International Journal of Chemistry Studies
ISSN: 2581-348X
Impact Factor: RJIF 5.44
www.chemistryjournal.in
Volume 2; Issue 4; July 2018; Page No. 19-24

Micellar liquid chromatography: Review

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Abstract
Micellar liquid chromatography (MLC) as a separation science technique remains hindered by reduced chromatographic efficiency compared to reversed phase liquid chromatography using hydro-organic mobile phases. The reduced efficiency is linked to the adsorption of surfactant monomers onto the stationary phase, resulting in a slow mass transfer of the analyte within the interfacial region of the mobile phase and stationary phase. The effect of various bonded stationary phases and silica pore sizes on efficiency in MLC was evaluated using an array of twelve liquid chromatography columns, including large-pore short alkyl chain, non-porous, superficially porous, and fluorinated stationary phases. The effect of organic micellar mobile phase additives was also evaluated using combinations of 1-propanol, 1-butanol, 1-pentanol, and triethylamine. A simplified equation for calculation of A′ and C′ terms from reduced plate height (h) versus reduced velocity (ν) plots was developed to compare efficiency data obtained with different columns and mobile phases. Surfactant adsorption isotherms were measured for five columns with three Micellar mobile phases to further understand the relationship between adsorbed surfactant, mobile phase additive, and column efficiency. Clear improvements in efficiency were observed with addition of 2% (v/v) triethylamine to 1-butanol modified aqueous micellar mobile phase in combination with the use of short alkyl chain, wide-pore silica columns, specifically, Nucleosil C4, 1000Å pore size. This finding is supported by lower amounts of surfactant adsorbed onto the stationary phase when triethylamine is present in the mobile phase compared to surfactant only, or 1-butanol modified mobile phase. In a separate series of experiments, elevated column temperatures were evaluated to determine the effect of temperature on efficiency. Efficiency improvements from 9% to 58% were observed for different columns over the temperature range of 40 to 70ºC. Finally, a quantitative method of direct injection of equine serum for detection of banned non-steroidal anti-inflammatory drugs in equestrian events was developed to take advantage of the observed enhancements in efficiency in the area of greatest benefit for MLC, the direct injection of physiological fluids.

Keywords: micellar liquid chromatography (MLC), surfactants and micelles, surfactant interactions with the stationary phase, surfactant adsorption, classification of models in MLC, optimization, applications

Introduction
Micellar liquid chromatography (MLC) is one of the many areas of liquid chromatography, which evolved from the studies of organized solutions. The solutions of surfactants above the critical micelle concentration (CMC) belong to most extensively studied organized solutions were used as mobile phases in MLC. The same organized solutions are being studied now as mobile phases in micellar electro kinetic chromatography. The starting point of MLC was pioneering by works of Armstrong more than 25 years ago [1]. The evolution of MLC is reflected in over 500 articles and reviews. The importance of MLC is confirmed by occurrence of the book "Micellar Liquid Chromatography" published by Berthod and Garcia-Alvarez-Coque [2], chapter in "Encyclopedia of Separation Science" [3] and a volume in "Comprehensive Analytical Chemistry" edited by Pramauro and Pellizzetti [4]. Theory and application of MLC is being developed by scientific groups in Spain, France, USA, Japan, Georgia, China, India, Pakistan, Iran etc. MLC today is extensive field of investigation that comprehended the problems of analytical chemistry, pharmacy and medicine, food and agricultural chemistry, chemo metrics and physicochemical studies. Several excellent reviews have appeared during the development of theory and practice of MLC [5, 7–19].

Most of them have been published in special issue of Journal of Chromatography A (1997, Vol. 780) that was aimed to collect most important achievements in micelle-mediated separation techniques [12–14, 18, 19]. Good review about all aspects of MLC has been published by Basova *et al.* [8] in Russian.

Surfactants and Micelles
As its name suggests, the liquid mobile phases used in MLC are solutions of surfactants at concentrations where micelles are formed. The unique nature of MLC is due to the use of the aqueous surfactant solutions. Surfactants belong to the class of compounds known as amphiphiles, or molecules having both a hydrophobic and hydrophilic component [20]. The hydrophobic component is generally referred to as the tail group and hydrophilic group is known as the head. The term surfactant comes from a contraction of "surface active agent" and is defined as a material which when present at low concentrations, adsorb onto the interface, or surface, of the system and thereby alters the interfacial free energies of the interface [21]. The concept of micelles in solution was

developed by James William McBain and coworkers at the University of Bristol in Bristol, England in the early twentieth century. In 1912, McBain developed a theory of "colloidal ions" in solution to explain the conductive properties of sodium palmitate solutions [22] and continued the argument for the existence of ionic micelles in soap in 1919 and 1920 [23, 24]. The "colloidal ions" later became known as micelles after a term borrowed from biology and popularized by G. S. Hartley in his book "Aqueous Solutions of Paraffin-Chain Salts, A Study in Micelle Formation" in 1937 [25]. Surfactants are generally classified by the charge of the hydrophobic head group: anionic, cationic, nonionic, and amphoteric or zwitterionic. The most commonly used household and industrial surfactants are anionic. The anionic surfactant dissociates in aqueous solutions to give a negatively charged surface active portion and an inactive cation, commonly Na+ or K+. The four main families of anionic surfactants are soaps, sulfonated compounds, alkyl sulfates and alkyl phosphates. In MLC, the most commonly used anionic surfactant is the alkylsulfate, sodium dodecyl sulfate (SDS), C12H25OSO3Na. Cationic surfactants are those that dissociate into a cationic amphiphile and an inactive anion, commonly Cl- or Br-. The most common cationic surfactants used in MLC contain a quaternary ammonium group, such as cetyl trimethylammonium bromide (CTAB), C16H33N(CH3)3 +, Br-. Non-ionic surfactants have a polar group such as an ether, alcohol, carbonyl or amino group as their hydrophilic portion. Zwitterionic surfactants contain both positive and negative charges on the same molecule.

The term "interface" refers to a boundary between two immiscible phases. The practically observed interfaces are: solid-vapor, solid-liquid, solid-solid, liquid-vapor, and liquid-liquid. The main impact of surfactants in modification of the interface is found when one of the phases is liquid, usually water [26]. The interfacial free energy is the minimum amount of work required to create that interface, and is measured per unit area by determination of the interfacial (surface) tension. A surfactant adsorbs to the surface at low concentrations, thereby changing, usually by lowering, the amount of work required per unit area required to expand the interface. Taking the most common air-water interface as an example, when an amphiphilic surfactant is dissolved in the bulk aqueous phase, the hydrophobic sections of the surfactant distort the structure of the phase by breaking the hydrogen bonds between the water molecules, causing an increase in the free energy of the solution. In order to lower the free energy, the surfactant is forced to the surface with the hydrophobic tail section oriented toward the air and the hydrophilic head group inside the water phase. This configuration creates a concentrated surfactant monolayer on the surface and lowering of the surface tension [21, 27].

Surfactant Interactions with the Stationary Phase

Liquid chromatography columns are almost always made with a silica based packing bonded with an R group to create the specific column chemistry desired. The silica particles are typically derivatized with chloro-silanes containing R groups that react with the surface hydroxyl groups (Si-OH) called silanols. After a given R group has been bonded to the silica surface, there remain unreacted, or residuals silanols. The residual silanols can have a pKa ranging from 4 up to 9.8 depending on the silica used, but will typically be negatively charged at the pH of most mobile phases. They also contribute to peak tailing of basic compounds due to interactions of the basic compounds with the silanols, and provide potential interaction sites for positively charged surfactant monomers, or repulsion of negatively charged surfactants. The silica particle size, surface area, porosity, and bonding density make up the most important physiochemical properties of HPLC columns. The particle size is a measured average diameter of the distribution of particles. Particle size has a large effect on chromatographic efficiency, as the particle size decreases, the efficiency typically increases. One drawback to the use of smaller particles is the effect on pressure within the HPLC column. As the particle size is halved, the observed pressure for the same flow rate is quadrupled.

The highly porous silica used in HPLC packing creates a large surface area that is equal to the sum of the internal pores of the silica particle and the outer surface, where the internal pores account for greater than 99% of the total surface area. The pores within a particle can be thought of as multi-branched cylindrical channels either partially or completely penetrating the particle. The pore size refers to the average diameter of the pores, usually expressed in angstrom, Å. Typical pore sizes used in most HPLC applications range from 70 – 150 Å, however many "wide-pore" stationary phases are commercially available ranging up to 4000 Å. Bonding density is described by the surface coverage in μmol/m2 of bonded R groups to silanols. A lower bonding density means that more residual silanols remain with additional contributions to peak tailing and surfactant adsorption.

Surfactant Adsorption

Surfactant interactions with the stationary phase have been extensively studied and determined that surfactant monomers tend to adsorb onto the stationary phase. Cyanopropyl, C18 and C8 derivatized silica stationary phases exposed to surfactant cetyltrimethylammonium bromide (CTAB) and SDS were studied by NMR by Levine and co-workers [28, 29]. They found that the hydrophobic alkyl tail of SDS was associated with the bonded layer of the C8 and C18 stationary phases, while the sulphate polar group of SDS protrudes out of the bonded layer. Conversely, the polar head group of CTAB incorporated within the bonded phase, with the tail group oriented outward [28].

For the cyano bonded phase, both the CTAB and SDS surfactants became tightly bound and entwined within the bonded phase through electrostatic interactions [29]. The adsorption of surfactants to the stationary phase will obviously have an effect on its properties, particularly the charge density of the surface and the interfacial tension of the stationary phase/mobile phase interface.

Mobile and stationary phase peculiarities in MLC

The same stationary phases and equipment are used in MLC and reversed-phase high performance liquid chromatography (RP-HPLC). However the separation conditions in MLC and RP-HPLC are quite differ. A main peculiarity of the MLC eluent is its micro heterogeneity, as the surfactant monomers are in dynamic equilibrium with self-assembled surfactant

microaggregates (micelles). The state of stationary phase in MLC is also peculiar in comparison with RP-HPLC, even if the same sorbents are used. Stationary phase is dynamically modified, because sorbent adsorb surfactant monomers and organic modifier molecules during the contact with micellar eluent. Adsorption may be accompanied by self-association of surfactants into surface microaggregates such as ordered layers, hemimicelles or admicelles. The more detail description of mobile and stationary phase peculiarities in MLC can be found in our previous review. Some new ideas on investigation of C18 stationary phase modified by SDS have been published recently by Yakovleva and Loginova [30]

Advantages of micellar eluents

Several advantages of micellar eluents in comparison with classical aqueous-organic eluents are presented below: 1) the possibility of simultaneous separation of charged and uncharged solutes; 2) direct injection of physiological fluids due to the capability of some micellar solutions (anionic or nonionic) to solubilize the protein matrix of samples; 3) compatibility of mobile phases with salts and water-insoluble compounds; 4) unique separation selectivity that is due to microheterogeneity of micellar eluents and dynamic modification of stationary phase; 4) robustness of results that is caused by stabilization of surfactant monomer concentration in the presence of micelles; 5) rapid gradient capability (shorter equilibration times); 6) enhanced luminescent detection that is due to the solubilization of solutes; 7) low cost of micellar eluents; 8) safety versus expensive and flammable solvents of chromatographic grade. [31-40]

Retention modeling

The dependence of solute retention factor on eluent composition is of interest in RP-HPLC as well as in MLC for several reasons. Firstly, the retention models are widely used for optimization of separation by different interpretive methods. Secondly, retention data are used to estimate octanol-water partition coefficients and biological activity by retention-structure, retention activity relationships. Thirdly, the study of retention for a wide range of solutes and separation conditions might provide insight into the fundamental basis of retention in different chromatographic modes.

Classification of models in micellar liquid chromatography

The three main groups of retention models can be differentiated: (i) conceptual retention models; (ii) mechanistic retention models; (iii) empirical retention models.

The conceptual retention models have the physicochemical background and can be derived by using main definitions of chromatographic retention and expressions that describe the processes in chromatographic column etc. The parameters of such models have clear physicochemical sense and some of them can be verified by independent experiments and other analytical methods. As a consequence, the conceptual retention models give the basis for more detail investigations of real processes in the column during the chromatographic separation. The mechanistic retention models include the parameters which have the physicochemical sense and even can be verified by non-chromatographic experiments, but their derivation is based on the several empirical limitations and/or assumptions about the processes in chromatographic column. The empirical retention models are used in interpretive optimization strategies. The aim of application of empirical retention models is an accurate fitting of retention of compounds by using minimal number of parameters. However, sometimes these equations can give the basis for derivation of physicochemical or mechanistic retention models with same functional dependences between variables.

Optimization of separation

The main goal of every practicing chromatographer is the resolution of all solutes of interest in a given probe. There are a large number of factors that affect separation in HPLC (e.g. column dimensions, packing characteristics, eluent composition, temperature, kind of buffer, pH, nature of solute-solvent interactions etc.). This, on the one hand, gives to analyst the unlimited resources for analytical methods developing, but, on the other hand, this means tremendous experimental work, more costs and delay in method development, because, at present, liquid chromatographic theory does not permit precise a priori prediction of how a separation will vary when the operating conditions are being changed. In spite of the fact, that trial-and-error method leads to non optimal separation conditions, needs considerable expenses of time and is very tedious, many of analysts that work in the field of chromatography are somewhat critical about application of chemometrics in chromatography. However, the complication of mixture composition compels to use mathematical evaluation of the data for method development and understanding of the obtained results.

Comparing of optimization strategies in RP-HPLC and MLC

Most approaches in RP-HPLC with respect to a systematic improvement of the isocratic separation of more complex mixtures are based on the following steps: first, the required elution strength is determined, preferably by means of gradient. Then, the selectivity of the mobile phase is changed, e.g. by changing the type of organic modifier, keeping the overall elution strength constant [41]. Strasters et al. [41] demonstrated that such sequential optimization is not feasible in MLC and that the complex retention behaviour necessitates a combined examination of the influence of all involved parameters.

In MLC the contribution of hydrophobic and electrostatic interactions to retention would not be the same for different compounds and is a function of their structural properties. Due to different types of interactions and the competing equilibria in MLC, one can expect any form of the selectivity behaviour (i.e. peak convergence, divergence, and crossover). However, for a large group of compounds (especially non-ionics), partition into the micelles and into the dynamically modified alkyl-bonded phase is directly related. Thus, it should be noted that the frequently observed phenomenon of elution order reversal in MLC because of a change in micelle concentration does not necessarily translate into a systematic enhancement [42-43]. It was previously observed, that for compounds with large water-micelle partition coefficients (e.g. hydrophobic compounds) the overall selectivity value is small and its

variation with micelle concentration is limited.

Software packages available for optimization of separation

Some of computer-assisted optimization methodologies are implemented in software packages that are commercially available. Most known programs for optimization in RP-HPLC are PREOPT-W [44–47]; DryLAB [48–50]; OSIRIS [51]; MICHROM Chrom Sword [52] among these programs, only MICHROM has been written for optimization of separation in MLC.

Applications of MLC

Micellar liquid chromatography continues to be used as a tool for the analytical chemist for a variety of applications, including the direct injection of serum and other physiological fluids, the modeling of physiological partitioning processes, and the analysis of pharmaceutical compounds. One of the most advantageous applications is the ability to directly inject physiological fluids. Micelles have an ability to solubilize proteins which enables MLC to be useful in analyzing untreated biological fluids such as plasma, serum, and urine. The main advantage of the use of MLC with these types of samples, is the great time savings in sample preparation. Alternative methods of analysis including reversed phase HPLC require lengthy extraction and sample work up procedures before analysis can begin. The majority of the published applications in MLC deal with direct injection of physiological fluids. In one example, Martinez *et al.* [53] found direct injection MLC to be highly useful in analyzing nine β-antagonist drugs, or beta-blockers, in urine samples with less than fifteen minute runtime.

Analysis of pharmaceuticals by MLC is also a common application. The selectivity and peak shape of MLC compared to commonly used ion-pair chromatography is much enhanced [54]. MLC mimics, yet enhances, the selectivity offered by ionpairing reagents for the separation of active ingredients in pharmaceutical drugs. For basic drugs, MLC decreases the excessive peak tailing frequently observed in ion pairing. Hydrophilic drugs, often unretained using conventional RP-HPLC, are retained using MLC due to ionic interactions with charged surfactant molecules that are adsorbed onto the stationary phase. 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 [54]. Other classes of basic drugs such as β-blockers [55, 56], phenethylamines [57], tetracyclines [58], and tricyclic antidepressants [59] have also been successfully separated using MLC.

The intent of the research presented in the following chapters is to provide continuous improvement for the applications listed above, and for those yet to come. By continuing to make advancements in micellar liquid chromatography, the perceived limitations may be overcome enough to broaden its scope to a wider audience of chromatographers and applications.

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