Research Article

Selectivity of Brij-35 in Micellar Liquid Chromatographic Separation of Positional Isomers

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Implementation of Brij-35, a nonionic surfactant, as a mobile phase for separation of positional isomers is investigated. Chromolith C-18 SpeedROD is used as a stationary phase. The effect of surfactant and organic modifier (propanol) concentration on the separation of some selected isomers is studied and evaluated in terms of linear solvation energy relationship (LSER). Shape selectivity is assessed by $\alpha$ value of sorbic and benzoic acid, which is found to be 1.339 by using mobile phase composed of 0.5% aqueous solutions of Brij-35 and propanol in 9:1. Isomers of parabens, nitroanilines, nitrophenols, and quinolinols are successfully separated using mobile phases composed of various percentages of surfactant and propanol. System constants for nonionic MLC using LSER analysis show that hydrogen bond basicity and dipolarity may be major contributors to selectivity, while excess molar refraction helps fine-tuning the separation which also imparts unique selectivity to nonionic surfactants as compared to ionic ones.

1. Introduction

Use of surfactants as mobile phases in HPLC above critical micellar concentration (cmc) gave birth to a new branch of chromatography, now known as micellar liquid chromatography (MLC) [1]. Since its first use for separation of PAHs in 1980 [2], a number of publications, books, and monographs appeared in the literature covering the applications and characterization of MLC systems [3–5].

The most commonly used surfactant in liquid chromatography and in overall analytical chemistry is sodium dodecylsulfate (SDS) [6], an anionic surfactant as compared to other cationic and nonionic surfactants [7]. In nonionic surfactants, Brij-35 (polyoxyethylene dodecyl ether) has found applications in liquid chromatography as it is non-UV absorbing, which is a drawback of other nonionic surfactants having aromatic ring into their structure.

Brij-35 is reported as mobile phase for the separation of a number of compounds [7]. In a previous report [8] by our group, we reported that isomers of propyl-parabens could be better separated by Brij-35 as compared to SDS. Takayanagi et al. [9, 10] have also reported the separation of various positional isomers using Brij-35 as a surfactant in capillary electrophoresis. Vlaseenko et al. [11] have studied the dissociation constants of hydroxybenzoic acids and parabens and found that addition of Brij-35 alters the dissociation of these compounds. Inspite of the number of publications on MLC using non-ionic surfactants as mobile phases, none of them is specifically focused on separation of isomers.

Separation of positional isomers is of importance from pharmaceutical perspective, due to differences in their complexation, toxicity, and reactivity in biological systems. Positional isomers cannot normally be separated through reverse phase HPLC with ODS columns commonly available in the laboratories with methanol/water or acetonitrile/water as mobile phases [12]. The objective of current study is to evaluate if Brij-35 could be used as mobile phase for separation of positional isomers and to find out the interaction responsible for selectivity in MLC using Brij-35. Here, we have investigated nonionic surfactants as mobile phase for separation of positional isomers using Chromolith C-18 column. The organic modifier (propanol) and surfactant (Brij-35) concentration on separation of some
Table 1: Retention time and resolution data for various positional isomers using hydro-organic and hybrid surfactant mobile phase composition.

| Analyte        | Solvent composition | \(^*t_R\) (R) | Solvent composition | \(^*t_R\) (R) |
|----------------|---------------------|---------------|---------------------|---------------|
| 2-nitroaniline | 2.87                | 88 : 12       | Brij35 (2% aq. soln.)/propanol | 4.57          |
| 3-nitroaniline | 2.28 (1.99)         | 99 : 1        | Brij-35 (1.5% aq. soln.)/propanol | 3.7 (2.56) |
| 4-nitroaniline | 1.99 (1.14)         | 90 : 10       | Brij35 (1.75% aq. soln.)/propanol | 3.43 (0.92) |
| 2-quinolinol   | 2                   | 99 : 1        | Brij-35 (1.5% aq. soln.)/propanol | 1.69          |
| 4-quinolinol   | 1.67 (1.53)         | 96 : 4        | Brij35 (1.75% aq. soln.)/propanol | 1.65          |
| o-cresol       | 2.03                | 90 : 10       | Bezoic acid 7:3 MeOH/water | 1.65          |
| p-cresol       | 1.96                | 96 : 4        | Sorbic acid 4:6 MeOH/water | 1.65          |
| m-cresol       | 1.96                | 90 : 10       | Bezoic acid 7:3 MeOH/water | 1.65          |
| m-nitrophenol  | 2.27                | 88 : 12       | Brij-35 (2% aq. soln.)/propanol | 1.83          |
| p-nitrophenol  | 2.07 (0.453)        | 99 : 1        | Brij-35 (2% aq. soln.)/propanol | 1.62 (1.35) |
| Dexamethazone  | 2.49                | 96 : 4        | Sorbic acid 4:6 MeOH/water | 4.24          |
| Betamethasone  | 2.52                | 99 : 1        | Sorbic acid 4:6 MeOH/water | 4.04          |
| Propylparaben  | 2.3                 | 96 : 4        | Sorbic acid 4:6 MeOH/water | 7.33          |
| iso-Propylparaben | 2.21 (0.32)   | 96 : 4        | Sorbic acid 4:6 MeOH/water | 6.47 (0.809) |
| Bezoic acid    | 1.78                | 90 : 10       | Sorbic acid 4:6 MeOH/water | 4.92          |
| Sorbic acid    | 2.24 (0.64)         | 90 : 10       | Sorbic acid 4:6 MeOH/water | 3.88 (1.73) |

\(^*t_R\) is retention time in minutes; in parenthesis “R” is resolution.

Positional isomers are studied. The findings are discussed and selectivity is accessed by using benzoic acid/sorbic acid selectivity ratio and reported in terms of LSER parameters, which are then correlated to understand the system selectivity.

2. Experimental

2.1. Instrumentation. A Hitachi 6010 liquid chromatograph fitted with a Hitachi L-4200 variable wavelength UV-Vis detector, a Rheodyne 7125 injector, and a Chromolith performance RP-18 e, 100 mm × 4.6 mm i.d. column (E. Merck, Darmstadt, Germany) was used. CSW32 software (Data Apex) was used for data acquisition and integration. The λ 220 nm (quinolinols), 254 nm (Methazones, parabens, cresols, and nitrophenols), and 320 nm (nitroanilines) were set, and flow rate was 1.0 mL/min for all experiments except nitrophenols and quinolinols, which is 2.0 mL/min.

2.2. Materials and Reagents. Brij-35 was purchased from Sumito Corporation, Tokyo, Japan, prepared in Millipore water and used without degassing and filtration; propanol (Fluka, Sigma-Aldrich, Buchs, St.Gallen, Switzerland) was mixed within system depending on percentage required. Benzoic acid, quinolinols, nitroanilines, parabens (Fluka, Sigma-Aldrich, Buchs, St.Gallen, Switzerland), sorbic acid (Merck, Frankfurt, Germany), beta-and dexamethasone (Glaxo SmithKline, Dungarvan, Ireland) and cresol (Riedel-Hansen, Sigma-Aldrich, Seelze, Lower Saxony, Germany) were used as received their stock solutions were prepared in propanol, and working solutions were diluted with running mobile phase. HPLC grade methanol was purchased from Fisher Scientific, Loughborough, UK.

3. Results and Discussion

3.1. Separation of Isomers. Table 1 shows the retention time and resolution data for various isomers including benzoic acid and sorbic acid for hydro-organic and hybrid non-ionic micellar mobile phase. It could be clearly observed that same mobile phase is not optimum for all separations and for most solutes studied here; separation is also possible with hydro-organic mobile phases but micellar mobile phase offers better resolution.

These separations were studied with mobile phase composed of aqueous solution of surfactant with concentration (1-2%) and modifier in the ratio 1–20% to the surfactant at pH 3 (adjusted with phosphoric acid). All the solutes were studied for 1.25, 1.5, 1.75, and 2% Brij-35 aqueous solution prepared in millipore water with various percentages of propanol. It was found that the increase in surfactant concentration can reduce the retention time but does not affect separation appreciably. So, for all pair of analytes, surfactant that provides better resolution was selected. Addition of organic modifier (1-propanol) imparts interesting changes in retention times and resolution. Figure 1 shows the effect of propanol concentration on retention times of isomers of nitroanilines, quinolinols, and parabens.

Propanol decreases the retention times in all cases, and more difference in retention time is seen at small
Figure 1: Effect of propanol concentration on retention of isomers of (a) quinolinol (Brij-35 1.5% aq. Soln.), (b) nitroanilines (Brij-35 1.75% aq. Soln.), (c) parabenes (Brij-35 1.5% aq. Soln.), and (d) parabens (Brij-35 1.75% aq. Soln.).

organic modifier content but resolution is better at higher concentrations. The decrease in retention is linear for quinolinols (Figure 1(a)) and nitroanilines (Figure 1(b)) though for nitroanilines not as good as for quinolinols. For parabens, a visible shift in retention time trend at 6% propanol (Figures 1(c) and 1(d)) is seen which is also observed with nitroanilines (visible when Figure 1(b) is expanded) and other compounds (data not shown). These findings are different in the previously reported data [7] that up to 20% propanol concentration micelles exist which may still be valid that this trend may be due to stripping of adsorbed surfactant from stationary phase at increasing propanol concentration. This study indicates that retention behaviour in MLC changes at 6% propanol with non-ionic Brij-35 surfactant when hybrid micellar mobile phase in HPLC with C-18 columns is used.

Better separation of isomers is observed at higher propanol concentration in most cases studied here as relatively sharp peaks appeared as compared to those at low propanol content (Figure 2). This may be due to decreased amount of adsorbed surfactant on C-18 stationary phase, which helps better mass transfer between stationary and mobile phase hence reduce diffusion of analyte.

It is also interesting to observe that o- and m-nitroanilines are better resolved with methanol/water while
m- and p-nitroanilines with hybrid surfactant system. Cresols could not be separated with either system; for rest of the studied isomers, better separations are obvious using hybrid surfactant mobile phases.

Selectivity of system was assessed by calculating a value of benzoic acid/sorbic acid $a_{BA/S}$. Highest $a_{BA/S}$ value of 1.40 was observed with Brij35 (1.5% aqueous solution)/propanol in the ratio of 90:10, while with 40:60 methanol/water the factor was 0.74. Also the elution order was reversed; with surfactant containing mobile phase sorbic acid elutes first, while with hydro-organic benzoic acid appears first.

3.2. Interactions Responsible for Separation of Isomers. In traditional hydro-organic chromatography, selectivity for separation of isomers is correlated with molecular order within stationary phase. Selectivity is enhanced with increasing hydrocarbon stationary phase, increased chain length, and decreased temperature [17]. Unique selectivity is observed with micellar liquid chromatography as compared to hydro-organic chromatography, that is, retention factor has linear relationship with homologous series in MLC in contrast to LC which is logarithmic. The phenomenon is explained on the basis of absence of free energy change in MLC for the formation of cavity in mobile phase for incorporation of solute with increasing hydrophobicity [18]. The linear increase in “$k$” is also ascribed for larger number of compounds eluted per unit time [14, 18].

In MLC, not only mobile phase contributes to the separation but surfactant modified stationary phase also plays a role. It is obvious in early reports on MLC that surfactants adsorb on stationary phases consequently modify their properties. Ionic surfactants adsorb on ODS surfaces and reaches plateau when surfactant-modified mobile phases are run. For nonionic surfactants, no plateau is observed and adsorption of surfactant is continuous with micellar mobile even above the cmc [19]. Quíñons-Torrelo et al. [20] have reported that nonionic surfactant impart interesting changes in polarity of stationary phase and termed the type of chromatography as Micellar Biopartitioning Chromatography (MBC). The same author further reported that this cannot afford separation of enantiomers, while in our work we can partially separate enantiomers of steroids; this shows that statement is valid for MBC only.

Linear solvation energy relation (LSER) is used to understand the types and relative strengths of chemical interactions that control retention and selectivity in various separation techniques [21]. LSER equation as proposed by Abraham is $\log k’ = c + eE + sS + aA + bB + vV$, where $k$ is retention factor, $c$ is constant, $E, S, A, B, V$ are solute descriptors independent of mobile and stationary phase used. $E$ is solute excess molar refraction, $S$ is dipolarity/polarizability, $A$ and $B$ are acidity and basicity and $V$ is MsGowan’s characteristic molecular volume. The lower case letters $c, e, s, a, b, v$ are the system constant reflecting the difference in solute interactions between mobile and stationary phase [22].

LSER has also been applied to MLC [13], where additional solute descriptors are added in the equation to explain the behaviour of ionic surfactants. In a recent review by Ruiz-Ángel [14], it is demonstrated that hydrophobicity coefficient “$v$” varies in narrow range with change in micellar mobile phase composition hence hydrophobic interactions are scarcely affected. LSER studies of MBC has shown to have pronounce effect by hydrophobicity as given by high coefficients for “$v$” [20]. This type of effect can also be observed in a report by Mutelet et al. [15], where $v$ values for Brij-35 (0.08 M), SDS (0.1 M), and CTAB (0.01 M), with 15% isopropanol as additive are 1.69 ± 0.13, 0.75 ± 0.08, and 0.56 ± 0.08, respectively.

Nonetheless, characterization of separation of isomers is explained on the basis of hydrophobicity value $v$ since most of the positional isomers have identical $v$ value for example, 2- , 3-, 4-nitroaniline. Berthod et al. [22] have discussed the utility of Abraham’s LSER model to characterize chiral recognition behaviour of teicoplanin aglycon chiral phases. Appreciably different patterns were found for chiral phases as compared to others, while it is worth noting in the report that $e$ and $s$ parameters besides $b$ and $v$ also play a role in fine tuning of separation mechanism.

The system constants using LSER approach are system specific, that is, applicable to given stationary and mobile phase. As for non-ionic liquid chromatography, system constants are already reported so to understand the isomer recognition mechanism of MLC Linear Solvation Energy Relationship parameters for various systems reported in the literature gathered (Table 2).

Systems listed in Table 2 are included here on the basis of columns and mobile phases used; ODS column is most commonly used while polar-endcapped and polar embedded columns were included bearing in the mind that non-ionic surfactants that modify the surface of ODS column may have any similarity. TAG columns are chiral selectors with multiple interactions which also could provide clue, and monolithic column is the part of current study and is most relevant. The LSER parameter; “$e$” is maximum for TAG phases with MeOH as mobile phase, 0 with monolithic columns with 10% MeOH and negative for Brij-35, “$s$” is negative for all systems except TAG phases with MeOH, while “$a$” and “$b$” are negative and “$v$” is positive throughout. Table 2 shows that excess molar refraction “$e$” is a discriminating factor for non-ionic micellar liquid chromatography, which is negatively related to log $k$ value. Such types of interactions are also reported by Quina et al. [16] while characterizing incorporation of non-ionic solutes in aqueous micelles using LSER analysis [23–25] and Altomare et al. [26] for estimating partitioning parameters of non-ionic surfactants. The “$e$” value for Brij-35 surfactant is 1.63 as compared to 0.76 for CTAB and 0.32 for SDS. The author has attributed this to polyoxyethylene head groups in Brij-35 that contribute to the polarizability of the micellar solubilization sites. Also the hydrogen bond basicity of micellar solubilization site is enhanced by the presence of ether oxygen of the polyoxyethylene headgroup. Jason et al. [23] have highlighted similar finding for other non-ionic surfactants, where molar excess refraction and basicity are major contributors to partitioning as compared to previous work on other systems. To check if similar factors contribute here, available molecular descriptors for some of the isomers are compiled in Table 3. It could be observed from Table 3 that dipolairizability is a major
Table 2: LSER system constants for various stationary and mobile phases reported in the literature.

| Column | Mobile phase | $e$  | $s$  | $a$  | $b$  | $v$  | Ref. |
|--------|--------------|-----|-----|-----|-----|-----|-----|
| ODS    | 50% ACN      | 0.181 | −0.527 | −0.417 | −1.646 | 1.492 | [13] |
| Polar Endcapped | 50% ACN      | 0.173 | −0.444 | −0.396 | −1.261 | 1.323 |
| Polar embedded | 50% ACN      | 0.146 | −0.339 | −0.079 | −1.581 | 1.320 |
| TAG    | 20% ACN      | 0.345 | −0.001 | −0.315 | −1.202 | 1.349 |
| TAG    | 25% MeOH     | 0.478 | 0.267 | −0.493 | −1.121 | 1.496 |
| Monolith C-18 | 50% ACN      | 0.07  | −0.33  | −0.52  | −1.55  | 1.49  | [15] |
| Monolith C-18 | 50% MeOH     | 0.30  | −0.67  | −0.41  | −1.77  | 2.10  |
| Monolith C-18 | 10% MeOH     | 0    | −0.51  | −0.26  | −2.18  | 3.83  |
| Monolith C-18 | 0.04 M Brij-35* | −0.165 | −0.087 | −0.191 | −1.284 | 1.080 | [16] |

*Mobile phase is 0.04 M Brij-35 with 0.01 M sodium dihydrogen phosphate at pH 7.4 adjusted with NaOH at 40°C.

Table 3: Abraham's LSER molecular descriptors for some of the isomers included in current study.

| Analyte          | A (acidity) | B (basicity) | S (dipolarity/dipolarizability) | E (excess molar refraction) |
|------------------|-------------|--------------|--------------------------------|-----------------------------|
| 4-nitroaniline   | 0.46        | 0.35         | 1.93                           | 1.22                        |
| 3-nitroaniline   | 0.4         | 0.36         | 1.71                           | 1.2                         |
| 2-nitroaniline   | 0.3         | 0.36         | 1.37                           | 1.18                        |
| o-cresol         | 0.52        | 0.30         | 0.86                           | 0.84                        |
| m-cresol         | 0.57        | 0.34         | 0.88                           | 0.82                        |
| p-cresol         | 0.57        | 0.31         | 0.87                           | 0.82                        |
| m-nitrophenol    | 0.82        | 0.26         | 1.72                           | 1.07                        |
| m-nitrophenol    | 0.79        | 0.23         | 1.57                           | 1.05                        |

4. Conclusions

Non-ionic micellar liquid chromatography offers different mode of interaction than hydro-organic or ionic micellar liquid chromatography. Better separation of positional isomers is possible with Brij-35/propanol hybrid mobile phase. Besides basicity, dipolarizability and excess molar refraction are responsible for fine-tuning of separation. This new face of non-ionic MLC opens field for many applications in separation of positional isomers.

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