h = the reduced plate height count (plate height/stationary phase particle diameter)

n = the reduced mobile phase linear velocity (velocity times stationary phase particle diameter/solute diffusion coefficient in the mobile phase)

A, B, and C are constants related to solute flow anisotropy (eddy diffusion), molecular longitudinal diffusion, and mass transfer properties respectively.

Berthod's use of the Knox equation to experimentally determine which of the proposed theories was most correct led him to the following conclusions. The flow anisotropy in micellar phase seems to be much greater than in traditional hydro-organic mobile phases of similar viscosity. This is likely due to the partial clogging of the stationary phase pores by adsorbed surfactant molecules. Raising the column temperature served to both decrease viscosity of the mobile phase and the amount of adsorbed surfactant. Both results reduce the A term and the amount of eddy diffusion, and thereby increase efficiency[15].

The increase in the B term, as related to longitudinal diffusion, is associated with the decrease in the solute diffusion coefficient in the mobile phase, DM, due to the presence of the micelles, and an increase in the capacity factor, k¢. Again, this is related to surfactant adsorption on the stationary phase causing a dramatic decrease in the solute diffusion coefficient in the stationary phase, DS. Again an increase in temperature, now coupled with an addition of alcohol to the mobile phase, drastically decreases the amount of the absorbed surfactant. In turn, both actions reduce the C term caused by a slow mass transfer from the stationary phase to the mobile phase. Further optimization of efficiency can be gained by reducing the flow rate to one closely matched to that derived from the Knox equation. Overall, the three proposed theories seemed to have contributing effects of the poor efficiency observed, and can be partially countered by the addition of organic modifiers, particularly alcohol, and increasing the column temperature.

Applications:

1. Micelles have an ability to solubilize proteins which enables MLC to be useful in analyzing untreated biological fluids such as plasma, serum, and urine.

2. Martinez et al. found MLC to be highly useful in analyzing a class of drugs called b-antagonists, so called beta-blockers, in urine samples.

3. Another application compared reversed phase HPLC with MLC for the analysis of desferrioxamine in serum. Desferrioxamine (DFO) is a commonly used drug for removal of excess iron in patients with chronic and acute levels. The analysis of DFO along with its chelated complexes, Fe (III) DFO and Al (III) DFO has proven to be difficult at best in previous attempts.

4. Analysis of pharmaceuticals by MLC is also gaining popularity. The selectivity and peak shape of MLC over commonly used ion-pair chromatography is much enhanced. MLC mimics, yet enhances, the selectivity offered by ion-pairing reagents for the separation of active ingredients in pharmaceutical

drugs. For basic drugs, MLC improves the excessive peak tailing frequently observed in ion-pairing.

5. Hydrophilic drugs are often unretained using conventional HPLC, are retained by MLC due to solubilization into the micelles. Commonly found drugs in cold medications such as acetaminophen, L-ascorbic acid, phenylpropanolamine HCL, tipepidine hibenzate, and chlorpheniramine maleate have been successfully separated with good peak shape using MLC.

6. Additional basic drugs like many narcotics, such as codeine and morphine, have also been successfully separated using MLC.

7. Another novel application of MLC involves the separation and analysis of inorganic compounds, mostly simple ions.

Conclusion

Since the technique was first reported on in 1980, micellar liquid chromatography has been used in hundreds of applications. This micelle controlled technique provides for unique opportunities for solving complicated separation problems. Despite the poor efficiency of MLC, it has been successfully used in many applications. The use of MLC in the future appears to be extremely advantages in the areas of physiological fluids, pharmaceuticals, and even inorganic ions. The technique has proven to be superior over ion-pairing and ion-exchange for many applications. As new approaches are developed to combat the poor efficiency of MLC, its application is sure to spread and gain more acceptances.

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