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Micellar liquid chromatography

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Abstract. Background and possibilities of practical applications of micellar liquid chromatography (MLC) are considered. Various retention models in MLC, the effects of the nature and concentration of surfactants and organic modifiers, pH, temperature and ionic strength on the MLC efficiency and selectivity are discussed. The advantages and limitations of MLC are demonstrated. The performance of MLC is critically evaluated in relationship to the reversed-phase HPLC and ion-pair chromatography. The potential of application of MLC for the analysis of pharmaceuticals including that in biological fluids and separation of inorganic anions, transition metal cations, metal chelates and heteropoly compounds is described. The bibliography includes 146 references.

I. Introduction

Studies of secondary chemical equilibria (acid – base, complexation, formation of ion pairs and solubilisation) in reversed-phase high-performance liquid chromatography (RP HPLC) have been gaining momentum in recent years since their consideration leads to a higher selectivity of separation. The application of surfactant solutions at concentrations above the critical micellar concentration (CMC) as mobile phases in RP HPLC has given birth to micellar liquid chromatography (MLC), which is an alternative to the classical RP HPLC with aqueous-organic eluents.[1–4]

Micellar liquid chromatography offers a number of advantages compared to other chromatographic methods. First, owing to the dual nature of surfactants possessing both hydrophobic and hydrophilic properties, it is possible to carry out simultaneous separation of a mixture of ionic and non-ionic sorbates. Second, owing to the ability of micelles to increase solubility of uncharged sorbates in water, they can act as organic modifiers; it is noteworthy that micellar eluents are inexpensive and non-toxic. Third, it is possible to influence effectively the selectivity of MLC because it depends on a large number of parameters: the nature of surfactants (*viz*., their types and charges) and concentrations, additives of various organic modifiers, ionic strength (salt additives) and pH of the mobile phase and the nature of the stationary phase. Fourth, biological fluids can be injected directly into a chromatographic column without preliminary precipitation of proteins, because both anionic and non-ionic surfactants are capable of solubilising proteins. This simplifies preparation of samples and accelerates analysis of various medicinal preparations. Fifth, the sensitivity of determination is increased since the intensity of luminescence, low-temperature phosphorescence and absorption are enhanced for many substances in the presence of surfactants. Sixth, the use of micellar gradients makes it possible to carry out fast separations because there is no need for column conditioning upon replacement of the mobile phase as the concentration of the free surfactant in solution is virtually constant after equilibration of the column with the surfactant at concentrations above CMC. Seventh, many authors have demonstrated the existence of a correlation between the retention and partition coefficients in the octanol – water system and biological activity. In this context, MLC may be useful for estimating hydrophobicity of sorbates and predicting biological activity.

A substantial drawback of this method is lesser efficiency of separation compared to that in classical RP HPLC. Two ways have been proposed to overcome this drawback: to use the so-called hybrid eluents, *i.e*., mobile phases with addition of small amounts of organic modifiers (usually, short-chain alcohols), or to raise the working temperature. The addition of alcohols to the micellar mobile phases leads not only to enhancement of the efficiency of separation, but also to changes in selectivity and to a faster analysis. Therefore, special attention has been paid in recent years to the MLC techniques using hybrid eluents.

Despite the fact that this method has been developing for only two decades (the first paper appeared in 1980[5]), several reviews[2,6–9] devoted to the discussion of basic theoretical problems of MLC and to the most recent achievements[7] have been published. However, in the Russian-language literature none of the numerous monographs or reviews devoted to HPLC make any mention of this method (except for Ref. 1 where only the notion of MLC is given).

The aim of this review is to fill this gap. The review generalises the retention models in MLC described in the relevant literature, discusses causes of low efficiency of separation and ways of its increase and the possibilities of modifying the selectivity of separation and gives examples of the use of MLC for the analysis

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and determination of inorganic, medicinal and biologically active compounds.

II. Retention models for micellar liquid chromatography

Micellar liquid chromatography is a variant of partition chromatography where the sorbate molecules are partitioned between three phases: stationary phase, micellar and non-micellar (aqueous) mobile phases. In MLC, three equilibria are taken into consideration: sorbate partition between the micelle and the aqueous phase characterised by the partition coefficient P_{MW}, sorbate partition between the micelle and the stationary phase with the partition coefficient P_{SM} and sorbate partition between the stationary phase and the mobile aqueous phase with the partition coefficient P_{SW}. Several equations have been proposed to relate the retention of sorbates in MLC to the concentration of micelles.[10–12] Armstrong and Nome [10] express this dependence as

$$\frac{V_s}{V_e - V_0} = \frac{V(P_{MW} - 1)}{P_{SW}}[M] + \frac{1}{P_{SW}}, \quad (1)$$

where V_s, V_e and V_0 are the volume of stationary phase, the volume of sorbate elution and the void volume of the column, respectively; V is the molar volume of the surfactant, [M] is the concentration of the surfactant in form of micelles in solution, which is calculated as $[M] = c - c_{cr}$, where c is the total surfactant concentration in solution and c_{cr} is the critical micelle concentration. The dependence $V_s/(V_e - V_0)$ on [M] should be linear, whereas the value of $V(P_{MW} - 1)$ is equal to the constant of sorbate association with the micelles [9] and may be obtained experimentally from the slope of the straight line and the magnitude of the intercept of this line on the ordinate.

Arunyanart and Cline Love [11] have proposed the equation relating the basic chromatographic parameter — capacity factor (k') — to the concentration of surfactant micelles.

$$\frac{1}{k'} = \frac{K_2}{\phi[L_s]K_1}[M] + \frac{1}{\phi[L_s]K_1}, \quad (2)$$

where K_2 is the constant of sorbate association with the micelle, ϕ is the phase ratio, $[L_s]$ is the ligand concentration in the stationary phase, and K_1 is the binding (adsorption) constant of sorbate from the mobile aqueous phase to the stationary phase.

In this case, the dependence of $1/k'$ on [M] should be linear, while the constants of sorbate–micelle association can also be easily derived from experimental data. Equations (1) and (2) are therefore equivalent.[13]

Proceeding from the general model of secondary equilibria in chromatography,[14,15] Foley [12] has deduced the equation

$$k' = \left(\frac{K_{SM}[M]}{1 + K_{SM}[M]}\right)k'_{SM} + \left(\frac{1}{1 + K_{SM}[M]}\right)k'_S, \quad (3)$$

where K_{SM} is the constant of sorbate–micelle association, k'_S and k'_{SM} are the capacity factors of the sorbate and the sorbate–micelle complex.

Transformation of Eqn (2) with consideration of the fact that the sorbate–micelle complex is not retained, since the micelle is a component of the mobile phase, gives the equations

$$k' = \left(\frac{1}{1 + K_{SM}[M]}\right)k'_S \quad (4)$$

or

$$\frac{1}{k'} = \frac{K_{SM}}{k'_S}[M] + \frac{1}{k'_S}. \quad (5)$$

Equation (5) is equivalent to Eqns (1) and (2).

It should be noted that the constant of sorbate–micelle association derived from any equation — (1), (2) or (5) — is called the constant of association per monomer. As the concentration of micelles is expressed by the equation

$$[M] = (c - c_{cr})/n, \quad (6)$$

where n is the aggregation number, the multiplication of the constant K_2/K_{SM} or $V(P_{MW} - 1)$ by n gives the constant of association per micelle.

In the studies of the retention mechanism of hydrophobic, poorly water-soluble alkylbenzenes in MLC with non-ionogenic surfactants polyoxyethylene [10]- or -[23]-lauryl ethers (Brij-22 or Brij-35, respectively), Borgerding [16] has modernised Eqn (1) and proposed a model of limited solubility. Assuming that $P_{MW} - 1 \approx P_{MW}$ and denoting the coefficient of sorbate partition between the stationary and mobile phase as $P_{SM} = P_{SW}/P_{MW}$, the equation

$$\frac{1}{k'} = \frac{1}{\phi}\left(\frac{V[M]}{P_{SM}}\right) + \frac{1}{\phi P_{SW}}, \quad (7)$$

was obtained, which in the extreme case (insolubility in water) is transformed into the equation

$$k' = P_{SM}\left(\frac{\phi}{V[M]}\right). \quad (8)$$

Analysis of Eqns (1) and (2) shows that as the concentration of micelles in the mobile phase increases the retention decreases ($1/k'$ increases), *i.e.*, the eluting strength increases with the concentration of micelles.

Equations (1) and (2) were both used for the determination of the constants of sorbate association with different surfactants in purely micellar as well as in hybrid eluents.[10,11,17–20] In both cases, straight lines with good correlation coefficients were obtained;[18] the values of association constants calculated by the two methods are also in good agreement.

It was noted that in the presence of the cationic surfactant cetyltrimethylammonium bromide the constants of association of a number of benzene and naphthalene derivatives with micelles are larger than those in the presence of the anionic surfactant sodium dodecyl sulfate,[18] which can be explained by the preferred electrostatic interactions between the positively charged head groups of cetyltrimethylammonium bromide micelles and delocalised electrons of the aromatic ring of sorbates. However, still higher values of the constant of sorbate–micelle association were obtained in the case of the non-ionogenic surfactant Brij-35, which is difficult to explain if one considers solely the hydrophobic interactions.

As it follows from the basic equilibria taken into consideration in the deduction of Eqns (1) and (2), the values of P_{MW} and K_2 depend only on the nature of the sorbate and the micellar system used and are independent of the nature of the stationary phase.[17] It should be noted that the error in the determination of dissociation constants also depends on the nature of the sorbate and is increased with its higher hydrophobicity [the P_{SW} values are large and the $1/P_{SW}$ value in Eqn (1) is small].

The addition of organic modifiers to a micellar system leads to changes in its characteristics (CMC and the aggregation number),[21,22] which should in turn result in the modification of sorbate–micelle interactions and, consequently, in changes in the retention.[21,22] The sorbate–micelle interactions are as a rule decreased in a medium modified by alcohols.[9] Indeed, the constants of sorbate–micelle association calculated for a number of benzene and naphthalene derivatives are larger for sodium dodecyl sulfate and cetyltrimethylammonium bromide in purely aqueous micellar solutions than in solutions containing 5 vol.% or 10 vol.% of n-butanol as the modifier, which is related, according to Garcia *et al.*,[20] to the competition between the sorbate and modifier molecules for the interaction with the

micelles. Addition of salts (*e.g.*, NaCl) to the system enhances the interaction between the sorbate and the sodium dodecyl sulfate micelle; in this case, the values of association constants are even higher than those in the purely micellar mobile phases.[20]

Thus, the ability of micelles for selective solubilisation of the sorbate or interaction with it is the basis of separation in MLC using grafted alkyl stationary phases. It is known that the molecules of monomeric surfactants are easily adsorbed on such stationary phases thereby modifying their properties and making a definite contribution to the retention and selectivity in MLC. However, the equilibrium adsorption of surfactants on the stationary phase was not taken into account in the deduction of Eqns (1), (2) and (5).

Hu *et al.*[23,24] have given an explanation of the mechanism of chiral recognition in the separation of enantiomers of 2,2′-dihydroxy-1,1′-dinaphthyl- and -1,1′-dinaphthyl-2,2′-diyl hydrophosphates by MLC with the use of different salts of bile acids (*e.g.*, sodium cholate) in the mobile phases. A three-phase system proved to be ineffective for this purpose. A new model of the heterogeneous stationary phase has been proposed: the ODS (octadecyl sulfate) stationary phase adsorbs micelles, and it is into these micelles that the analytes are distributed. Based on the existence of the following equilibria in the system

$$\begin{aligned}
&A_m + L_s \overset{K_1}{\rightleftharpoons} AL_s\,,\\
&A_m + M_m \overset{K_2}{\rightleftharpoons} AM_m\,,\\
&AM_m + L_s \overset{K_3}{\rightleftharpoons} AL_s + M_m\,,\\
&A_m + M_s \overset{K_4}{\rightleftharpoons} AM_s\,,\\
&AL_s + M_s \overset{K_5}{\rightleftharpoons} AM_s + L_s\,,\\
&AM_m + M_s \overset{K_6}{\rightleftharpoons} AM_s + M_m\,,
\end{aligned} \tag{9}$$

where A, M and L are the sorbate, the micelle and the solvated stationary phase, respectively, whereas the subscripts 'm' and 's' refer to the mobile and stationary phases, respectively, the following equation was deduced

$$\frac{1}{k'} = \frac{V_m}{K_3K_4V^L[L_s] + K_4V_s^M[M_s]} + \\ + \frac{V_m^M[M_m]}{K_3V^L[L_s] + V_s^M[M_s]}\,, \tag{10}$$

where V^L, V_s^M, V_m and V_m^M are the volumes of solvated stationary phase, micelles adsorbed on the stationary phase, micelles of solvent and micelles in the mobile phase, repectively; $[M] = c - c_{cr}$.

The retention mechanism depends on the concentration of a co-solvent (acetonitrile) in the mobile phase: at low concentrations of acetonitrile, its partition between the micelles in the mobile and stationary phases is dominant. At higher acetonitrile concentrations, the mechanism of chiral recognition is determined by the partition between the bulk of mobile phase solution and the micelles adsorbed on the stationary phase. However, consideration of the equilibrium of micelle adsorption on the stationary phase did not result in the modification of the general relationship between the retention and the concentration of micellised surfactant: as in the case of Eqns (1) and (2), the dependence of $1/k'$ on [M] is linear.

In the deduction of equations, neutral organic compounds, mainly aromatic hydrocarbons, were used as model sorbates. The distribution of a sorbate in a micelle is determined by the nature of the micelle and the balance of hydrophilic and hydrophobic properties of the sorbate. The micelle has a lamellar structure. In aqueous solution, it consists of a hydrocarbon nucleus, a water–hydrocarbon layer the thickness of which is usually 2 or 3 CH_2 groups and corresponds to the depth of penetration of water molecules inside the hydrocarbon part of the micelle or, conversely, of the hydrocarbon chains inside the aqueous phase (here, the hydrophilic–hydrophobic balance is established), a layer of hydrated polar groups and a heterogeneous layer of solution (the diffusive part of the double electrical layer in the case of ionic micelles) adjoining the micelle.[22] Since the micelle is regarded as the macroscopically homogeneous medium, it cannot be indicated unequivocally where the sorbate is localised. As the existence of a correlation between the capacity factors and partition coefficients in the octanol–water system [8,9] has been demonstrated for many neutral organic compounds, it is believed that the basic interactions are hydrophobic; in this case, the sorbate is either in the hydrophobic hydrocarbon nucleus or in the layer nearest to it. In the course of partition of charged organic sorbates, in addition to hydrophobic interactions, electrostatic interactions should also be observed, including those with the stationary phase surface modified due to the adsorption of monomeric surfactants.

The retention of monoprotonic,[25–28] diprotonic and zwitterionic (amino acids and peptides) [26–28] compounds in MLC have been systematically studied. The retention of monoprotonic compounds is described [26] by the equation

$$k' = \frac{\phi L_s(K_s^{HA} + K_s^A - K_a/[H^+])}{1 + K_m^{HA}[M] + (1 + K_m^A - [M]K_a/[H^+])}\,, \tag{11}$$

where K_s^{HA}, K_s^A, K_m^{HA} and K_m^A are the binding constants of a weak acid HA and the conjugate base A with the stationary phase ligands L_s and the micelle M, K_a is the constant of HA dissociation in aqueous solution, [M] is the concentration of micelles calculated as the stoichiometric concentration of surfactant minus CMC. Equation (11) allows prediction of the retention at any concentration of micelles and protons.

For the diprotonic sorbates in the mobile phase, the following equilibrium is established:

$$HABH^+ \overset{K_{a1}}{\rightleftharpoons} H^+ + {}^-ABH^+ \overset{K_{a2}}{\rightleftharpoons} 2H^+ + {}^-AB\,.$$

Each particle interacts with a micelle with the equilibrium constants K_{mc}, K_{mz} and K_{ma}, respectively, and with the stationary phase with the equilibrium constants K_{sc}, K_{sz} and K_{sa}, respectively. The following equation was proposed for calculating the capacity factor

$$k' = \left[\frac{k'_c(1 + K_{mc}[M]) + k'_z(1 + K_{mz}[M])K_{a1}}{[H^+]} + \right. \\ \left. + \frac{k'_a(1 + K_{ma}[M])K_{a1}K_{a2}}{[H^+]^2}\right] \Bigg/ \\ \Bigg/ \left[\frac{1 + K_{mc}[M] + (1 + K_{mz}[M])K_{a1}}{[H^+]} + \frac{(1 + K_{ma}[M])K_{a1}K_{a2}}{[H^+]^2}\right]\,, \tag{12}$$

where k'_c, k'_z and k'_a are the capacity factors of the ions $HABH^+$, ${}^-ABH^+$ and ${}^-AB$, respectively; K_{ma} is the apparent dissociation constant in a micellar solution. Equations (11) and (12) are useful for studying the dependence of selectivity on pH of the mobile phase. They were deduced assuming the occurrence of only hydrophobic interactions of various particles (charged, uncharged, zwitterionic) with the micelle and the mobile phase with the consideration of secondary acid–base equilibria in the mobile phase.[26] Sorbate–micelle interactions in micellar chromatography of zwitterions were studied by Zibas.[27] Carmelo [28] used the example of cephalosporin to study the retention mechanism in the micellar liquid chromatography of zwitterions.

Since the hydrophobicity of inorganic anions is usually low and the main interactions in such systems are electrostatic, an ion-exchange model [29] was proposed for explaining the mechanism of separation of transition metal cations in MLC. In this model, ionic micelles (and the stationary phase coated with sodium dodecyl sulfate) are regarded as ion exchangers; retention is determined by the concentrations of micelles and counter-ions. For the ion exchange equilibrium

$$2\,Na_m^+ + M_s^{2+} \overset{K_{ie}}{\rightleftharpoons} 2\,Na_s^+ + M_m^{2+}$$

(Na^+ ions are counter-ions in the sodium dodecyl sulfate micelle, whereas the subscripts 'm' and 's' refer to the phases of micelles and solution, respectively; K_{ie} is constant), the following equation was obtained

$$\log K_{MW}^{app} = \log([Na_m^+]^2 K_{ie}) - 2\log[Na_s^+], \quad (13)$$

where K_{MW}^{app} is the apparent coefficient of sorbate partition between the aqueous and micellar phases. The value of K_{MW} was calculated using Eqn (11) proposed earlier by Okada [30, 31] for the retention in the exclusion MLC and simplified assuming that the inner volume of the ODS stationary phase is negligibly small

$$\frac{1}{V_r - V_0} = \frac{(K_{MW} - 1)vc_m + 1}{K_{SW}V_s}, \quad (14)$$

where V_r, V_0 and V_s are the volume of sorbate retention (elution), the void volume of the column and the stationary phase volume, respectively; v is the partial molar volume of micelle; c_m is the concentration of micelles equal, as in all the cases discussed above, to the surfactant concentration minus CMC; K_{MW} and K_{SW} are the coefficients of sorbate partition from the aqueous phase into the micelle and the stationary phase, respectively. Equation (14) is analogous to Eqn (1).

In order to minimise partition of sorbates into the stationary phase, it is recommended to supplement the eluent with salts and also with tartaric acid, which is a complexation agent.[32] If the retention fits the ion-exchange model, the dependence of $\log K_{MW}^{app}$ on $\log [Na_s^+]$ should be linear, and this was confirmed for all metals, the slope of the straight line being equal to 2.

If complexation of metal ions with tartrate ions in the mobile phase (the complexes ML with the stability constant β_L and MHL^+ with the stability constant β_{HL}) is taken into consideration, Eqn (13) is transformed into (15) (K_2 is the constant of tartaric acid dissociation).

$$\frac{1}{K_{MW}^{app}} = [Na_s^+]^2\left(1 + \beta_{HL}[HL^-] + \frac{\beta_L[L^{2-}]}{K_{ie}[Na_m^+]^2}\right) = [Na_s^+]^2\left[\frac{1 + (\beta_{HL}[H^+]/K_2 + \beta_L)[L^{2-}]}{K_{ie}[Na_m^+]^2}\right] \quad (15)$$

In this case too, a linear dependence of $\log K_{MW}^{app}$ on $\log [Na_s^+]$ was obtained for all metal ions, which implies that complexation does not influence the partition of sorbate into the micellar phase. This is understandable since in the presence of micelles the ionisation constants of weak acids (pK_a) increase,[21] which leads to a lower concentration of the reactive ligand L^{2-} in the system. Thus for tartaric acid at pH 4.35, 50% of L^{2-} is present in the aqueous solution, whereas in the presence of sodium dodecyl sulfate its content is as low as 4%.[29]

A mechanism based on the stoichiometric ion-exchange model for anions in the system with a cationic surfactant was proposed by Okada and Shimizu.[33] Based on the existence of equilibria of the analyte A and the counter-ion C

$$A_{aq}^- + C_s^- \overset{K_{ies}}{\rightleftharpoons} A_s^- + C_{aq}^-,$$

$$A_{aq}^- + C_m^- \overset{K_{iem}}{\rightleftharpoons} A_m^- + C_{aq}^-,$$

$$Surf\,C_m \overset{K_d}{\rightleftharpoons} Surf_m^+ + C_{aq}^-,$$

where the subscripts 'aq', 's' and 'm' refer to the aqueous solution, the stationary phase and the micellar phase, respectively; K_d is the equilibrium constant of the counter-ion transition from the micelle to the aqueous phase; K_{ies} and K_{iem} are the constants of ion-exchange equilibrium between the solution – resin and solution – micelle systems, the following equations were deduced

$$\frac{\phi}{k'} = \frac{[C_{aq}^-]}{K_{ies}[C_s^-]}\left(1 + \frac{K_{iem}c_m}{[C_{aq}^-] + K_d}\right), \quad (16)$$

$$\frac{n_s^c}{(V_r - V_0)[C_{aq}^-]} = \frac{1}{K_{ies}}\left(1 + \frac{K_{iem}c_m}{[C_{aq}^-]}\right), \quad (17)$$

where n_s^c is the number of counter-ion moles in the stationary phase, which is equal to the number of moles of hexadecyltrimethylammonium bromide adsorbed on the stationary phase; c_m is the concentration of micelles; V_r and V_0 are the volume of analyte retention and the void volume of the column, respectively. The concentration of the counter-ion in the aqueous phase $[C_{aq}^-]$ is determined as the sum of concentrations of the added counter-ion C^- (due to dissociation of micelles) and of the counter-ions of monomeric surfactants ($c - c_{cr}$). The retention of ions in ionic micellar chromatography can be described by the stoichiometric ion-exchange model, and it formally fits the pseudophase model where the concentration of the added counter-ion is sufficiently high; in this case, the concentration of the counter-ion formed upon dissociation of micelles may be neglected.

The model of micellar exclusion chromatography [30, 31] is based on Eqn (1) and takes into account that the micelles of cetyltrimethylammonium and dodecyltrimethylammonium chlorides [30] or sodium dodecyl sulfate can penetrate partly into the stationary phase (external solution). Micelles do not penetrate into the inner part of the stationary phase, but the latter contains surfactant monomers (internal solution) and ions are retained there owing to ionic interactions. In this case, the retention is described by the equation

$$\frac{1}{V_r - V_e} = \frac{(K_{MW} - 1)Vc_m + 1}{V_i + V_sK_{SW}}, \quad (18)$$

where V_r, V_e, V_i and V_s are the volumes of sorbate retention, external solution, inner solution and stationary phase, respectively; K_{MW} is the coefficient of sorbate partition (ratio of concentrations) between the micellar phase and the internal solution phase; K_{SW} is the coefficient of sorbate partition (ratio of concentrations) between the stationary phase and the internal solution phase; V is the molar volume of surfactant; c_m is the concentration of micelles in the eluent. The dependence of $1/(V_r - V_e)$ on c_m is linear for both anions (IO_3^-, I^-, Br^-, NO_3^-, NO_2^-) [30] and cations (Pb^{2+}, Zn^{2+}, Co^{2+}).[31]

Thus, there are two basic models for describing MLC, *viz.*, Eqns (1) and (2); they describe adequately the dependence of sorbate retention on the concentration of micellised surfactant and are based on three equilibria: sorbate partition between the micellar mobile phase and the aqueous mobile phase, between the micellar mobile phase and the stationary phase and between the stationary phase and the aqueous mobile phase. The major types of interactions are hydrophobic in the case of organic uncharged and charged sorbates and electrostatic for transition metal cations. The stationary phase is always modified due to the adsorption of monomeric surfactant molecules; this effect, however, was taken into account only qualitatively. In many cases, irreversible surfactant adsorption on various commercial silica gels with grafted C_{18} groups was observed.[34–36] As shown in the example of cetyltrimethylammonium bromide, the amount of surfactant sorbed depends irreversibly on the sorbent character-

istics, the type of eluent and temperature.[36] In the case of sodium dodecyl sulfate, 6% of this compound initially adsorbed on the C_{14} stationary phase is sorbed irreversibly.[35] On the whole, irreversible adsorption is more probable for monomeric stationary phases with a high density of grafted ligands and surfactants with a small polar head group (when both the surfactant and the grafted ligand have the alkyl chain length of more than 8 carbon atoms).[35] In the early stages of MLC studies it was believed that the surfactant adsorption is arrested after reaching CMC. In 1989, Borgerding *et al.*,[37] showed that more than 58% of the total amount of sodium dodecyl sulfate is adsorbed on Resolve C_{18} when its concentration in the mobile phase varies in the range of 2 to 70 CMC. For Brij-22 and Brij-35, more than 50% of the total amount of adsorbed surfactant is sorbed on Resolve C_{18} from solutions with the initial concentration > 50 CMC. Thus, the adsorption of both non-ionogenic surfactant and anionic surfactant continues after attaining CMC.

In their recent study, Hu and Haddad[38] tried to take into account the surfactant adsorption on the stationary phase surface. They also used a three-phase model. The stationary phase consisted of a surfactant adsorbed on an ODS sorbent. The sorbate localised in the extramicellar mobile phase A_m can bind to the surfactant on the stationary phase S_s in the form of an AS_s complex in conformity with the equation

$$A_m + S_s \overset{K_1}{\rightleftharpoons} AS_s ,$$

and to the surfactant in the mobile phase S_m in the form of a complex AS_m in accordance with the equation

$$A_m + S_m \overset{K_2}{\rightleftharpoons} AS_{sm} .$$

Direct transfer to the stationary phase of the sorbate bound to the surfactant in the mobile phase AS_m can also take place by the equation

$$AS_m + S_s \overset{K_3}{\rightleftharpoons} AS_s + S_m.$$

The capacity factor can be expressed by the equation

$$k' = \frac{K_1 V_s^S [S_s]}{V_m + K_2 V_m^S [S_m]} , \qquad (19)$$

where V_s^S, V_m and V_m^S are the volumes of surfactant adsorbed on the stationary phase bulk, of the solvent and of surfactant in the mobile phase, respectively.

It follows from Eqn (19) that

$$\frac{1}{k'} = \frac{V_0'}{K_1 W_s} + \frac{K_2 V_m^S [S_m]}{K_1 W_s} , \qquad (20)$$

where $W_s = V_s^S[S_s]$ is the mass of surfactant adsorbed on the stationary phase; $V_m = V_m^S - V_0$. The dependence of $1/k'$ on $[S_m]$ (determined as the total surfactant concentration in the mobile phase) should be linear. The content of sodium dodecyl sulfate on the stationary phase was determined (16.5%) and found to be almost equal to the carbon : silicon ratio in the initial sorbent (17.1%). In other words, the octadecyl groups of the sorbent are covered to a large extent with the adsorbed surfactant, and the stationary phase may be regarded as the adsorbed surfactant. The dependence of $1/k'$ on $[S_s]$ is linear for the model sorbents studied, *viz.*, benzyltriethylammonium chloride and phenylalanine. It should be noted that Eqn (20) is analogous to Eqn (2), the only difference consisting in that Eqn (2) includes the surfactant concentration in the form of micelles in solution (minus CMC), while Eqn (20) contains the overall surfactant concentration in solution.

Consideration of some other equilibria (acid – base equilibria in the mobile phase or adsorption of micelles on the stationary phase) does not modify the dependence of retention on the concentration of micelles. Electrostatic interactions (ion exchange between metal cations and the cations of the diffusive part of the double electrical layer) are taken into consideration in the MLC model in which retention is dependent on both the concentration of micelles and the concentration of counter-ions.

III. Efficiency of micellar liquid chromatography

One of the major drawbacks of the MLC method is its low efficiency compared to that of RP HPLC. There are several explanations of this phenomenon in the relevant literature. One of these[39] suggests that the main factor of the band blurring is the resistance to the sorbate mass transfer because of the poor wetting of the stationary phase. Yarmchuk *et al.*[40] presume that the resistance to the sorbate mass transfer exists due to both low rates of the sorbate efflux from the micelle and its slow desorption from the stationary phase. When constructing their model, these authors[40] took into account kinetics of sorbate adsorption onto, and desorption from, the stationary phase, on the one hand, and kinetics of the sorbate influx to, and efflux from, the micelle into the bulk of the non-micellar mobile phase, on the other. Having calculated the rates of all processes, they have shown[40] that the rate constants of the influx into the micelle are appreciably higher than the rate constant of the adsorption on the stationary phase (silica gel with the C_1-grafted radicals Supelcosil LC 1) because of the differences in the distance to be covered by the sorbate. Thus 0.10 M sodium dodecyl sulfate solution contains 9.03×10^{17} micelles cm^{-3}. Consequently, in order to bring the sorbate from the bulk of solution to the surface of the stationary phase, it is necessary to encounter about 180 micelles, whereas an appreciably shorter distance must be covered for a repeated collision with the micelle. In order to pass from the micelle to the stationary phase, the sorbate should have a definite orientation relative to the stationary phase. No definite orientation is required for the reverse transition; the sorbate can enter the micelle at any point. The rate constants of these four processes depend on the nature of the sorbates.

Relatively small and well water-soluble molecules, having large rate constants of the influx into, and the efflux from, the micelle can move quickly in the bulk of solution, enter and leave micelles multiply before they reach the stationary phase surface. For such hydrophilic sorbates, the efficiency (mass transfer) is not limited by micellar equilibria. For the more hydrophobic sorbates the affinity to the micelle is higher and the rate constants of their efflux from the micelle are much lower (*e.g.*, for pyrene the efflux rate constant is 1000 times as low as that for benzene). Consequently, the hydrophobic sorbates remain in the micelle for an appreciably longer time, decreasing the mass transfer between the micelle and the stationary phase. Since there is an antiparallel correlation between the rate constants of the sorbate efflux from the micelle and the constants of desorption from the stationary phase, the hydrophilic sorbates can return more often to the bulk of aqueous mobile phase and be thereby close to equilibrium. The consequence of the differences in the rates of these processes for the hydrophilic and hydrophobic sorbates is fairly good efficiency for the former and smeared peaks for the latter.

In order to increase the efficiency and to improve the shape of peaks, it was proposed to add small amounts of organic modifiers to the mobile phase and to operate at higher temperatures.[40] Yarmchuk *et al.*[40] explain enhanced efficiency in the presence of 2 vol.% – 5 vol.% of alcohols by the better mass transfer in the mobile phase owing to the increase in the rate of sorbate efflux from the micelle since the non-micellar part of the mobile phase becomes less polar. These authors relate the higher efficiency after an increase in temperature [for small sorbates of the benzene and phenol type the number of theoretical plates (N) is increased by 50% – 100% at 50 – 60 °C] to both upgraded micellar kinetics and lower viscosity of the mobile phase. Addition of 3% of propanol to the mobile phase and an increase in temperature to 40 °C make it possible to attain efficiencies close to that of RP HPLC with aqueous – organic eluents.[39,41] Enhanced efficiency is promoted

by the decrease in the surfactant concentration.[40] Indeed, Borgerding *et al.*[42] have shown that the use of elevated surfactant concentrations can result in the total loss of efficiency. Another factor influencing the height equivalent to a theoretical plate (HETP) is the linear rate.[40] For small molecules (benzene), the derived dependence was almost analogous to that of usual RP HPLC, whereas for large molecules (naphthalene, anthracene) the efficiency drop with the increasing rate was appreciably larger. Yarmchuk *et al.*[40] recommend to operate at low flow rates.

The low efficiency of MLC is related [36, 43–45] to the surfactant adsorption on the stationary phase surface, which results in poor mass transfer in the stationary phase.[43, 44] Direct relationship between the amount of surfactant in the stationary phase and the decrease in efficiency was observed.[43]

Based on the determination of diffusion coefficients for a series of sorbates (2-naphthol, *p*-nitroaniline, *p*-nitrophenol, naphthalenedisulfonic and naphthalenetrisulfonic acids) in the absence and the presence of sodium dodecyl sulfate and methanesulfonate in the mobile phase, Borgerding and Hinze[43] attempted to elucidate which of the mass transfer processes (in the mobile or the stationary phase) is causative of poor efficiency in MLC. All sorbates may be divided into two groups: the sorbates which interact actively with the micelles (2-naphthol, *p*-nitroaniline and *p*-nitrophenol) and those which do not interact and are repelled or excluded from the micelles (sodium 2,6-naphthalenedisulfonate, sodium 4,5-dihydroxynaphthalenedisulfonate and sodium 1,3,6-naphthalenetrisulfonate). The sorbates of the first group have noticeably lesser diffusion coefficients in the micellar mobile phases. Thus the diffusion coefficients of 2-naphthol and sodium 1,6-naphthalenedisulfonate are 6.0×10^{-6} and 6.71×10^{-6} cm^2 s^{-1} in water, 5.67×10^{-6} and 4.73×10^{-6} cm^2 s^{-1} in 0.1 M $MeSO_3Na$ solution, and 0.90×10^{-6} and 4.0×10^{-6} cm^2 s^{-1} in 0.1 M sodium dodecyl sulfate solution. If the band blurring determined the slow mass transfer in the micellar phase, one would observe different values of efficiency for the two groups of sorbates. Besides, no appreciable variation of N was noticed at different surfactant concentrations in the mobile phase. All this indicates that the most probable factor eliciting the deterioration of the efficiency is the resistance to mass transfer in the stationary phase. The MLC efficiency is strongly dependent on the flow rate. For example, in the case of *p*-nitroaniline the value of N for a 300×4 mm column with the cyanopropyl stationary phase increases from 400 to 1300 upon the decrease in the mobile phase flow rate from 1.5 to 0.25 ml min^{-1}. However, this cannot be regarded as the proof in favour of only a decelerated process of mass transfer in the mobile phase because, according to the van Deemter equation, both members contributing to HETP owing to the resistance to the mass transfer in the mobile and the stationary phases are proportional to the linear flow rate.

Borgerding *et al.*[42] believe that the enhancement of efficiency in MLC with the micelles of ionic surfactants occurs owing to changes in the mobile phase: the repulsion barrier decreases because of the lower density of the electrical charge on the surface of ionic micelles. Indeed, the addition of alkanes has no effect on the surface density of charge and does not lead to higher efficiency for strongly hydrophobic sorbates. Addition of alcohols does not result in higher efficiency of MLC with non-ionogenic surfactants, *e.g.*, Brij-35.

In order to decrease the resistance to the mass transfer and to improve the MLC efficiency, Berthod and Roussel[46] proposed to use hybrid mobile phases. The addition of short- and medium-chain alcohols induces desorption of surfactants from the stationary phase, which leads to the improvement of mass transfer into the stationary phase (and from the stationary phase) and also ensures better wetting.[37, 39, 41, 42] This effect is enhanced with increase in the modifier concentration and hydrophobicity.[19, 37, 47] A study[41] carried out on a broad set of sorbates showed that the higher efficiency of the column is observed not for all sorbates; nonetheless, it was concluded that the cause of higher efficiency is the decrease in the amount of surfactant sorbed on the stationary phase.

Another mechanism of the effect of alcohols on the efficiency was proposed by Armstrong *et al.*[44] In their opinion, the use of hybrid eluents makes possible changes in the structure of the stationary phase itself from the block-like to the brush-like one, which can also improve the mass transfer into the stationary phase (and from the stationary phase). It is noted therewith that the use of hybrid eluents increases appreciably the efficiency in the case of hydrophobic rather than hydrophilic sorbates.[35, 43–45]

As there is no consensus about the cause of poor efficiency in MLC, investigators come continuously back to this problem.[35, 45, 48] Thus investigation of the effect of the nature of the organic modifier (ethyl acetate, n-propanol) on the efficiency of a column packed with different sorbents with the grafted C_1, C_8, C_{18} or cyanopropyl groups in the elution by mobile phases with the surfactant concentrations above or below CMC made it possible to reveal a correlation between the column efficiency and the extent of wetting of the surface of grafted sorbents with an organic solvent.

Bailey and Cassidy[45] studied the effect of sodium dodecyl sulfate concentration (0–0.35 M) at the ionic strength of 0–0.3 M ($NaClO_4$) and of n-propanol (0–0.8 M) on the column efficiency. In the absence of micelles in the mobile phase, the efficiency of the column varies insignificantly. This suggests that the decrease in the amount of sodium dodecyl sulfate adsorbed on the stationary phase is not the cause of the efficiency increase in the presence of alcohols, contrary to what was thought by some investigators.[35, 39, 41, 42] In the absence of micelles, at low alcohol concentrations (not exceeding 0.13 M), the column efficiency for benzaldehyde and acetophenone reaches its maximum, and this may be attributed to modification of the structure of the stationary phase surface. However, at high alcohol concentrations, which are commonly used for achieving better efficiency in MLC, the column efficiency remains approximately constant, which may be indicative of the absence of modification of the stationary phase surface in the presence of n-propanol. Studies of the factors that influence the mass transfer in the mobile phase in the range of sodium dodecyl concentrations from CMC to 0.3 M showed that at concentrations of 0.005–0.012 M this dependence passes through a maximum, the position of which depends on the sorbate hydrophobicity. At higher concentrations the efficiency decreases, which is consistent with the data reported by Yarmchuk *et al.*[40] In the presence of n-propanol, the maximum is broader and less clearly pronounced, whereas the column efficiency increases from 200–2200 to 700–3700 theoretical plates. These results led the authors to conclude that mass transfer in the stationary phase is not the main cause of low efficiency in MLC; this is apparently related to the processes of micellisation. In a micellar system, two oppositely directed processes take place continuously: desintegration of micelles and their formation. At the same time, two basic processes occur in the micellar system: rapid exchange of the surfactant monomer with the micelle [the lifetime of the surfactant molecule in the micelle is of the order of 10^{-7} s (see Ref. 22)] and the process of formation and disintegration of micelles [the half-life of the micelle is 10^{-3}–1 s (see Ref. 22)]. The latter can in turn be split into two processes: (1) a series of stepwise additions of single molecules to the surfactant aggregates with formation of micelles and (2) association of premicellar aggregates with formation of micelles. Either of these processes can influence the sorbate mass transfer between the eluent and the stationary phase. The addition of alcohols can influence these processes and enhance the kinetics of mass transfer in the mobile phase.

Villanueva Camanas *et al.*[49] studied the decrease in the efficiency due to the addition of alcohols to the mobile phase containing sodium dodecyl sulfate micelles in the elution of L-DOPA, 2-methyl-DOPA, norepinephrine, adrenalone, dopamine, isoproterenol on Spherisorb ODS-2 C_{18}. Analogous regularities were revealed earlier in the elution of amines.[50, 51] It is presumed that the mass transfer is brought about through the

following mechanisms: (1) direct transfer from the micelles to the stationary phase, which is opposed by the electrical repulsion between them and (2) transfer through a continuous aqueous pseudophase. In the presence of alcohol, the polarity of the continuous aqueous pseudophase is decreased. On the one hand, this increases the rate of transfer of hydrophobic sorbates through this pseudophase, while on the other hand, the solubility and the rate of transfer of highly hydrophilic catecholamines is decreased, which accounts for the decrease in the efficiency.

Analysis of the band blurring in MLC was performed [35, 52] using the Knox equation

$$h = Av^{1/3} + B/v + Cv\,, \tag{21}$$

where $h = H/d_p$ is the reduced height equivalent to a theoretical plate, H is the height equivalent to a theoretical plate, d_p is the size of stationary phase particles, $v = ud_p/D_m$ is the reduced linear flow rate, u is the linear flow rate of the mobile phase, D_m is the coefficient of sorbate diffusion in the mobile phase, A is the coefficient which characterises the quality of the column packing and the decelerated mass exchange in the mobile phase outside the particles, B is the coefficient accounting for the contribution to the band blurring from the longitudinal diffusion and C is the coefficient describing the decelerated mass exchange in the stationary phase.

The dependences of h on v were determined [33] on one column using first an aqueous–organic mobile phase, then the micellar mobile phase and then again the same aqueous-organic phase. Two types of alkylated silica gels were used, *viz*., a monomeric C_{18} sorbent and a C_{14} sorbent with high density of grafted alkyl groups (3.5 μmol m^{-2}), and two types of surfactants, *viz*., sodium dodecyl sulfate (anionic surfactant) and Brij-35 (non-ionogenic surfactant). An increase in h was shown to occur mainly due to the higher values of coefficient A: for benzene with the aqueous methanol as a mobile phase, $h = 4.9$ at $v = 10$, whereas in the presence of sodium dodecyl sulfate at $v = 10.9$ the contribution from the coefficient A is 2.36 (48% methanol) and 6.9 (63% methanol), respectively. In micellar eluents, the contribution from the coefficients B and C also increases resulting in a stronger band blurring and lower efficiency. The decrease in the diffusion coefficient of sorbates upon their inclusion into the micelles induces the increase in B. The coefficient of sorbate diffusion in the stationary phase depends on the nature of the sorbate and decreases as the hydrophobicity increases; highly hydrophobic sorbates are transferred from the micelle directly into the stationary phase by-passing the extramicellar aqueous phase in which they are poorly soluble. The value of coefficient C increases in a micellar system (*e.g*., up to 700% for propylbenzene). The decrease in the mass transfer coefficients may also be associated with poor wetting.[2, 39] The most important reason, however, is believed to be the surfactant adsorption on the stationary phase.[16, 17, 43, 44, 50]

In the first place, adsorption of a surfactant on the stationary phase surface [a C_{18} sorbent adsorbs 70 mg g^{-1} of Brij-35, and a C_{14} sorbent adsorbs 140 mg g^{-1} (or 2.3 μM m^{-2}) of sodium dodecyl sulfate] leads to a decrease in the pore volume, obstructs the smallest pores with diameter < 7 nm and reduces appreciably the surface area of the stationary phase and its permeability.[35] The consequence of this is the increase in the coefficient A values. Secondly, adsorption of a surfactant increases thickness of the organic layer of the stationary phase and decreases the mass transfer rate and the diffusion coefficients in the stationary phase and the coefficients of sorbate diffusion in the mobile phase.[16, 42, 43, 46] This results in the increased values of the coefficients B and C. The surfactant adsorbed on the stationary phase can be eluted with a pure solvent; however, the adsorption may prove to be partially irreversible.[32–34] Following operation with the use of micellar phases, the efficiency of a column drops drastically as is evident from the values of the coefficients A, B and C in the Knox equation for benzene. This is also the consequence of changes in the porosity and permeability of the stationary phase.

Mobile phase	A	B	C
Methanol–water (30 : 70)	1.1	3.1	0.22
1.4% Sodium dodecyl sulfate in water	3.2	20	0.2
Methanol–water (30 : 70) after operation with sodium dodecyl sulfate	5.7	4	−0.7

Lavine and Hendayana [52] studied the effect of temperature in the range of 25–45 °C on the dependence of h on v for acetophenone, nitrobenzene, benzene, methyl benzoate and toluene in MLC with an aqueous solution of sodium dodecyl sulfate. The values of the coefficients A, B and C for *p*-nitrophenol in 0.02 M sodium dodecyl sulfate solution were found to be 2.34±0.14, 7.43±0.69 and 0.074±0.013 at 25 °C and 1.06±0.13, 11.4±0.65 and 0.026±0.010 at 45 °C, respectively. The decrease in the coefficient B at 35 °C (5.16±0.66) is anomalous and inexplicable.[52]

Noticeable enhancement of chromatographic efficiency with the increase in temperature occurs owing to the decrease in values of both the coefficients A (flow anisotropy) and C (mass transfer in the stationary phase). The decrease in A values with the increase in temperature may be explained by the decrease in the constant of sorbate partition between the micelle and the bulk of solution [K_2, Eqn (2)] and, consequently, by the decrease in the proportion of sorbate bound to the sodium dodecyl sulfate micelles in the mobile phase. A decrease in the eddy diffusion is eventually attained. The decrease in the values of the coefficient C with the increase in temperature may be explained by the decrease in the adsorption of a surfactant on the stationary phase surface and the higher rate of sorbate mass transfer between the mobile and stationary phases. In addition, the fluidities of the grafted alkyl chains and the adsorbed surfactant are increased at higher temperature, which also decreases the resistance to the mass transfer in the stationary phase.

The values of the coefficient B increase considerably with temperature due to both the lower viscosity of bulk of the solvent and transition of part of the sorbate from the micelle to the solution.

In the example of separation of 12 polycyclic aromatic hydrocarbons on Nova-Pak C_{18}, the influence of the nature and the content of the alcohol and of the column temperature on the efficiency of separation in MLC with the use of sodium dodecyl sulfate was studied.[53] The number of theoretical plates increases with the increase in the surfactant concentration in the mobile phase up to 0.13 M. Upon transition from methanol to 2-propanol, the peak becomes more symmetrical, and one observes an increase in the efficiency for less hydrophobic sorbates (fluorene and chrysene). For more hydrophobic dibenzo[*ac*]anthracene, more symmetrical peaks were obtained with n-butanol, although in this case the changes in the efficiency were insignificant. It should be noted that in contrast to conventional HPLC, in MLC the number of theoretical plates is commonly calculated by the following formula [54]

$$N = \frac{[41.7(t_R/w_{0.1})^2]}{(B/A + 1.25)}\,, \tag{22}$$

where t_R is the retention time, $w_{0.1}$ is the peak width at its 10% height, B/A is the factor of peak asymmetry.

An increase in temperature leads to insignificant enhancement of the efficiency and appreciable improvement of the shape of peaks. In this case, the effect of the flow rate on N at different temperatures depends on the sorbate hydrophobicity.

Studies of the effect of alcohols as modifiers of the mobile phase showed that the addition of small amounts of propanol or pentanol led to higher efficiency because of considerable decrease in the values of the coefficients A and C in Eqn (21).[52] An increase in the coefficient B upon addition of an alcohol seems to occur due

to the lower viscosity of the mobile phase and a shift of sorbate equilibrium in the micelle–solvent volume system. Consequently, the mechanisms of the influence of the increase in temperature and addition of alcohols on the separation efficiency in MLC are similar in many respects.

Thus, Okada [32] as well as Lavine and Hendayana [52] related the deterioration of MLC efficiency to an appreciable increase in the coefficients A and C in the Knox equation. Flow anisotropy, resistance to mass transfer in the stationary phase and molecular diffusion contribute to the band blurring. To enhance the efficiency in MLC, it is recommended to use hybrid eluents.

IV. Selectivity of micellar liquid chromatography

The selectivity of separation in MLC is influenced by the type and concentration of the surfactant, the nature and concentration of the organic modifier, pH, ionic strength, temperature and the nature of the stationary phase. Of these factors, the effects of surfactant and modifier concentrations have been studied most extensively. In MLC, the eluting strength of purely micellar eluents is small, it increases with increase in the micelle concentration in the mobile phase.[47]

1. Effect of surfactant concentration

Foley [12] deduced Eqn (23) for calculating the optimum concentration of micelles [rather than of the micellised surfactant as in Eqns (1) and (2)]

$$\mathrm{pM_{opt}} \approx \log K_{\mathrm{SM}} - \tfrac{1}{2} \log \frac{k'_s}{k'_1}, \qquad (23)$$

where $\mathrm{pM_{opt}} = -\log [\mathrm{M}]$, K_{SM} is the constant of sorbate association with the micelle calculated per micelle rather than per monomer, k'_s is the factor of sorbate capacity (the extreme value), k'_1 is the sorbate capacity factor at a surfactant concentration of 1 mol litre^{-1}.

The optimum pM values for a broad spectrum of sorbates and three surfactants (sodium dodecyl sulfate, cetyltrimethylammonium bromide and Brij-35) were calculated using Eqn (23). The concentrations of surfactants calculated from Eqn (23) fall into a wide range and are the optima as regards selectivity rather than retention.

For dihydropyridines on a C_{18} stationary phase, an increase in the surfactant concentration induces a trend towards changes in the retention mechanism from three partition equilibria to direct transfer from the micelles to the stationary phase.[55, 56]

Madamba-Tan *et al.*[57] used isocratic data to deduce the equation for predicting the retention times in the gradient regime of elution with a gradient of micelle concentration

$$t_{\mathrm{R}} = (t_0/b)\{-1/k'_0 + [(1/k'_0)^2 + 2b(1-f)]^{1/2}\} + t_0 + t_{\mathrm{D}} \qquad (24)$$

where t_0 is the dead time of the column; b is the parameter of the gradient steepness; k'_0 is the capacity factor in the initial mobile phase; t_{D} is the delay time, *i.e.*, the time interval before the gradient does reach the upper part of the column; f is the part of the column through which the sorbate band has already passed and which is calculated from the equation

$$f = t'_{\mathrm{D}}/t_{\mathrm{R}} = \frac{[t_{\mathrm{D}}(1 + 1/k'_0)]}{t_{\mathrm{R}}}. \qquad (25)$$

2. Effect of organic modifier concentration

The addition of an organic solvent to micellar eluents leads to simultaneous increase in the eluting strength and selectivity for some ionic and non-ionic sorbates.[47, 58, 59] The increase in selectivity is related to the existence of competitive partition in MLC and to the unique capacity of micelles for solubilising separately molecules of sorbates and organic solvents.

Subsequent studies of the role of organic modifiers and micelles in the control of the eluting strength and selectivity in MLC in the example of small peptides, phenols, substituted benzenes and benzoic acids in a sodium dodecyl sulfate–water–2-propanol system confirmed simultaneous increase in the eluting strength and selectivity;[60] in this case, the selectivity varies systematically and monotonically as the concentration of 2-propanol in the mobile phase increases. The concentration of micelles has the opposite effect on selectivity compared to that of 2-propanol. For those ion pairs the selectivity of which decreases as the concentration of 2-propanol increases, one observes enhancement of selectivity with the increase in concentration of micelles and *vice versa*. Thus, although the eluting strength of the mobile phase is increased with the increase in the concentration of micelles and organic solvent, the effects of these parameters may be quite different and even opposite. Micelles and 2-propanol compete for the interaction with sorbates, which influences the selectivity and retention.

The dependence of retention on the volume proportion (φ) of organic modifier is described by the equation [47]

$$\ln k' = -S_{\mathrm{hyb}}\varphi + \ln k'_0, \qquad (26)$$

where S_{hyb} is the parameter of solvent strength, $\ln k'_0$ is the apparent (extrapolated) coefficient of sorbate capacity in a purely micellar mobile phase.

Equation (26) is analogous to Eqn (1) used for describing changes in the retention with the variation of the volume proportion of the organic modifier in RP HPLC. Combination of Eqns (26), (1) and (2) gives relations (27) and (28).

$$\ln(P_{\mathrm{SW}}\Phi) = -S_{\mathrm{s}} + \ln(P^0_{\mathrm{SW}}\Phi), \qquad (27)$$

$$\ln(K_{\mathrm{MW}}[\mathrm{M}] + 1) = -S_{\mathrm{m}} + \ln(K^0_{\mathrm{MW}}[\mathrm{M}] + 1), \qquad (28)$$

where S_{s} and S_{m} are the parameters which characterise the sensitivity to changes in the sorbate partition from the bulk of solution into the stationary phase and the micelles with variation of φ; K^0_{MW} is the constant of sorbate binding to a micelle, P^0_{SW} is the coefficient of sorbate partition between the mobile and stationary phases for aqueous micellar eluents, *i.e.*, in the absence of an organic modifier.

The parameter S_{hyb} depends on S_{s} and S_{m}

$$S_{\mathrm{hyb}} = S_{\mathrm{s}} - S_{\mathrm{m}}. \qquad (29)$$

Several conclusions can be drawn from Eqns (27)–(29). First, the magnitude of the solvent strength parameter in hybrid eluents is smaller than that in aqueous-organic eluents. Second, S_{m} and, consequently, S_{hyb} depend on the micelle concentration. For two sorbates (a and b) and at two concentrations of organic solvent (φ_1 and φ_2) in the mobile phase, the following equation is valid

$$\ln \alpha_2 - \ln \alpha_1 = -(S^b_{\mathrm{hyb}} - S^a_{\mathrm{hyb}})(\varphi_2 - \varphi_1), \qquad (30)$$

while combination of Eqns (26), (29) and (30) gives Eqn (31).

$$\ln \alpha = (dS_{\mathrm{m}} - dS_{\mathrm{s}})\varphi + \ln \alpha_0, \qquad (31)$$

where $\alpha = k'_b/k'_a$ is the selectivity coefficient ($k'_b > k'_a$), α_0 is the selectivity coefficient in a purely aqueous micellar eluent, dS_{m} and dS_{s} are the differences in S_{m} and S_{s} for compounds a and b.

It follows from Eqn (31) that the selectivity ($\ln \alpha$) depends linearly on the volume proportion of the organic modifier in the mobile phase and decreases or increases monotonically depending on the $dS_{\mathrm{m}}/dS_{\mathrm{s}}$ ratio for two sorbates. Thus for the majority of substituted benzenes, amino acids and peptides, the reverse dependence between retention and S_{hyb} is observed: the more strongly compounds are retained, the smaller is the S_{hyb} value. In this case, for most aromatic compounds, amino acids and proteins a direct dependence between retention and S_{m} and a reverse dependence between retention and S_{s} are observed. The influence of the sorbate structure on the value of S_{hyb} is reflected mainly on changes in S_{s}.

The influence of the concentrations of an organic solvent and micelles on different equilibria occurring in the system can clearly be seen if Eqn (1) or (2) is transformed into (32).

$$\alpha = \left(\frac{P^b_{SW}}{P^a_{SW}}\right) \Big/ \left(\frac{K^a_{MW}[M]+1}{K^b_{MW}[M]+1}\right) = \frac{\alpha_{SW}}{\alpha_{MW}}, \quad (32)$$

where α_{MW} is the parameter characterising the selectivity of binding of sorbates to the micelles, α_{SW} is the parameter characterising the selectivity of partition into the stationary phase.

The values of P_{SW} and K_{MW} decrease as the concentration of 2-propanol increases; however, the extent of decrease in these parameters is different for different sorbates, which leads to changes in selectivity. The concentration of micelles, in turn, influences the selectivity at a fixed concentration of an organic modifier mostly through the parameter α_{MW}: as the concentration of micelles increases the parameter α_{MW} decreases faster than the parameter α_{SW}, which is independent of [M]. The selectivity is increased because of this effect.

3. Effect of the nature of the organic modifier

The nature of the organic modifier of the mobile phase influences the selectivity of separation in MLC; however, this effect is not the same as that in RP HPLC. Usually lower alcohols from methanol to pentanol [9, 35, 39, 41, 42, 47, 55, 57, 58, 60, 61–70] and also a pentanol–heptanol mixture (3 : 1) [71] are used as modifiers in MLC. Diols,[61] dipolar aprotic solvents,[61] acetonitrile and tetrahydrofuran [67] were also used for this purpose. The principles of the choice of solvent selectivity in RP HPLC are based on the Snider triangle of selectivity, but this approach proved to be inapplicable to MLC.[67] The selectivities of separation attainable with 2-propanol, acetonitrile and tetrahydrofuran belonging to different types of solvents are close,[67] whereas those with 2-propanol and n-butanol belonging to the same type exhibited strong differences.[9, 65, 67] For example, in a model mixture of 15 polyaromatic hydrocarbons (PAH) in the absence of the modifier (0.15 M sodium dodecyl sulfate) in the mobile phase, many compounds are co-eluted, particularly the most hydrophobic ones.[65] In the presence of 15 vol.% methanol, the peaks of all compounds are well resolved. The quality of separation with 2-propanol instead of methanol is similar; in this case, the time of analysis is reduced from 45 to 25 min. However, the addition of only 7 vol.% of n-butanol degrades separation.

On the one hand, the addition of alcohols to the micellar mobile phase modifies appreciably the partition of sorbates between the micelles and the bulk of aqueous phase which becomes less polar.[64] On the other hand, alcohols decrease the amount of surfactant sorbed on the stationary phase and solvate the grafted hydrocarbon phase. Considering that n-butanol is endowed with a higher solvation capacity than methanol or 2-propanol, this should exert stronger effect on the partition of sorbates over three phases. This effect is particularly noticeable for more hydrophobic compounds.

The parameter S_{hyb} reflects the extent of solvation of sorbates by organic solvents, localisation of the molecules of sorbates and organic solvents in the micelles [9] and hence, should depend on the nature of both the sorbate and the surfactant. Khaledi *et al.*[47] have found that for 14 aromatic compounds belonging to different classes the values of S_{hyb} in the cetyltrimethylammonium bromide micellar phase, modified by methanol, 2-propanol and n-butanol, vary in the order $S_{\text{n-butanol}} > S_{\text{2-propanol}} > S_{\text{methanol}}$, which coincides with the increase in the eluting strength of these modifiers in RP HPLC. An analogous sequence was obtained by Rodriguez Delgado *et al.*[65] for 15 PAH in the mobile micellar phase of sodium dodecyl sulfate modified with the same alcohols.[65] The higher values of the parameter S_{hyb} for 2-butanol indicate that it interacts with the micelles more actively, solvates the sorbates more effectively and can compete better with the micelles for the interaction with sorbates than does methanol.

Variation of S_{hyb} as a function of surfactant concentration is also different for different modifiers: as the concentration of sodium dodecyl sulfate in the mobile phase increases from 0.06 to 0.14 M, the ΔS_m varies by $\sim$0.2 for methanol, $\sim$0.4 for 2-propanol and $\sim$2.0 for n-butanol.[65]

Less hydrophobic sorbates interact strongly with the micelles (*e.g.*, anthracene with cetyltrimethylammonium bromide [47] or naphthalene, acenaphthylene, fluorene and anthracene with sodium dodecyl sulfate [65]). They are less accessible to such polar solvents as methanol. Consequently, the addition of modifying alcohols will have a weaker effect on their retention compared to that in RP HPLC. The interaction of sorbates with the micelles decreases as the hydrophobicity of alcohols increases. This effect is particularly well manifested for hydrophobic sorbates (having large partition coefficients in a water–micelle system) because of the competition between this alcohol and the micelles for the interaction with the sorbate.

The order of the variation of S_{hyb} as a function of the nature of the alcohol used proved to be the same in the systems with both cetyltrimethylammonium bromide [47] and sodium dodecyl sulfate.[65]

It should be noted that the proportion of an organic modifier added to hybrid eluents does not usually exceed 10 vol.%. At larger contents of organic solvents, the retention mechanism can be changed to reach eventually that typical of RP HPLC; in this case, the system will lose all its advantages in selectivity. It was shown [58, 59] that up to 20 vol.% of 1-propanol could be added to the micellar eluent without formation of the usual aqueous-organic system.

Based on the equations deduced for RP HPLC, Madamba-Tan *et al.*[71] have developed a new approach to the gradient elution with organic modifiers in MLC. When such gradients are used, equilibrium is established rapidly owing to a narrow interval of organic modifier concentrations in the gradient. This results in the reduction of the analysis time. Practical applications of propanol and acetonitrile gradients have been described.[72] For example, separation of dansylated and bisdansylated amino acids in the isocratic regime requires 67 min. A gradient of 2-propanol (from 3 vol.% to 15 vol.%) in 5 min with subsequent isocratic elution for another 20 min allows separation of these compounds (the total separation time is 32.5 min, including the time necessary for the equilibrium onset).

According to Eqn (26), the retention of sorbates in MLC decreases as the concentration of organic modifier increases. This holds for the most systems studied in a narrow range of modifier concentrations (3 vol.%–15 vol.% of 2-propanol).[67] However, the dependence of $\ln k'$ on φ is not linear for some amino acids and alkylbenzenes with mobile phases containing sodium dodecyl sulfate or cetyltrimethylammonium bromide [47] and for some benzene and naphthalene derivatives in a sodium dodecyl sulfate–*n*-butanol–water system. For some other compounds, the dependence of $\ln k'$ on φ was linear only for the mobile phases supplemented with methanol.[72] For this reason, a new model was proposed [72] for describing the variation of the sorbate retention time in MLC with changes in the organic solvent concentration. In the example of separation of a mixture of catecholamines on a column packed with Spherisorb ODS-2, the dependences of k', $1/k'$ and $\log k'$ on [M] and φ were approximated using different polynomials up to the second power inclusive using the factor planning method (the factors planned were the surfactant concentration, the micelle concentration [M] (up to 0.15 mol litre^{-1}) and the propanol concentration φ (up to 10 vol.%). The dependence of k' on [M] and φ was shown to be a poorly expressed asymmetric hyperbolic surface with an extremum situated near the maximum values of [M] and φ. The addition of 10 vol.% of propanol and 0.035 M sodium dodecyl sulfate led to a 58%–78% decrease in k', whereas the increase in surfactant concentration in a purely micellar eluent from 0.035 to 0.15 M resulted in a 81% reduction of k'. The least approximation error was obtained using the equation

$$1/k' = A[M] + B\varphi + C[M]\varphi + D, \quad (33)$$

where [M] is the micelle concentration, φ is the volume proportion of the organic modifier and A, B, C and D are constants.

Equation (33) gives a satisfactory description of the retention of amino acids, peptides, phenols and some other aromatic compounds (the coefficient of correlation between the calculated and experimental k' values is above 0.996).

The following equation was also proposed to describe retention:

$$1/k' = A[\mathrm{M}] + B\varphi + C[\mathrm{M}]\varphi + D\varphi^2 + E, \quad (34)$$

where A, B, C, D and E are coefficients.

For catecholamines, amino acids and phenols the coefficients of different members of Eqns (33) and (34) are the same, the contribution of the term $D\varphi^2$ to Eqn (34) is insignificant. Equation (33) gives too low values for the most hydrophobic surfactants. Equation (34) provides a better description of the retention.[70] The following equation was proposed

$$k' = \left[\frac{K_{\mathrm{SW}}(1 + K_{\mathrm{SD}}\varphi)}{1 + K_{\mathrm{AD}}\varphi}\right] \Big/ \left[\frac{1 + K_{\mathrm{AM}}(1 + K_{\mathrm{MD}}\varphi)}{(1 + K_{\mathrm{AD}}\varphi)[\mathrm{M}]}\right], \quad (35)$$

where K_{AD}, K_{MD} and K_{SD} are constants reflecting the relative variation of the sorbate concentration in the aqueous phase, in the micelles and in the stationary phase, respectively, in the presence of a modifier compared to that in the purely micellar solution. Garcia Alvarez-Coque *et al.*[70] examined separation of nine sorbates on a column packed with Spherisorb ODS-2 (C_{18}) with a propanol-containing mobile phase of sodium dodecyl sulfate in order to assess the applicabilities of Eqns (33), (34) and (35) for the description of retention. The best results were obtained using Eqn (35). Thus in the description of retention of pyrene, Eqn (35) gives a substantially higher ($> 50\%$) accuracy than does Eqn (33). Analogous mean relative deviations of the calculated k' values from the experimental ones were obtained for other sorbates. For the purely micellar eluents, Eqn (33) is transformed into well-known Eqn (2).

Thus, the simultaneous influence of both the surfactant and the organic modifier concentrations on the retention time was again demonstrated. It is these two factors that play the crucial role in the optimisation of separation conditions in MLC.

Strasters *et al.*[58] have proposed an iterative-regressive strategy for the choice of optimum conditions for the separation of model mixtures of ionogenic compounds: 13 amino acids and peptides with a sodium dodecyl sulfate – 2-propanol – water mobile phase and 15 phenols with a myristyltrimethylammonium bromide – 2-propanol – water mobile phase. Prediction of the retention of sorbates proved to be possible on the basis of only five experiments even when the values of parameters were far apart in the parametric space. The time of analysis was decreased 3 – 7-fold and the selectivity was enhanced.

In order to solve the problems of three-parametric optimisation in MLC where the variables are the concentrations of a surfactant, an organic modifier and pH of the mobile phase, a Turbo-Pascal (version 5.5) programme has been developed.[59] In the proposed model, it is necessary to derive 15 initial chromatograms in order to plot the function of response – criterion of separation (the resolution of a pair of components which are most difficult to separate and the total time of analysis are regarded as the separation criterion). This model is applicable for optimising the separation of a mixture of 9 amino acids and peptides using a hybrid eluent containing a phosphate buffer, sodium dodecyl sulfate and 2-propanol. Rukhadze *et al.*[73] have established the conditions for the separation of a mixture of five barbiturates by the three-factor method using the micelles consisting of sodium dodecyl sulfate and a mixed organic modifier [pentanol – heptanol (1 : 3)] at pH 2.5; the separation time was 40 min.

Potential advantages of the application of artificial neuronal computational networks instead of classical statistical methods for simulating the dependence of the retention time on the concentrations of surfactant and organic modifier have been demonstrated using a mixture of 27 dihydropyridines[68] or a mixture of 23 benzene derivatives and PAH.[69] The effects of the network architecture (the binding mode of individual neurons), the nodal activation function, the number of layers and the mode of the data processing were studied. The linear activation function was shown to be optimal. It is necessary to use networks with the recurrent bonds; the best results were obtained using the logarithms of capacity factors as the output data.[69] In the case of dihydropyridines, 1 – 3 neurons and recurrent networks were used.[68] Different transmission functions (two sigmoidal, one logarithmic and one linear) were activated in the node of the hidden layer; of these the logarithmic function proved to be the optimum. The optimum number of nodes in the hidden layer was equal to three. The use of the magnitude reciprocal to the capacity factor enhances the performance of neuronal networks. It was shown[68] that there are appreciable differences between the possibilities of simulation of the retention of dihydropyridines with the aid of neuronal networks and by means of empirical equations.

4. Effect of the nature of the surfactant

The selectivity of separation in MLC may be influenced not only by the variation of the surfactant concentration but also by changes in the nature of the surfactants (*e.g.*, the alkyl chain length and charge). Thus a mixture of six vanillins was successfully separated using a mobile phase of 0.02 M sodium dodecyl sulfate solution (pH 3).[74] However, when cetyltrimethylammonium bromide was used, the retention time of four of six vanillins proved to be longer than in the case of sodium dodecyl sulfate, although in the latter case the mobile phase contained an appreciably larger number of micelles (the CMC of cetyltrimethylammonium bromide at 30 °C and of sodium dodecyl sulfate at 25 °C in distilled water are 0.9 and 8 mmol litre^{-1}, respectively). Thus, the interaction of the vanillins studied with the stationary phase modified by cetyltrimethylammonium bromide is stronger than that with the stationary phase modified by sodium dodecyl sulfate. Analysis of the regression dependences carried out with the aid of Eqn (2) showed that when sodium dodecyl sulfate is used, the elution order of the sorbates correlates with the magnitude of $\Phi[\mathrm{L_s}]K_1$, and hence the selectivity is determined mostly by the stationary phase – sorbate interactions. It should be noted that for a number of compounds the retention time also correlates with K_2. A decrease in selectivity with the increased concentration of sodium dodecyl sulfate, and hence with the quantity of micelles in the mobile phase, also indicates that the sorbate – micelle interaction begins to play a more important role. For cetyltrimethylammonium bromide, the order of elution correlates with changes in both $\Phi[\mathrm{L_s}]K_1$ and K_2, *i.e.*, the selectivity of separation is determined by the interactions of sorbates with both the micelles and the stationary phase.

The observed differences in selectivities are determined, in the opinion of some investigators,[33, 44, 75] by the nature of the surfactant interaction with the stationary phase and by the properties of the modified surface. It follows from the models of surfactant adsorption that in the adsorption of sodium dodecyl sulfate the hydrophobic alkyl radical is inserted into the stationary C_{18} phase, whereas the hydrophilic polar heads are directed towards the bulk of mobile aqueous phase and form a hydrophilic layer, which results in a decrease in the stationary phase polarity. In the case of cetyltrimethylammonium bromide, the head groups seem to be localised nearer to the surface owing to additional hydrophobic interactions of the *N*-methyl groups of ammonium salts with alkyl radicals of the stationary phase. It is possible that they are partially incorporated into the grafted phase. A part of cetyltrimethylammonium bromide can be sorbed irreversibly; the positively charged head groups of the surfactant can form strong hydrogen bonds with the residual silanol groups of the stationary phase and at the sites of defects in the grafted layer. All this leads to an increase in the stationary phase hydrophobicity. Indeed, when aqueous-organic eluents are used before and after work with the micellar phases, the retention times of

compounds where sodium dodecyl sulfate is used are decreased, which points to a decrease in the hydrophobicity of the mobile phase, but they are increased with the use of cetyltrimethylammonium bromide, which suggests an increase in its hydrophobicity. Lavine *et al.*[74] presume that the irreversible adsorption of cetyltrimethylammonium bromide leads to a reduced activity of the residual silanol groups, which compensates for the increase in the stationary phase polarity associated with the presence of the charged head alkylammonium groups on the surface. On the whole, the stationary phase becomes less polar and more hydrophobic. Since compounds under separation (vanillins) are weak acids ($pK_a = 7.4-8.9$), they are characterised by specific interactions (hydrogen bonding) with the negatively charged layer of the head groups of sodium dodecyl sulfate, which results in higher selectivity than that in the case of cetyltrimethylammonium bromide.

Lavine *et al.*[76] have shown that the micelles of cetyltrimethylammonium bromide possess better selectivity with regard to compounds containing acidic functional groups. This is explained by the existence of a secondary chemical equilibrium which includes proton transfer from the ionogenic compound to the water molecules in the Stern layer of the surfactant micelle. The decrease in K_a from 0.5 to 3.0 can lead to the incorporation of the guest molecule into the cationic micelle.[77]

The surfactants containing two or more hydrophobic 'tails' in the monomer, *e.g.*, didodecyldimethylammonium bromide or dihexadecyl phosphate, form vesicles. The selectivity of separation with the use of vesicles in the mobile phase differs from that in MLC, which is associated with the increase in the number of sites accessible for solubilisation from five (in a micelle) to nine (in a vesicle).[78]

5. Effect of pH

The effect of pH on selectivity is determined by the occurrence of different secondary equilibria in the mobile phase and the surfactant-induced shift of the ionisation constants of sorbates.[26] The influence of pH on the selectivity of separation of amino acids (*e.g.*, tryptophan and phenylalanine) was studied in aqueous, ion-pair and micellar eluents. The maximum selectivity was observed for the mean pK_a value of a pair of sorbates. The optimum selectivity α_{opt} may be calculated by the equation

$$\alpha_{opt} = \frac{2SS_m + SS_m^{1/2}(SS_m K_i^* + K_j^*)/(K_i^* K_j^*)^{1/2}}{2SS_m + SS_m^{1/2}(SS_m K_j^* + K_i^*)/(K_i^* K_j^*)^{1/2}}, \quad (36)$$

where SS_m is the mean self-selectivity (the ratio of capacity factors of two sorbate forms [14]), K_i^* and K_j^* are the apparent dissociation constants of sorbates i and j in aqueous, non-aqueous and micellar media.

The selectivity of separation of this pair of amino acids is a maximum in aqueous medium, ion-pair chromatography and micellar liquid chromatography at pH 2–2.5, ~4 and 5–5.5, respectively.

It should be noted that the surfactant-induced shifts of pK_a values do not necessary result in enhanced selectivity. For example, for compounds having different pK_a values and hydrophobicity (isomeric chlorophenols), the micelles influence both parameters.[79]

6. Effect of the nature of the stationary phase

The effect of the nature of the stationary phase is usually considered in MLC as a variation of the column efficiency due to different adsorption of surfactants. Alkylated silica gels C_1, C_8, C_{14} and C_{18} with different densities of grafted alkyl groups and cyanopropyl phases were studied. Yang and Khaledi[80] have demonstrated the advantages (higher selectivity and shorter analysis time) of fluorinated, chemically bound stationary phases compared to the alkyl ones for the separation of mixtures of amino acids, peptides, sulfonamides, phenols and alkylphenols using the mobile phases containing anionic and cationic surfactants.

Model mixtures of vanillins were separated on columns packed with Apex IC_{18} and Apex IC_8 (5 μm) using the same mobile phases (0.02 M surfactant solution).[74] Both stationary phases provide effective separation upon elution with sodium dodecyl sulfate solutions; in this case, the retention is determined by the interaction of sorbates with the stationary phase. The retention times of sorbates on the C_8 phase are longer than those on the C_{18} phase, despite the fact that the latter is more hydrophobic and contains 13.3% of carbon (the C_8 phase contains 9.0% of carbon). This may be due to poorer sorption of sodium dodecyl sulfate on the C_{18} phase because of the higher density of grafted groups.[81]

The use of mobile phases containing the cetyltrimethylammonium bromide micelles also results in a stronger retention on the C_8 phase than on the C_{18} phase; however, in this case an additional effect consists in the alteration of the order of elution of some compounds. The low adsorption level on the C_8 phase could be the cause of the stronger retention than that on the C_{18} silica gel; this should not however result in the reversal of the elution order. The higher activity of the residual silanol groups on the C_8 sorbent compared to that on the C_{18} sorbent can neither explain the observed phenomenon since the elution order of sorbates in the methanol–water system is the same on both stationary phases. For this reason, Lavine *et al.*[74] have presumed that small premicellar aggregates of cetyltrimethylammonium bromide are formed inside the stationary phase and modify the retention of sorbates.

To explain the differences in selectivity observed in MLC, the interaction of sodium dodecyl sulfate and cetyltrimethyl- and dodecyltrimethylammonium bromides with Apex I C_{18},[82] Apex I cyanopropyl and Apex I C_8[83] was studied using HPLC and ^{13}C NMR. It was shown that the surfactant is adsorbed on the stationary phase and that this provides for a selective separation of isomeric vanillins. The use of sodium dodecyl sulfate makes it possible to achieve better separation of hydrophilic compounds because it forms a hydrophilic, negatively charged layer on the stationary phase surface. During adsorption of ammonium surfactants, the head groups are incorporated, at least partly, into the grafted phase owing to hydrophobic interactions of their methyl groups with the C_{18} radicals of the stationary phase.[82] In the separation of a mixture of hydrophilic compounds with the aid of these cationic surfactants, the best results were obtained at high concentrations of the surfactant in the mobile phase, when the sorbate–micelle interactions are advantageous. The length of the hydrocarbon chain of the stationary phase is an important factor in the separation with the use of cetyltrimethyl- and dodecyltrimethylammonium bromide micelles: the behaviour of hydrophilic aromatic compounds on the C_8 and C_{18} phases is different.[83] It was noted[83] that when sodium dodecyl sulfate as well as cetyltrimethylammonium bromide are employed, the retention times of some ionogenic compounds are increased. This unusual phenomenon may be explained by strong interactions of the polar groups of the surfactant and the nitrile groups.

7. Effect of temperature

The effect of temperature is the least studied aspect of MLC. It is obvious from general speculations that the temperature should be higher than the Kraft point, which corresponds to the lower temperature limit for the existence of micelles (23 °C for cetyltrimethylammonium bromide[73]). Researchers usually operate at higher temperatures (40–60 °C, most often at 40 °C) mainly because elevated temperatures enhance the chromatographic system efficiency and reduce the time of analysis.

The effect of temperature on the retention in MLC was investigated in thermodynamic terms.[63,84] The retention of anthracene and its derivatives on C_{18} in a sodium dodecyl sulfate–1-propanol–phosphate buffer (pH 2.1) system was studied in the temperature range 303–353 K.[84] The results

obtained were interpreted using two models. There is no correlation between the enthalpies calculated in both models and the physicochemical properties of sorbate molecules. In the model based on the Van't Hoff equation without consideration of individual equilibria, the values of standard entropies correlate with the polarity of sorbate molecules.

Villanueva Camanas *et al.*[49] did not succeed in enhancing the efficiency of separation of some 6-mercaptopurine-based medicinal preparations by using sodium dodecyl sulfate and varying the temperature from 20 to 60 °C. They noted the influence of both organic modifiers (alcohols, acetonitrile) and temperature on the resolution owing to changes in selectivity; however, these parameters affect little the efficiency and their variation has insignificant practical application. As the studies devoted to a systematic investigation of the effect of temperature in MLC are very scarce, it is impossible to draw any generalising conclusions. It may only be said that it is advisable to use elevated temperatures in MLC for decreasing the viscosity of mobile phases. This increases the column efficiency and stabilises the operation of pumps the working pressures of which are restricted.

8. Effect of ionic strength

There are no separate studies concerning the investigation of the effect of ionic strength in MLC. Since the addition of salts leads to a lower CMC and, consequently, to an increase in the micelle concentration, the effect of ionic strength on selectivity may be described as the influence of the increasing concentration of micelles.

The selectivity of separation in micellar chromatography differs from that in conventional ion-exchange[85] and ion-pair chromatography. In MLC, after the column equilibration with 10^{-4} M cetyltrimethylammonium bromide solution, anions are eluted with 0.025 M NaCl solution in the following sequence: IO_3^- < NO_3^- < Br^- < NO_3^- < I^- (see Ref. 30). The use of eluents containing high concentrations of an organic modifier (lower alcohols or acetonitrile) leads to degradation of micelles (in the presence of > 43.5% of methanol no micelles are formed at all[86]), so that retention is controlled only by the extent of surfactant adsorption on the stationary phase, and the selectivity becomes the same as that in ion-exchange or ion-pair chromatography.

On the whole, it should be noted that studies of the effects of various factors on the selectivity in MLC should consider all three of the equilibria established in the system. In the first place, there is an interaction of sorbates with micelles, which is characterised by the association (binding) coefficient K_2, and with the stationary phase characterised by the partition coefficient P_{SW}; they have opposite effects on retention.

V. Retention and structure of sorbate molecules

In RP HPLC, three types of simulation of retention parameters (or their functions of parameters characterising specific features of the sorbate structure and influencing its chromatographic behaviour) are used, *viz.*, simple additive models, a retention–property correlation and a retention–structure correlation.[1] However, in ion-pair chromatography the complexity of the processes occurring prevents construction of models which describe the effect of structural characteristics of sorbates on retention. Nonetheless, there are indications[1] that the parameters of retention models may be related to the contributions from different structural fragments and to the Gantsch π-constants of hydrophobicity. It would seem that in MLC where interactions are more numerous than in RP HPLC and ion-pair chromatography, construction of such models is not feasible. However, the existence of a clear-cut dependence of solubilisation on hydrophobic and hydrophilic properties of sorbates has stimulated the attempts to apply correlations of the retention–property type, which relate the capacity factor and the partition coefficient in a 1-octanol–water system (P_{OW}).

Thus Khaledi and Breyer[87] have revealed that the values of k' correlate well with the log P_{OW} for 35 related compounds on the phenyl phase with a cationic surfactant; however, the dependence of log k' on log P_{OW} forms a plateau at high values of the partition coefficient, *i.e.*, for hydrophobic compounds. For aromatic compounds on the stationary C_8 and C_{18} phases, a better correlation was obtained for the dependence of log k' on log P_{OW}, as in RP HPLC. The existence of a linear correlation $\log k' = f(\log P_{OW})$ was established for monosubstituted benzenes with sodium dodecyl sulfate, cetyltrimethylammonium bromide and Brij-35 as the mobile phases,[88] for a number of phenols and other monosubstituted benzenes with alcohol-modified mobile phases of sodium dodecyl sulfate and cetyltrimethylammonium bromide[76] and for a series of surfactants with mobile phases containing sodium dodecyl sulfate, cetyltrimethylammonium bromide and Brij-35.[89] A correlation between the log P_{OW} and the logarithms of the constant of sorbate association with the micelle was established.[76, 87, 90] A linear correlation was found to exist between the log k' (the capacity factor in the absence of micelles) and log P_{OW}.[87] The dependence of k' and log k' on log P_{OW} was studied by MLC for a series of benzene derivatives and surfactants on a stationary C_8 phase.[91]

Estimates of k', k'_m (k' at zero micelle concentration) and P_{MW} were derived for 26 1,4-dihydroxypyridines, and it was shown that they correspond to the hydrophobicity scale.[92] The best hydrophobicity scale is the $k' - \log P_{OW}$ correlation for the mobile phase of 0.031 M sodium dodecyl sulfate containing 3% of n-propanol; this phase is described by the equation $k' = 48/3 \log P_{OW} - 98.49$. The differences between the experimental and calculated values of log P_{OW} for 20 of the 26 compounds studied were found to be less than 3%.

A linear dependence was shown to exist between the free energy of transfer of a compound from the aqueous to the micellar phase in a water–octanol system for benzene and naphthalene derivatives in micellar[18] and hybrid[20] eluents, as well as for substituted phenols.[93] The slope of the straight lines is virtually independent of the nature of the surfactant (sodium dodecyl sulfate, cetyltrimethylammonium bromide or Brij-35) or of the presence of an organic modifier.[20] This indicates that in all cases the reactions of sorbates with the micelles are based on hydrophobic interactions. For alkyl-substituted phenols, the value of the free energy of transfer of the aliphatic hydrocarbon groups is less negative for the sodium dodecyl sulfate micelles than in the octanol–water system, whereas for the phenols containing the OR, COR and COOR substituents the contribution to the hydrophobicity is larger in the presence of sodium dodecyl sulfate than in the octanol–water system.[93] These differences may be due to the fact that solubilisation of different sorbates can occur in the micelle at different sites which differ in dielectric constants, local microviscosity, steric factors and the occurrence of electrostatic interactions with the ionic head groups of surfactants for charged sorbates.

The second group of correlations relates retention to the structure of sorbates described with the aid of various descriptors,[1] the simplest of which is the number of the carbon atoms in the molecule (n_C). In the example of n-alkylbenzenes and alkylphenols in sodium dodecyl sulfate and cetyltrimethylammonium bromide-containing systems, including those with propanol added,[19] it was shown that in contrast to RP HPLC where the dependence of log k' on n_C is linear, in MLC with both purely micellar and hybrid eluents only the dependence of k' on n_C is linear, whereas the dependence of log k' on n_C is better described by the quadratic function. Analogous dependences were derived in the studies of the retention of alkylbenzenes in the mobile phases containing Brij-22 or Brij-35.[16] Alkylphenols in the micellar mobile phases of sodium dodecyl sulfate are an exception. For this group of compounds the dependence of log k' on n_C is linear, while the dependence of k' on n_C is quadratic, as in RP HPLC.[19] For phenols and a hybrid sodium dodecyl sulfate mobile phase, the differences in k' and log k' are too small to allow one to

state with certainty which of the dependences is better approximated by the straight line. The cause of this phenomenon is not sufficiently clear. Khaledi *et al.*[19] proposed two possible explanations. The first explanation is based on the difference in the location of various sorbates in (or on) the micelle since the heterogeneous nature of the micelles creates a unique situation where different sorbates in the same mobile phase have microenvironments differing in polarity. The second explanation relies on the model of interaction indices,[94,95] according to which the dependence of $\log k'$ on the number of carbon atoms for aqueous-organic eluents is described by the equation

$$\log k' = (\log \gamma) n_C^2 + (\log \alpha) n_C + \log \beta , \quad (37)$$

where $\log \alpha$ and $\log \beta$ are constants and $\log \gamma$ is expressed by the Eqn (38)

$$\log k' \gamma = \frac{\Delta V_x \Delta I_x \Delta C_x C_M I_M}{2.3RT}, \quad (38)$$

where ΔV_x and ΔI_x are the changes in the sorbate volume and in the interaction index in a homologous series, ΔC_x and C_M are the proportionality constants for the sorbate and the mobile phase, I_M is the index of interaction for the mobile phase.

Usually the value of $\log \gamma$ for aqueous–organic eluents is so small that the first term of Eqn (37) may be neglected. It is probable that in MLC the product of $\Delta V_x \Delta I_x$ is somewhat larger for some homologues and this causes the departure of the dependence of $\log k'$ on n_C from linearity.

Nonetheless, Khaledi *et al.*[19] have succeeded in revealing linear dependences of the logarithms of sorbate–micelle association (binding) constants (K_2) and of partition coefficients of sorbates between the extramicellar mobile phase and the stationary phase (P_{SW}) on n_C. Analysis of these dependences showed that the K_2 values for homologues are larger in the cetyltrimethylammonium bromide micelles than in the sodium dodecyl sulfate micelles, which points to a stronger interaction of sorbates with the cetyltrimethylammonium bromide micelles owing to the availability of hydrophobic environments. The slope of these straight lines is the measure of free energy calculated for the transfer of one methylene group from the bulk of solution into the micelle (dependence of K_2) or into the stationary phase (P_{SW}), and this slope is steeper for cetyltrimethylammonium bromide than for sodium dodecyl sulfate, which is indicative of the higher affinity of the methylene group of cetyltrimethylammonium bromide due to its larger hydrophobicity.

Hinze and Weber[96] proposed a more rigorous approach to the rationalisation of the non-linearity of the dependence of $\log k'$ on the number of the carbon atoms in the surfactant molecule in MLC in their paper entitled 'Why the relationship between the logarithm of k' and homologue number in micellar chromatography is not linear'. In RP HPLC, it is implied that each CH_2 increment in the chain of a homologue makes a constant contribution to the free energy of sorbate transfer between the mobile and stationary phases. In MLC, the retention of small-sized polar sorbates that are well soluble in water and poorly soluble in micelles is basically determined by the partition between the surfactant-modified stationary phase and the non-micellar mobile phase. In a homologous series, compounds become less water-soluble as their size increases, and in the extreme case of total insolubility in water the sorbates are partitioned between the chemically similar micellar phase and the surfactant-modified stationary phase. For this process, the free energy of one methylene group transfer from the mobile to the stationary phase (ΔG^m) is virtually equal to zero. As in MLC the ΔG^m value depends on the homologue size, the dependence of $\ln k'$ on the number of methylene groups in the sorbate (n^m) should not be linear.

$$\frac{1}{k'} = \left(\frac{V_m}{V_s}\right) \exp\left[\frac{\Delta G^{fg}_{WS} + n^m \Delta G^m_{WS}}{RT}\right] + \quad (39)$$

$$+ \left(\frac{V_x}{V_s}\right) \exp\left[\frac{\Delta G^{fg}_{XS} + n^m \Delta G^m_{XS}}{RT}\right]$$

where the subscripts 'X', 'W' and 'S' refer to the micellar, aqueous and stationary phases; V stands for the corresponding volumes of phases and the superscripts 'fg' and 'm' refer to the functional and methylene groups, respectively. The dependence of $\ln k'$ on n^m is described by the equation

$$\ln k' = -\ln[(1-\Phi_x)\exp(a + n^m b) + \Phi_x \exp(c + n^m d)] , \quad (40)$$

where $\Phi_x = V_x/V_m$ is the ratio of the volumes of micelles and stationary phase; a, b, c and d are constants. The parameters of a non-linear regression were calculated, the free energies of the methylene group transfer from the non-micellar part of the mobile phase (pure water or water containing 5 vol.% of alcohols) into the surfactant-modified stationary phase [parameter b, Eqn (40)] and the free energy of the methylene group transfer in the micelle–stationary phase system [parameter d, Eqn (40)] were estimated for alkylbenzenes with the use of sodium dodecyl sulfate–1-pentanol–water and Brij-35–water systems and also for methyl esters of fatty acids with the use of a sodium dodecyl sulfate–1-propanol–water system.

Recent years witnessed the appearance of more complex models which relate retention to the sorbate structure and which are based on the statistical analysis and the method of analysis of basic components.[97,98] In the example of 17 surfactants with three different micellar mobile phases (sodium dodecyl sulfate, cetyltrimethylammonium bromide, Brij-35) it was shown that retention can be adequately described using only two of the nine descriptors: L/B (ratio of the maximum molecule length to its width) and one of the topological descriptors.[97] The contribution from the topological descriptors is most important in the case of cationic and anionic surfactants, whereas the electrostatic interactions are particularly substantial in the case of sodium dodecyl sulfate. Correlation equations of the types $\log k' = f(F, L/B, c)$ and $\log k' = f(n, L/B, c)$, where F is the correlation factor, c is the surfactant concentration in the micellar mobile phase, n is the number of π-electrons in the molecule, were derived. These equations are easier to use because the values of the corresponding descriptors can be calculated directly from the structural formulae of compounds. Analogous correlations can also be established for the partition coefficients P_{SM}, P_{SW} or P_{MW} since they can be calculated from experimental chromatographic data using Eqn (1) or (2).[76] For sodium dodecyl sulfate, correlations of the type P_{SM} (P_{SW} or P_{MW}) $= f(L/B, F)$ were obtained; however, the regression is deteriorated in the sequence $P_{SM} > P_{MW} > P_{SW}$ because of the increased uncertainty of calculations.[97]

In the hybrid mobile phases which contain sodium dodecyl sulfate with additions of methanol, 2-propanol or n-butanol the retention of unsubstituted PAH is described by the equation considering the size and shape of molecules.

$$\log k' = aF + bL/B + c, \quad (41)$$

where a, b and c are constants.[98]

This model does not however describe methyl-substituted PAH. Therefore, new models were proposed which include electronic parameters allowing consideration of the distribution of electronic density in the molecule, *viz.*, dipole moments (DPMON) and the share of the non-polar unsubstituted surface area (NUSA/TSA) [Eqn (42)].

$$\log k' = aF + bL/B + c\text{DPMON} + d\text{NUSA/TSA} + e, \quad (42)$$

where a, b, c, d and e are constants. Equation (42) gives a satisfactory description of the retention of both unsubstituted and substituted surfactants upon addition of < 20 vol.% of methanol or 2-propanol or < 6 vol.% of n-butanol.

The influence of molecular structure on the constants of the sorbate–micelle and sorbate–stationary phase binding has been studied.[99,100] The existence of a clear-cut linear dependence between the constants of the sorbate–micelle and sorbate–

stationary phase binding was demonstrated.[100] Fairly good quantitative dependences of the constants of sorbate–micelle and sorbate–stationary phase binding on solvatochromic properties were obtained.[99,100] The correlation between k' and solvatochromic parameters is better in the presence of tetradecyltrimethylammonium bromide micelles, whereas the correlation between log k' and solvatochromic properties is better in the presence of sodium dodecyl sulfate micelles; the addition of 7% of 2-propanol or 5% of butanol does not modify the strong correlations revealed for tetradecyltrimethylammonium bromide.[99] An important role in the retention of neutral sorbates is played by both the process of cavity formation and the hydrogen bonding. The examples considered above indicate that such models not only allow prediction of the retention of various sorbates but are also helpful for revealing interactions of sorbates with stationary and mobile phases.

Guermouche *et al.*[101] studied theoretical aspects of the retention of 15 aromatic compounds in MLC using the zwitterion surfactant n-dodecyl 3-(*N*,*N*-dimethylamino)propane-1-sulfonate (DDDMAPS) and calculated the constants of equilibrium partition of sorbates using Eqn (1). Compared to the cationic and anionic surfactants in the DDDMAPS-containing mobile phases, the lowest values of K_{SW} and K_{MW} were obtained, which leads to larger capacity factors at a constant surfactant concentration. Sorbates are retained owing to hydrophobic interactions with the stationary phase and electrostatic interactions with the surfactant in the micelles as well as in the modified stationary phase. Molecular interactions were studied using the method of linear dependences of solvation energy.[101] The sorbate size and basicity were shown to be the dominant factors affecting the retention. The dipolar environment in the zwitterionic DDDMAPS is larger and this influences the partition of sorbates: acidic sorbates are more easily bound to this surfactant.

A correlation between the retention and biological activity was established using models of the retention–property type. Breyer *et al.*[102] revealed a clear-cut correlation between k' and biological activity, measured as log $1/c$, where c is the concentration required for a 50% inhibition of growth, for 26 *para*-substituted phenols. The advantage of this approach consists in that it uses a single parameter, *viz.*, retention in MLC, whereas normally three descriptors are used for estimating biological activity: log P_{OW}, acid dissociation constant and resonance parameter. Owing to the unique properties of surfactant micelles in MLC, both hydrophobic and electronic interactions are combined in one parameter, *viz.*, k'. The nature and acidity of the stationary phase influence the correlation between k' and log $1/c$; the optimum system is cetyltrimethylammonium bromide supplemented with 10 vol.% of 2-propanol.

Escuder-Gilabert *et al.*[103] studied qualitative and quantitative correlations between the retention and the anesthetic strength, equianesthetic concentration, duration of action, toxicity and the half-life of anesthetics. Clear-cut correlations between k' and the anesthetic strength of compounds were established.

Deriving correct correlations requires reliable determination of the dead time (t_0) which is problematic in MLC. In MLC, the magnitude of t_0 is measured with respect to the introduction of water,[93,104] salt solutions ($NaNO_3$,[25,37] NaI and KI[11,19]) and acetonitrile.[66]

Torres Lapasio *et al.*[105] proposed four different criteria for determining the characteristic time at the beginning of chromatography (the time of the exit of the first peak, first perturbation) that may be called reference time.

1. Search for the first basic maximum or minimum.

2. Formation of groups reducing noises to a definite scale.

3. Formation of groups for scaling the slopes of the basal line noises.

4. First-derivative method consisting in the detection of an important change in the basal line slope upon the occurrence of a considerable perturbation. The best results are given by the noise-scaling criterion; in this case it is advisable to measure the reference times upon addition of standard solutions or micellar solutions of sorbates. Determination of the reference time using a large number of different mobile phases has shown that it does not change noticeably as the mobile phase composition is modified.

VI. Analytical application of micellar liquid chromatography

The development of the theory of MLC was based on the studies of different organic compounds: non-ionogenic (substituted benzenes, phenols, naphthalenes, anthracenes, *etc.*) as well as ionogenic (amino acids and peptides), whereas practical uses of this method lie in the analysis of medicinal preparations (Table 1). Such an analytical application reflects several advantages of MLC: selectivity and capacity for simultaneous separation of ionic and non-ionic components of samples, direct introduction of biological samples without preliminary precipitation of proteins or extraction of drugs, as well as the possibility of fluorescence enhancement or stabilisation. Simplification of the sample preparation for the analysis of biological specimens is achieved owing to solubilisation of proteins on micellar aggregates since the protein–micelle aggregates have a large size and are not retained by the stationary phase. Low detection limits make it possible to detect specific compounds not only in pharmaceutical formulations but also in urine.

The possibility of enhancement of sensitivity of determination of some compounds in the presence of surfactants has been demonstrated. Thus the oxidation current of acetaminophen is increased in the presence of sodium dodecyl sulfate micelles.[108] An electrochemical detector with a cell of the 'reflecting wall of the three-electrode configuration' type with a carbon-fibre microelectrode was designed for detecting acetaminophen. The compatibility of the separation by MLC with amperometric detection was first demonstrated by Khaledi and Dorsey;[126] dopamine was determined using a glassy-carbon electrode.[124]

Steroids were determined in urine by the MLC method and detected using the terbium-sensitised fluorescence.[106] The micellar environment promotes an efficient Forster energy transition from donors (steroids) to acceptors [terbium(III) ions]. This method is highly sensitive and allows determination of as little as 100 pg of steroids in urine. This detection technique is appreciably more sensitive than the spectrophotometric detection,[114] which is applicable only for the analysis of initial pharmaceutical preparations and is unsuitable for the analysis of biological samples.

The luminescence [sensitised by a terbium(III)–surfactant complex] in the inverted micelles in non-aqueous solutions (ethyl acetate or its mixture with hexane) was used for the detection of theophylline;[120] the presence of paraxanthin does not interfere with this determination due to the low efficiency of energy transfer to the lanthanide ion. This method minimises the fluorescence quenching induced by the use of inverted micelles in non-aqueous solvents.

Examples of the use of micellar mobile phases in the inorganic analysis are not numerous: separation of inorganic anions;[30,32,85] cations of transition metals;[9,31] chelates;[127–131] heteropoly-acids;[132,133] different forms of arsenic,[77,134,135] mercury,[77,135] selenium[77] and tin;[77,136] organic and inorganic compounds containing heavy metals.[137]

Thus the separation of five anions on a column (250×5 mm) packed with ODS (5 μm) using 1.36×10^{-1} M aqueous cetyltrimethylammonium chloride as the eluent at a flow rate of 1.5 ml min^{-1} was effected within 10 min.[85] Detection was carried out at 205 nm. The order of elution: $IO_3^- < NO_2^- < Br^- < NO_3^- < I^-$ is analogous to the selectivity sequence for a typical, strongly basic anion exchange resin.

The use of micellar exclusion chromatography on a column packed with the Asahipak GC-310H sorbent, which is a polyvinyl alcohol gel, proved to be effective for the separation of both anions[30] and cations.[32] In the separation of anions the elution order varies with the modification of hydrophobicity of the

Table 1. Determination of components of medicinal preparations by MLC using sodium dodecyl sulfate.

Components of medicinal preparations	Organic modifier	Detector	Detection limit	Object of analysis	Ref.
Steroids	acetonitrile	FL	100 pg	urine	106
Amiphenazole, amiloride, amphetamine, clostebol, ephedrine, phenylpropanolamine, methanedienone, methoxyphenamine, nandrolone, spironolactone	–	SP	0.07 – 10.95 μg ml^{-1}	"	107
Acetaminophen	n-propanol	EC	0.02 μg ml^{-1}	"	108
Clenbuterol	n-butanol	SP	–	"	109
Diuretics	n-propanol	SP	1.7 – 2.9 μg ml^{-1}	"	110
Caffeine, theophylline, theobromine	"	SP	0.36 – 1.23 μg ml^{-1}	"	111
N-(1-Benzo[*b*]thien-2-yl)ethyl-*N*-hydroxyurea, its *N*-dehydroxylated metabolite	"	SP	0.08 – 0.1 μg ml^{-1}	"	112
Sulfonamides	"	–	–	urine, milk	113
Amiloride, bendroflumethazide, chlorothalidone, spironolactone, triamerene	n-pentanol	–	–	pharmaceutical preparations pills	114
Diuretics	n-propanol	SP	0.029 – 0.55 μg ml^{-1}	"	115
Catecholamines	"	SP	2 – 16 μg ml^{-1}	capsules, pills	48
Anabolic steroids	n-pentanol [a]	SP	6.4 – 860 ng ml^{-1}	pills, suspension, gel, solution for injections	116
Proteinic amino acids	n-propanol	SP	(1.3 – 1.5) × × 10^{-7} mol litre^{-1}	pharmaceutical preparations	117
Diuretics	–	–	–	the same	118
β-Blockers (atenolol, metoprolol, oxyprenolol, diuretics (amiloride), bendroflumethazide, hydralazine	n-propanol	SP	–	pills, capsules	119
Theophylline	"	SP	6 μg ml^{-1}	pills	120
6-Mercaptopurine, 6-thioguanidine	–	–	–	serum	121
4-Formylaminoantipyrine, 4-aminoantipyrine, 4-methylaminoantipyrine	n-pentanol	SP	10.54 μg ml^{-1} – – 17.0 ng ml^{-1}	plasma	122
(R)- and (S)-Naproxenes	–	–	–	human liver microsomes	123
Dopamine	–	EC	–	–	124
Theophylline	ethyl acetate, hexane	FL	9 ng ml^{-1}	–	125

Note: FL, fluorescent; SP, spectrophotometric; EC, electrochemical (amperometric) detectors, respectively; [a] microemulsion.

surfactant alkyl chain: with 0.06 or 0.01 M cetyltrimethylammonium chloride mobile phase, the anions are eluted in the sequence $IO_3^- < I^- < Br^- < NO_2^- < NO_3^-$, whereas in the presence of dodecyltrimethylammonium chloride this order is $IO_3^- < Br^- < NO_2^- < I^- < NO_3^-$ (see Ref. 30).

Using micellar exclusion chromatography, Okada [29] separated Pb(II), Co(II) and Cu(II) in 0.05 M sodium dodecyl sulfate containing 0.08 mol litre^{-1} of NaCl (pH 4.0) and also a mixture of cations of rare-earth elements (Yb, Ho, Dy, Tb and Pr) and of transition metals [Cu(II), Pb(II), Zn(II), Ni(II), Co(II) and Mn(II)] in the gradient mode of elution with a mixture of 0.025 M sodium dodecyl sulfate and 0.08 M α-hydroxyisobutyric acid (pH 4.0) or with 0.025 M sodium dodecyl sulfate and 0.08 M tartaric acid (pH 4.3).[31] Separation of 8 transition metal cations [Fe(III) < Cu(II) < Zn(II) < Ni(II) < Pb(II) < Co(II) < Cd(II) < Mn(II)] was performed in 20 min on a column with Inertsil ODS-2 with an aqueous mobile phase containing 0.0945 mol litre^{-1} of sodium dodecyl sulfate and 0.0684 mol litre^{-1} of tartaric acid (pH 4.02).[29]

Miura *et al.*[128] determined the distribution constants of 2-(2-thiazolylazo)-4-methylphenol (TAMP) and 2-(2-thiazolylazo)-5-dimethylaminophenol (TAAP) in solutions of non-ionic surfactants [poly(oxyethylene)-4-nonylphenyl ether (PONPE), poly(oxyethylene)oleyl ester (POOE), Triton X-100, POL-10]; these constants were found to decrease as the length of the oxyethylene chain increased. Rapid elution of TAMP and TAAP is achieved using surfactants with shorter oxyethylene chains because the reagents have the higher affinity to the micellar phase. The retention time is reduced as the number of oxyethylene units in PONPE and POOE decreases.

PONPE-20 was used for the separation of metal chelates with TAAP.[127, 128] Of the nine chelates studied, only the V(V) chelate is eluted selectively, the peak of Fe(III) chelate overlaps with the peak of the solvent front, while the reagent itself and other complexes are strongly retained (and some of them are possibly degraded) in the column.[127] Probably, the use of hybrid eluents (supplemented with organic modifiers) would yield better results. Separation of Cu(II), Zn(II), Ni(II) and Co(II) takes 45 min using a mobile phase containing 0.006 mol litre^{-1} of sodium dodecyl sulfate, 0.002 mol litre^{-1} of 8-hydroxyquinoline-5-sulfonic acid and pH gradients of 3.5 → 3.9.[131] Determination of aluminium with a detection limit of 1 ppb in the form of chelates in human serum with 8-hydroxyquinoline was performed on a column packed with CAPCELL PAK MF ph-1 with an eluent containing 20 mass % of acetonitrile, 0.01 M sodium dodecyl sulfate, 10^{-4} M EDTA, 0.01 M *N,N*-bis(2-hydroxyethyl)-2-aminoethanesulfonic acid-based buffer (pH 7.0) with fluorescent detection (370 – 504 nm).[130] The contents of Ni(II), Co(II) and Cu(II) in metal alloys were determined using chelates with sodium diethyldithiocarbamate and the mobile phase containing 0.03 M cetyltrimethylammonium bromide, 10^{-4} M sodium diethyldithiocarbamate and 45% of 1-propanol (spectrophotometric detection).[129] The detec-

tion limits of Ni(II), Co(II) and Cu(II) were 68.1, 67.2 and 35.4 pg, respectively; the separation time did not exceed 6 min.

Compatibility of MLC with the mass-spectrometric determination with the use of induction plasma was demonstrated in the example of determination of arsenic using the cetyltrimethylammonium micelles.[135] In this case, the detection limits (90–300 pg) were comparable with that in ion-pair chromatography (0.4–1.2 ng cm^{-3} at a loop volume of 250 mm^3 with detection by atomic-fluorescent spectrometry with preliminary generation of hydrides[138]) but they were inferior to that in ion-exchange chromatography (20–91 pg[139]). This may be due to the larger volumes of peaks and a complex composition of the mobile phase and, especially, to the influence of sodium ions from buffer solution which can suppress ionisation of arsenic.

A series of studies[77, 135, 136] were devoted to the use of vesicles in MLC. If a micelle is a closed monolayer, then a vesicle is a closed bilayer. The cavity is filled with the substance which is contained in the environment.[22] To be able to form vesicles the surfactant molecules should contain two (or more) hydrophobic hydrocarbon radicals with 16–18 carbon atoms. These requirements are met, for example, by didodecyldimethylammonium bromide[135, 136] and dihexadecyl phosphate.[75, 136] These mobile phases can be used in combination with selective detection techniques, such as atomic-emission spectrometry with induction plasma (also after formation of hydrides), mass-spectrometry with induction plasma, atomic-absorption spectrometry of cold vapour and atomic-absorption spectrometry with electrothermal atomisation.

Interesting results were obtained in MLC of heteropolyanions:[132, 133] a mixture of $BW_{12}O_{40}^{5-}$, $SiW_{12}O_{40}^{4-}$ and $SiMo_{12}O_{40}^{4-}$ was separated within 10 min and a mixture of $SiW_{11}FeO_{39}^{5-}$, $BW_{12}O_{40}^{5-}$ and $SiW_{11}O_{39}^{8-}$ within 7 min.

However, the results of the application of MLC in inorganic analysis, even though being interesting *per se*, are basically inferior to those achieved in ion-pair or ion-exchange chromatography.[140–142]

VII. Comparison of the possibilities of ion-pair and micellar liquid chromatography

As the role of micelles is similar to that of organic modifiers owing to their ability to increase solubility of uncharged sorbates in water, MLC is usually compared to RP HPLC. Separation of uncharged and hydrophobic compounds, for which RP HPLC is widely used, is the field in which MLC is inferior as regards its efficiency, eluting strength and selectivity. The efficiency of columns in MLC is appreciably lower than that in RP HPLC and decreases as hydrophobicity of compounds increases. The micellar eluents are weak eluents; therefore, the retention times of hydrophobic sorbates are long. Selectivity is also low because of the similarity of processes which occur in the mobile and stationary phases,[19, 66] although in a number of instances the reversal of the elution order is observed.[143–145] All of these characteristics of the chromatographic system can be enhanced by the introduction of organic modifiers into the eluent; however, MLC will not all the same be competitive with RP HPLC in the separation of hydrophobic sorbates.

An advantage of the MLC method is the possibility of separating mixtures of ionic and non-ionic sorbates. Another method allowing solution of this problem is ion-pair chromatography. These methods have much in common: both use surfactants; electrostatic interactions play a substantial role (though if in ion-pair chromatography this role is crucial and determines retention, in MLC it is secondary), while organic modifiers regulate the eluting strength and selectivity of separation. Also, there are differences in principle between these two methods. Micellar solutions are considered in MLC as macroscopically homogeneous systems composed of two phases: the micelles and the bulk of aqueous solution. In the ion-pair chromatography, the mobile phases are homogeneous and consist solely of surfactant monomers. Furthermore, the amount of surfactant adsorbed on the stationary phase increases with the concentration of the ion-pairing agent in the mobile phase. Descriptions of MLC by different authors are contradictory: some believe that the amount of adsorbed surfactant is almost constant after it exceeds CMC, while others argue that the surfactant adsorption remains substantial at concentrations several tens of times that of CMC.

Thorough comparison of the possibilities of both methods was carried out in the example of separation of amino acids and peptides.[66, 146] The separation was performed using 2-propanol-modified sodium dodecyl sulfate-containing mobile phase. Investigation of the dependence of retention on the alcohol concentration showed that in the case of ion-pair chromatography the parameter of the solvent strength S correlates well with the logarithm of capacity factor in a purely aqueous phase ($\ln k'_0$). The selectivity of separation drops with the increasing content of the organic solvent in the mobile phase. Such dependences are also characteristic of RP HPLC. Totally different regularities were observed in MLC: variation of S proved to be reciprocal to changes in $\ln k'_0$, whereas the separation selectivity of most sorbate pairs increased as the content of the organic solvent in the mobile phase increased.

In MLC and ion-pair chromatography, the surfactant concentration has different effects on the retention of sorbates. In ion-pair chromatography the retention time of oppositely charged sorbates (at pH 2.5, the amino acids and peptides studied have a partial positive charge) increases with the concentration of the ion-pairing agent due to the larger amount of surfactant adsorbed on the stationary phase surface and forms a plateau upon saturation of the stationary phase. In MLC, the retention time is decreased as the total surfactant concentration in the system increases. In ion-pair chromatography there is no clear-cut dependence of selectivity on the ion-pair reagent concentration; though it is usually increased with the increase in concentration, particularly when sorbates differ in their charges. In MLC, the selectivity is as a rule enhanced as the micelle concentration increases. In ion-pair chromatography, the eluting strength of the mobile phase decreases as the ion-pairing agent concentration increases, whereas in MLC it increases with the concentration of micelles. Thus, the enhancement of selectivity and eluting strength occurs in parallel in MLC and antiparallel in ion-pair chromatography.

Many investigators noted better reproducibility of the retention characteristics in MLC compared to that in ion-pair chromatography. In the latter case, one often has to operate in the range where the retention is strongly dependent on the ion-pairing agent concentration; therefore, even minor changes in the mobile phase composition lead to a fairly appreciable variation of retention. Both methods were shown to have lower column efficiency compared to that in RP HPLC. Thus a new 25-cm long column packed with a C_{18} sorbent had an efficiency of 14 000 theoretical plates in the elution with an acetonitrile–water mixture (25 : 75). In the presence of sodium dodecyl sulfate, its efficiency dropped to 5500 theoretical plates in both MLC and ion-pair chromatography. Nonetheless, the ion-pair chromatography has an advantage compared to MLC as regards the efficiency owing to the variation of the hydrophobic nature of the ion-pairing agent.

It should be noted in conclusion that MLC is an interesting method with the unique possibilities of selectivity variation; its theory has been well developed. The micellar liquid chromatography is an alternative to the RP HPLC method. Analytical chemists consider this method as the most useful in the analysis of biological specimens.

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