Basic concepts and recent advances biology essay

Science, Biology

Abstract:

Micellar liquid chromatography is the one of the very important analytical technique. It contains mobile phase added with surfactants above its critical micellar concentration and the stationary phase is modified with surfactant monomers. So, micelles alter the solubilising capability of the mobile phase. This forms diverse interactions with major implications in retention and selectivity. It is alternative to conventional reversed phase liquid

chromatography. It allows direct injection of physiological fluids, analysis of pharmaceutical compounds, physiological partitioning processes. MLC has proved time saving technique as compared to other analytical methods like HPLC and ion-pairing. So the popularity of MLC is increasing day by day. This article contains some concepts of surfactants and micelles like micelle formation, factors affecting micelle formation. We also focused on the particularities of micellar mobile phase, stationary phase, partition coefficient and retention behavior. Key words: MLC, micelles, surfactants, partition coefficient and retention behavior

1] Introduction

Micellar liquid chromatography is the alternative to conventional reversed phase liquid chromatography in which surfactants are added to mobile phase above its critical micellar concentration. It confers analytical procedures with greater accuracy and a lower cost. MLC is versatile in nature because of the wide variety of interactions that are established among the eluted solutes, the stationary phase, the aqueous phase and micelles. MLC allows analysis of wide variety of compounds. There are many benefits of addition of surfactant in mobile phase. Some of them are modification of the interactions established inside the column, reduction of the necessary amount of organic solvent in the mobile phase, which can be recycled due to low evaporation. MLC is a type of reversed-phase liquid chromatographic (RPLC) systems, in which stationary phase is a non-polar and mobile phase is a polar aqueous. However in conventional RPLC hydro-organic mobile phases are homogeneous, but in MLC micellar solutions are heterogeneous, being composed of two distinct media: the amphiphilic micellar aggregates

(micellar pseudophase) and the surrounding bulk water or aqueous-organic solvent that contains surfactant monomers in a concentration approximately equal to the CMC. On the other hand, surfactant monomers are adsorbed on the stationary phase, and thus creating a structure similar to an open micelle, reducing silanophilic interactions. The nonionic surfactants causes change only in the polarity of the stationary phase. But, ionic surfactants, a net charge (positive or negative) appears on its surface with major implications. Recently a new micellar chromatographic mode has been described: high submicellar chromatography [1, 2, 3] in which the organic solvent content is high and surfactant forms micelles. This mode complements low submicellar chromatography and incresases number of applications of this chromatographic technique. Fundamental studies into MLC have not only served to develop the technique but also establish it’s theoretical basis which enables its use in diverse applications.

2] Basic concepts of surfactants and micelles

I: mat2761-f1. jpgThe mobile phase in MLC contains surfactants which form micelles. Surfactants are known as amphiphiles, because they have both a hydrophobic and hydrophilic nature [4, 5]. The hydrophobic nature is given by the tail of the molecule whereas the hydrophilic nature is provided by the head. The surfactant is the surface active agent. Surfactants are classified into various classes viz. anionic, cationic, nonionic, and amphoteric or zwitterionic. This classification is according to the charge of the hydrophobic head group. The anionic surfactant is that which dissociates in aqueous solutions and give a negatively charged surface active portion and an inactive cation, commonly Na+ or K+ where the cationic surfactant

dissociate in an aqueous solution and gives positively charged surface active portion and negatively charged inactive anion. The most commonly used anionic and cationic surfactants in MLC are the SDS and CTAB respectively. Nonionic surfactants contain polar group like an ether, carbonyl, amino group or alcohol as hydrophilic portion. Zwitter ionic surfactants have both positive and negative charges on the same molecule [6]. Interface is the boundary which separates two immiscible phases. Surfactants influence most when one of the interphase is water [7]. The interfacial free energy is the minimum amount of work required to that interface, and is measured per unit area by determination of the interfacial (surface) tension. A surfactant by adsorbing and by lowering, the amount of work required per unit area required to expand the interface. For example, air-water interface when an 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 a lowering of the surface tension [8, 9].

3] Micelle Formation

As there is increase in the concentration of surfactant in solution, there is saturation at interphase with monomers. So, it is required to decrease the free energy and surface tension. The physiological properties of solution change suddenly at CMC. The concept of CMC was determined by Bury and

coworkers in 1929 -30 [10, 11] as well as Hartley in 1936 [12]. CMC i. e. critical micelles concentration is the point at which micelles start to form. As soon as the micellar aggregates form, the surface tension and free energy is lowered. In solution there is dynamic equilibrium between micelles and monomers. The value of CMC is different for every surfactant. Because the aggregation number i. e. number of monomers that form micelle is different. In aqueous solution the hydrophobic tails of micelles face outward whereas the hydrophilic heads face inward. At concentration very near to the CMC, mostly the micelles formed in spherical shape. In case of ionic surfactant as the concentration increases, other shapes of micelles also can be formed in the sequence of spherical, cylindrical, hexagonal, and lamellar . The thermodynamics of micelle formation is usually thought in terms of a phase, or pseudo-phase model approach. Micelles are considered as a pseudo-phase because of their dynamic nature. During micellization, there is not only rapid interchange of surfactant monomers in a given micelle, but also formation and breakup of the micellar aggregates constantly itself. The rate of exchange of individual surfactant in a given micelle is at the order of 10-8 to 10-4 seconds. The micellar aggregate has a typical lifetime on the order of 10-3 to 1second [13, 14]. There is electrostatic repulsion of the polar heads and the attraction between the alkyl chains in ionic surfactants. During micellization interactions included are1) The surfactant hydrophobic part with water2) The surfactant hydrophobic interactions with each other in the micelle core3) The surfactant hydrophilic head groups with each other4) The hydrophilic head group solvation in water. Based on these interactions, two simple models are generally used to express the mechanism of micelle

formation: AN. S↔ SAN ……. ……..(1) andAN. S↔ mS + SAN ……. (2)whereAN = The aggregation number, or number of monomers of a surfactant Sm = The number of free surfactant monomers in solutionSAN = The micelle comprised of the surfactant S. An equilibrium between surfactaSnt monomers and micelles is assumed, giving an equilibrium constant for both equations of:…..(3)ΔG° i. e. the standard free energy, of micelle formation can be expressed as:…….(4)whereR is the gas constant and T is temperature in degrees Kelvin. From equation 4, it can be derived: ……(5)The first term on the right side of equation may be neglected when there is sufficiently large AN, usually larger than 50, which leads to the final approximation at the CMC of :……(6)Equation (7) will give a negative value ΔG and hence it is proved that formation of micelle is aspontaneous process. ΔS°i. e. The change in entropy can be expressed as:…..(7)As the temperature increases, the value of CMC also increases. So ΔS is generally positive, and so spontaneous. The nonpolar surfactant tails face to nonpolar interior of the micelle which gives higher freedom of motion to the nonpolar hydrocarbon chains within the micelle structure as opposed to the bulk solution [15]. So the entropy of the water molecules is increased and this is the most important reason behind this spontaneous process. It is very hard to ensure the value of CMC for a given surfactant. The CMC values for various surfactants have been determined by a variety of techniques after the study for many decades. Many physiochemical changes like surface tension, conductivity, osmotic pressure, dye absorbance and turbidity observed at the point of micelle formation, which make the measurement of the CMC for given surfactants relatively easy. Surface tension measurement

is very common and easy way to determine CMC values. Because the surface tension suddenly decreases when micelles start to form spontaneously at the CMC. This allows the determination of the CMC. Mukerjee and Mysels in 1971 and 10 Van Os, Haak, and Rupert in 1993 [16], compiled CMC values for surfactants at various conditions [17] with an similarly large number of values. A selection of CMC and AN values for surfactants commonly used in MLC is shown in following table:

Type

Name

CMC

AN

Anioniccholic acid sodium salt142-4deoxy cholic acid sodium salt52-10glycol alcoholic sodium salt132sodium dodecyl sulphate8. 2762taurocholic acid, sodium salt10-154sodium tetra decyl sulphate2. 1CationicCetyl trimethyl ammonium chloride178Cetyl trimethyl ammonium bromide1. 3Dodecyl trimethyl ammonium bromide1450Zwitter ionic3-[(3-Cholamidopropyl)Dimethylammonium]-1-Propanesulphonate(Chaps)3-[(3-Cholamidopropyl)Dimethylammonium]-2-Hydroxy-1-Propanesulphonate(Chapso)N-Dodecyl-N, N-Dimethylammonio-3-Propane Sulphonate8108113. 3Non ionicN-Dexyl-B-DglucopyranosideTrition X-100Polyoxyethylene(23)Dodecanol(BRIJ35)Polyoxyethylene(20)-Sorbitane Monooleate(TWEEN80)Polyoxyethylene(20)-Sorbitane Monooleate(TWEEN80)2. 20. 241400. 10. 010. 059

4] Factots affecting micelle formation

Many factors affect the process of micelle formation and the CMC of a given surfactant. These factors include the hydrophobic and hydrophilic groups, counter ion effects from ionic surfactants, electrolytic effects from added salts, effects of added organic solvents, and temperature. One of the very important affecting factor is hydrocarbon chain length of the surfactant on CMC values. Hydrophobicity is proportional to the hydrocarbon chain length. Therefore as hydrocarbon chain length increases CMC decreases. Klevins in 1953 developed the equation for calculating CMC of a homologous series, which may be given as [18]:……8where A is the constant specific of a homologous series and temperature, whereas B is the constant approximately equal to log 2, or B ≅ 0. 30, and n is the number of carbon atoms in the chain [8, 15]. The CMC values also depend on ionic or nonionic nature of hydrophilic head group of surfactant monomer. A nonionic head group decreases the CMC whereas ionic part increases. In case of ionic surfactants, the degree of interaction is affected by the counter-ion of the bulk solvent. The CMC decreases with increasing ion binding. The ion binding is directly proportional to the polarizability and charge of counter ion, and inversely proportional to hydrated radius. Thus, the decreasing order of CMC of any given surfactant will becomeLi+ > Na+ > K+ > Cs+ > N(CH3)4 + > N(CH2CH3)4 + > Ca2+ ≅ Mg2+ . The CMC value of cationic surfactants like dodecyltrimethylammonium halides decreases when charge no of counterion increases. So the decreasing order becomes: F- > Cl- > Br- > I-. So, an increase in charge from ±1 to ± 2 or ± 3 the CMC decreases sharply [19]. The CMC for most surfactants especially ionic also decreases when

electrolytic salts are added. Because the addition of salts causes the reduction in the electrostatic repulsion of the polar heads. Corrin described the relationship between salt concentration and CMC which is given by equation [20]: Log CMC = -a log Ci + b ……(9)where a and b represents the constants of given surfactant at a particular temperature whereas Ci is the total concentration of the added salt counter ion. The effect of addition of electrolytes is negligible for nonionic zwitter ionic surfactants as compared to ionic surfactants [21, 22]. In MLC short chain alcohols are usually added. This decreases the CMC of given surfactant. A little quantity of organic solvent is added often to a micellar mobile phase which improve efficacy and resolution of compound. But, micelles may be dispersed if very high concentration of the organic solvent is used, since it depends upon hydrophobic effects for its formation. Generally the micelle size, CMC and aggregation number of ionic surfactants are decreased by the addition of alcohols . The effects of addition of alcohols on various properties of micelles have extensively studied by Zana et al[23]. Alcohols partially solubilise in micelle and produce mixed surfactant and alcohol micelle. It produces most of the desire effects. Alcohols reduce the electrostatic repulsion between head groups by orienting themselves between surfactant chains. So micellisation becomes easy and cmc is also reduced [24]. But, this effect will be observed upto 20% of alcohols only. Above that cmc is increased. At greater concentrations, ~20% or above, of methanol and ethanol, an increase in CMC is observed. The effect of addition of propanol at 23% in potassium dodecanoate is completely disruption of micelles. Organic additives, like urea and dioxane, cause a little increase the CMC after

addition [25-31]. One very important factor affecting the formation of micelle is temperature. This effect is different for ionic and nonionic surfactants. In case of ionic surfactants, as temperature increases the solubility of surfactants also increases due to formation of micelle. Krafft point is the lowest temperature at which micelles start to form at a minimum surfactant concentration. As the alkyl chain length of surfactant increases the Krafft point will also increased. The Krafft point will get changed with changes in counter ion. As temperature increases, the degree of hydration of the polar head group decreases and equilibrium shifts to micelle formation. On the other hand, interaction between water molecules get reduced due to reduced hydration and reduction in the free energy which shifts the equilibrium away from micelle formation. The Krafft point is not applicable to nonionic surfactants. In its place, there is a cloud point. It is the temperature at which the nonionic micellar solution becomes turbid. It also becomes biphasic as the temperature rises. The cloud point decreases with increase in concentration increases [6, 7].

5] Micellar Mobile Phase

For ionic surfactants micelles provide two site of interaction viz. hydrophobic and electrostatic. The micelles have three sites of solubilisation -the core which is hydrophobic in nature, the surface which is hydrophilic in nature, and the palisade layer i. e. the region between the surfactant head groups and the core. In the micelles there is a microenvironment which is different from that of bulk solvent and solutes associated with these micelles experience this environment [32]. Mostly separations in MLC are carried out with hybrid micellar mobile phases which is a buffered medium that contains

micelles, molecules of organic solvent, surfactant monomers and water. The organic solvent reduces the polarity of the aqueous solution and alters the micelle structure. The separation mode is predominantly micellar in nature, which is perturbed by the organic solvent. So there may be change in micellar parameters, such as the CMC and surfactant aggregation number. The type of organic solvent and surfactant decides maximal allowable concentration of organic solvent. Because high percentage of organic solvent can also disrupt the micelle structure.

5. 1] pH of the Mobile Phase.

The packing materials for MLC is same as classical RPLC, having a limited working pH range of 2. 5–7. 5. Appropriate pH values depend on the nature of the analytes and the surfactant selected. Phosphoric or Citric acid buffers are used to fix the pH of the micellar mobile phase [33, 34]. Potassium salts are not recommended for mobile phases containing SDS as potassium dodecyl sulphate because it presents a high Krafft point which results into precipitation from aqueous solutions at room temperature [33].

5. 2] Organic Solvents:

Types and Concentration.

The polarities of the analytes decide which organic solvent modifier should be selected for MLC. For polar analytes, 1-propanol, 2-propanol, or acetonitrile are used for sufficiently short retention times (below 20 min), whereas for nonpolar compounds stronger solvents as 1-butanol or 1-pentanol are needed. These organic solvents can also used for compounds with high affinity for the surfactant adsorbed on the stationary phase [34]. It

should be noted that the two latter alcohols give rise to micro emulsion formation at sufficiently high concentration [35]. The lower organic solvent consumption reduces cost and toxicity, which helps for " green chemistry". Organic solvent in the micellar media decreases evaporation of micellar mobile phases and thus it can be preserved in the laboratory for a long time without significant changes in their composition. However, In practice, the amount of organic solvent that can be added is limited by its solubility. High organic solvent concentration causes the disaggregation of micelles and the only free surfactant molecules are remained in mobile phase. The organic solvent contents that preserve the integrity of micelles are below 15% for propanol and acetonitrile, 10% for butanol, and 6% for pentanol [36]. These values are quite less as compared with those needed in classical RPLC.

6] Modified Stationary Phase

6. 1] Surfactant Adsorption. The most widely used stationary phase in MLC is alkyl-bonded C18, but other columns like C8 or cyanopropyl may be selected. SDS, CTAB, Brij -35 these surfactants very strongly modify alkyl-bonded phase when incorporated into the mobile phase. They adsorb on the porous RPLC packing and drastically affect the chromatographic retention, which leads to change in various surface properties of the stationary phase like polarity, structure, pore volume, and surface area. Surfactant molecules reduce the volume of stationary phase pores too [37]. The addition of ionic compounds in micellar mobile phase causes not only pH buffering but also gives ionic strength. Salt addition changes the amount of adsorbed ionic surfactant because of the reduction in both electrostatic repulsion and surfactant CMC, and the enhancement of hydrophobic interactions [38].

Surfactants coat the bonded-stationary phase. And thus full similar coating would make the stationary phases all similar. According to solid-state NMR studies of SDS, the hydrophobic tail of SDS associated to C18 alkyl-chain bonded to the silica stationary phase whereas the sulphate head group oriented away from the surface [39]. This creates a negatively charged hydrophilic layer affecting the penetration depth of solutes into the bonded phase. In the case of CTAB with alkyl-bonded phases, surfactant adsorption leads to a more hydrophobic stationary phase because the cationic trimethylammonium head group is partially incorporated into the bonded phase, associated to free silanols . In contrast, on cyano-bonded phases, both charged surfactants (SDS and CTAB) are adsorbed head down with their tails projected outwards, creating thus pseudo-alkyl bonded phases . The smaller surface charge of the modified cyano-bonded phases is responsible for the more important role of solute–micelle interactions for charged solutes.

6. 2] Use of large-pore stationary phases

Absence of organic solvent in mobile phase causes elimination of micelles from the pores, in which > 99% of the stationary phase resides [40]. 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) [41], even high concentrations of micelles are not sufficient to elute moderately to highly hydrophobic compounds. In the case of non-ionic surfactants that form large micelles, steric effects are the most likely reason for micellar exclusion from small-pore materials. With ionic surfactants (such as SDS and CTAB) that form smaller charged micelles, both electrostatic and

steric effects are probably responsible for micellar exclusion: the resulting charge on the stationary phase surface within the pores gives rise to a Donnan-like potential that tends to repel like species from the pores. In order to determine whether large-pore stationary phases may overcome the lack of strength in MLC, several C8 and C18 stationary phases ranging from100 to 4000Å pore size were investigated with micellar mobile phases of non-ionic (Brij-22), anionic (SDS) and cationic (dodecyl trimethylammonium bromide i. e. DTAB) surfactants [40]. 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.

7] Interactions of MLC :

There are two types of interaction between surfactant monomer and stationary phase.[42, 43]:(a) Hydrophobic interaction: In which the alkyl tail of the surfactant monomer is in contact with the nonpolar ligand of the stationary phase and the ionic head group of surfactant monomer directed away from it. In this way, the stationary phase got some ion exchange ability with charged solutes (Fig. 1 (a;(b) Silanophilic interaction: Where the ionic

head group of the surfactant would be adsorbed on stationary phase. Thus the stationary phase becomes more hydrophobic. (Fig. 1 (b». There is also a possibility of competition between surfactant and solute for stationary phase. (45) So, MLC is more complex than conventional RPlC with hydro - organic giving rise to no of interactions like electrostatic, hydrophobic and sterlc(?). Micelles provide hydrophobic as well as electrostatic sites for interactions with solutes [44], this leads MLC to determine almost any compound [43].

8] Partition coefficient

In micellar liquid chromatography the solute get partitioned between stationary phase, bulk mobile phase, and micelle. So, three different partition coefficients exist for solute. First partition coefficient is between stationary phase and bulk mobile phase which is represented as Psw. The second equally important partition coefficient of solute is between bulk mobile phase and micelle, represented as Pmw. And the Third solute partition coefficient is stationary phase and micelle, represented as Psm. (45)Armstrong and Nome [45] first time developed the partition equation in MLC by using the three-phase model i. e. bulk aqueous, micellar pseudo-phase stationary phase which accounts for uncharged solutes in RPLC. The equation can be written as:.....(10)Where Ve = retention volume of the solute , Vs= The volume of the stationary phase, V = The partial specific volume of the surfactant in the micelle, and V m= The volume of the mobile phase,[M] = The micellized surfactant concentration, i. e., (total surfactant concentration minus the CMC) in moles per liter; and P sw is the partition coefficients of the solute between the stationary phase and water, and Pmw is partition coefficient of solute between the micelle and water respectively. The term Psw is obtained

by plotting Vsj(Ve - V m) versus [M]. The term Vsj(Ve - V m) can be measured whereas value of [M] is known. Psw the partition coefficients of the solute between the stationary phase and water can be determined by the intercept and the ratio of the slope over intercept provides the value of PMW i. e. the partition coefficient of solute between the micelle and water can be calculated from when v is provided. But this obtained value for PMW is for per monomer surfactant. This value is then multiplied by aggregation no to obtain true value of PMW whereas the quantity PSM is obtained from the j of the other two partition coefficients:

9] Retention mechanism

This article contents various theoretical approaches to describe the retention behavior of binding solute viz. Armstrong and Nome [45], and the equilibrium approaches of Arunyanart and Cline-Love [46], and Foley [47]. 9. 1] Armstrong and Nome modelThis model include transitions among 3 environments i. e. water, micelles and stationary phase in micellar chromatographic system. In above equation Ve represents the total volume of mobile phase needed to elute a given solute from the column(1) Where, Vs is the volume of the active surface on the stationary phase, V0 is the column void volume, ϕ is= Vs/V0 i. e. phase ratio and the partial specific volume of monomers of surfactant in the micelle is represented by v [44] and it is required to calculate the value of Pmw.

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9. 2] Arunyanart and Cline-Love model

K

In this equation KAS is the association equilibria of solute [S] in bulk aqueous solvent (A) and stationary phase binding sites (S), whereas KAM is the association equilibria of solute in bulk aqueous solvent (A) and with monomers of surfactant in the micelle (M) [46].

9. 3] Foley model

Foley put forward the idea of treating the retention factor the retention factor.[47]This model resembles previous two models as the retention factor of free solute (k0) has coincide with PWS in Armstrong and Nome model model and KAS in Arunyanart and Cline-Love model whereas KAM coincide with KAM in Arunyanart and Cline-Love. 10] Applications

(Thomas david)

Micellar liquid chromatography is used for various applications. It includes direct injection of serum and other physiological fluids, the analysis of pharmaceutical compounds. It also has major role in physiological partitioning processes [48-57]. MLC has proved time saving as compared to other analytical methods like HPLC and ion-pairing. Because it does not require sample extraction or any other sample preparation. The peak shapes obtained are superior too [58]. The MLC reduces the excessive peak tailing of basic drugs which is very common in ion pairing whereas hydrophilic drugs are retained which are unretained in HPLC. Another very important application of MLC is the analysis of physiological samples. Micelles solubilize proteins which make possible MLC to analyze urine, serum, and plasma [59]. Martinez et al. analyze nine beta-blockers, in urine samples within fifteen

minute runtime by directly injecting the urine sample in MLC [60]. MLC also succeeded in separating β-blockers [61, 62], phenethylamines[63], tetracyclines [64], and tricyclic antidepressants [65] . The scope of MLC is gradually getting widen.

11] Recent advances :

Brij-35, a non ionic surfactant is studied for its selectivity in separation of positional isomers by N. Memon et al. in 2012. There is different way of interaction of non ionic MLC as compared with ionic or hydro-organic MLC. LSER i. e. Linear solvation energy relationship is also evaluated by studying the effect of concentration of surfactant and organic solvent on the separation of some selected isomers. Additionly dipolarizability, excess molar refraction and basicity such parameters are responsible for separation. Non-ionic MLC has shown new way for separation of many positional isomers [66]. A. U. Kulikov developed and validated a MLC method for the analysis of sesquiterpenic acids separated from root and rhizome of Valeriana officinalis and valerian dry hydroalcoholic extract without using gradient elution technique which is very common in hplc . This is very important advantage of MLC which allows separation of compounds having different hydrophobicity in a single run without the gradient elution. This is very useful application of MLC [67]. M.-L. Chin-Chen determined the biogenic amine spermine in anchovy sauce after derivatizing it with 3, 5-dinitrobenzoyl chloride. Direct injection of samples (after filtration) was used avoiding any tedious extraction and purification step. This is another interesting advantage of the MLC technique [68].