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Electrophoretic separations of twelve phenothiazines and N-demethyl derivatives by using capillary zone electrophoresis and micellar electrokinetic chromatography with non ionic surfactant

D.C. Le[a], C.J. Morin[b], M. Beljean[c], A.M. Siouffi[a], P.L. Desbène[b,*]

[a] *UMR 6180 Chirotechnologies Catalyse et Biocatalyse, Université Aix-Marseille III, Faculté des Sciences et Techniques St Jérôme, Avenue Escadrille Normandie Niemen, 13397 Marseille Cedex 07, France*
[b] *Laboratoire d'Analyse des Systèmes Organiques Complexes, UPRES EA 3233 SMS-IRCOF et IFRMP, Université de Rouen, 55 Rue Saint Germain, 27000 Evreux, France*
[c] *Centre Hospitalier Spécialisé du Bon Sauveur, 93 Rue Caponière – 14000 Caen, France*

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Abstract

We focused our work on the separation of phenothiazines that are important drugs used for the treatment of psychic diseases. For a better understanding of the metabolism of these solutes, we wanted to separate not only a mixture of 12 phenothiazines but also a mixture containing phenothiazines and their N-demethyl metabolites by capillary electrophoresis. Separations in capillary zone electrophoresis were performed using 3×10^{-2} mol/L H_3PO_4 (pH 2.5) but the obtained resolutions were not entirely satisfactory especially with regard to phenothiazine – N-demethyl derivative pairs. To improve the obtained results, we have performed separations by using micellar electrokinetic chromatography. In this approach, we used a running electrolyte containing 3×10^{-2} mol/L H_3PO_4 electrolyte (pH 2.5) and octaethylene glycol monododecyl ether ($C_{12}E_8$) as neutral surfactant. By introducing 2×10^{-3} mol/L $C_{12}E_8$ in the electrolyte, 11 out of 12 phenothiazines have been baseline separated. With respect to the separation of a mixture containing 3 phenothiazines and their 3 demethyl derivatives, we obtained an excellent separation by using a running electrolyte prepared with 7.5×10^{-4} mol/L $C_{12}E_8$ and 3×10^{-2} mol/L H_3PO_4.

Keywords: Capillary electrophoresis; Phenothiazine; MEKC; Non ionic surfactant

1. Introduction

Phenothiazines and their derivatives, which were generally called the phenothiazines, are an important group of pharmaceuticals. Many compounds of this group are used for the treatment of psychic diseases [1]. Others possessing antihistaminic or antiemetic properties have diverse clinical uses [2]. The dosage related to phenothiazines depends on the used molecule and on the required effect. For many phenothiazines, the active forms directly responsible of their desirable effects are in fact their metabolites resulting from diverse metabolism procedures [3,4]. In human body, many metabolites of phenothiazines originate from different ways of metabolism. One of these ways, the N-demethylation of phenothiazine by P450 cytochrome suppresses one N-bonded methyl group to form the N-demethyl metabolite. It was proved that N-demethyl metabolites of levomepromazine, chlorpromazine, fluphenazine, perphenazine and methoxypromazine were the active compounds having certain pharmaceutical effects similar to that of respective phenothiazine [3]. N-Demethyl levomepromazine was found to have important roles on sedative and muscle relaxation properties of levomepromazine [4]. In consequence of their important applications, phenothiazines and their metabolites have been studied in different domains of pharmaceutical

* Corresponding author. Tel.: +33 2 32291570; fax: +33 2 32291539.
E-mail address: paul-louis.desbene@univ-rouen.fr (P.L. Desbène).

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research. In many cases, the utilization of a very performant analytical method is required to analyze them in biological liquids or to study their metabolism. In such a context, capillary electrophoresis (CE) appears a very interesting tool. Therefore several separations and enantioseparations of phenothiazines have performed by CE [5–11]. In capillary zone electrophoresis (CZE), the influence of pH on the electrophoretic behaviour of 13 structurally related phenothiazines was thoroughly studied and the authors of this work have concluded that it was preferable to select buffer pH in the range of 2.5–3.5 to separate these phenothiazines [5]. Thus, almost all the separations and enantioseparations of phenothiazines were carried out in this acid zone of pH. Nevertheless, even in these conditions, CZE did not lead to satisfactory resolutions if no modifier is introduced within the running electrolyte to increase the difference of electrophoretic mobilities [4]. With this aim, most of published works focused on the utilization of cyclodextrins (neutral or charged) in the electrolyte to form hydrophobic interactions with these compounds in order to perform the separation or enantioseparation. For example, a separation of phenothiazines was carried out with hydroxypropyl-β-cyclodextrin (HP-β-CD) and β-cyclodextrin (β-CD) in phosphate buffer at pH 3.0 [6]. Enantioseparations of phenothiazines were carried out using different kinds of cyclodextrins as chiral selectors: β-CD [7,8], HP-β-CD [7,8], γ-cyclodextrin [7,8] and carboxyethyl β-cyclodextrin [9]. In contrast, the utilization of micellar electrokinetic chromatography (MEKC) was surprisingly rare for the separation of phenothiazines. We only found in the literature a MEKC method to separate phenothiazines using simultaneously two cationic surfactants, a fluorinated cationic surfactant and cetyltrimethylammonium bromide, as micelle forming agent, with Tris–acetic acid as background electrolyte [10]. A publication describing a cyclodextrin-modified MEKC method to separate enantiomers of optically active phenothiazines was also published [11]. This method used citrate buffer (pH 3.5) containing a cationic surfactant, tetradecyltrimethylammonium bromide, into which, either β-CD or either HP-β-CD was added. In the two above-mentioned methods, the analyses were carried out with reversed electroosmotic flow because of the presence of cationic surfactants in the running electrolyte. Moreover, by studying the literature we were surprised by the lack of works in capillary electrophoresis with regard to the separation of phenothiazines and their metabolites, like N-demethyl compounds, despite undeniable importance of such works on learning metabolism mechanics. In such a context, we decided to estimate the ability of CZE and MEKC to separate in acidic medium the phenothiazines and their N-demethyl metabolite. In a first approach, we studied the limitations of CZE for the separation of these solutes. In a second approach, we focused our work on MEKC because its potentiality was not yet really studied in the particular case of phenothiazines. In this paper, we develop a new MEKC method for the separation of phenothiazines involving a non ionic surfactant to form micelles. Neutral surfactants such as Brij, Tween or alkylglucosides [12,13] were previously used in MEKC but in order to form either mixed micelles with ionic surfactants or in situ charged micelles via complexation. Nevertheless, neutral micelles can be used for separating charged compounds. For instance, Desbène and Fuchic [14] used this last strategy to carry out enantioseparations of amino acids by using nonyl-β-D-glucopyranoside to form chiral micelles. In this study, we chose neutral surfactant for several reasons. First, in acid zone of pH, basic compounds like phenothiazines were positively charged like micelles formed by cationic surfactants. In opposite, micelles formed by non ionic surfactant brought no charge and their interaction with positively charged analytes such as phenothiazines was therefore not constrained by unfavourable electrostatic repulsion. Secondly being uncharged, non ionic surfactant did not increase the ionic strength of the running electrolyte. Consequently, the applied voltage can be maintained at a high value without problem linked to the increase of Joule heating. Consequently, a broad range of surfactant concentration can be used without decreasing the electrical field. Lastly, in acidic medium (pH 2.5), electroosmotic flow was roughly suppressed if a fused silica capillary is used for the separation. In these conditions, uncharged micelles rested immobile inside capillary whereas positively charged micelles migrate in the same direction of cationic solutes. So at pH 2.5, interactions between phenothiazines and neutral micelles have stronger influence on the migration of phenothiazines than that caused by cationic surfactants. So, the use of non ionic surfactant might improve the resolution of phenothiazines more efficiently than that of cationic surfactant. In this first approach, we did not want to use polydispersed neutral surfactants like Brij or Tween for a best understanding of the separation process. Besides, the interactions between the hydrophobic core of the micelles and the solutes had to be strong enough to modify the separation. These interactions partly depend on the alkyl length of the surfactant. So, taking into account that sodium dodecyl sulfate has been successfully employed in most of MEKC applications, we decided to use the same alkyl length than this ionic surfactant. Consequently, among various neutral surfactants, we chose octaethylene glycol monododecyl ether ($C_{12}E_8$) to perform the separations.

We studied in our experiments twelve phenothiazines (levomepromazine, chlorpromazine, cyamemazine, prochlorperazine, trifluoperazine, fluphenazine, promethazine, acepromazine, alimemazine, methopromazine, triflupromazine, and thioridazine) and the N-demethyl derivatives of three phenothiazines: N-demethyl levomepromazine (DM levomepromazine), N-demethyl chlorpromazine (DM chlorpromazine) and N-demethyl cyamemazine (DM cyamemazine). All these compounds, depicted in Fig. 1, were basic ones with different substituents bonded to the phenothiazine ring at 2- and 10-positions. Moreover, the substituent at 10-position is an aliphatic side chain containing either an amino group or an alkyl piperazine group or a piperidine moiety.

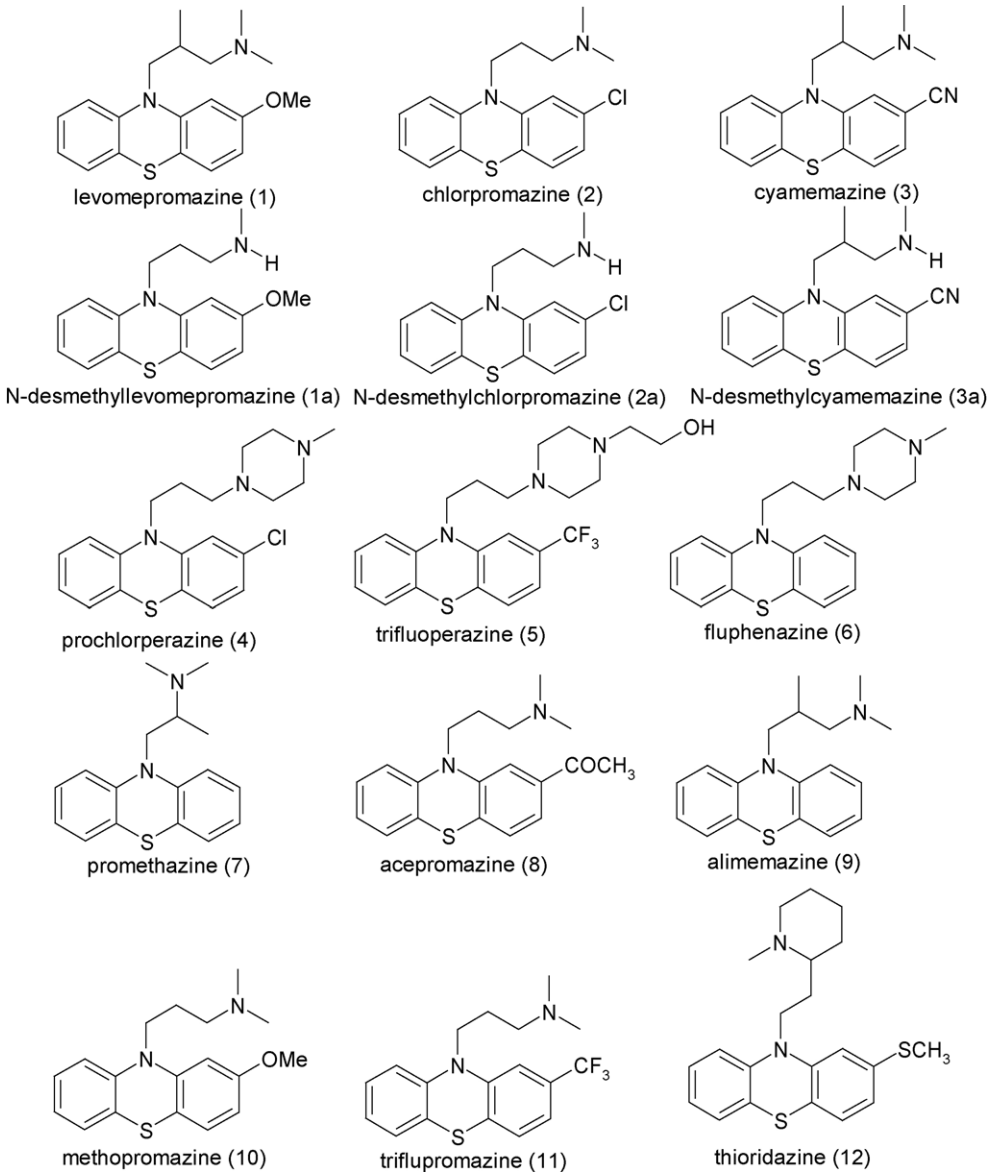

Fig. 1. Structure of phenothiazines and N-demethyl phenothiazines analyzed in this work.

2. Experimental

2.1. Chemicals

The studied phenothiazines (purity ≥99%) came from different origins. Alimemazine, methopromazine, chlorpromazine, N-demethyl chlorpromazine, prochlorperazine, levomepromazine, N-demethyl levomepromazine, cyamemazine and N-demethyl cyamemazine were provided by Aventis (Aventis France, Paris, France). Fluphenazine and triflupromazine came from Bristol–Myers–Squibb (Bristol–Myers–Squibb France, Rueil Malmaison, France). Acepromazine, thioridazine, trifluoperazine and promethazine came respectively from Sanofi-Synthelabo (Sanofi-Synthelabo France, Paris, France), Novartis (Novartis France, Rueil Malmaison, France), Theraplix (Theraplix France, Paris, France) and Celltech Pharma (Celltech Pharma France, Paris, France). Orthophosphoric acid (purity >99%) and octaethylene glycol monododecyl ether (purity >98%) were purchased from Fluka (Sigma–Aldrich–Fluka France, L'Isle d'Abeau Chesne, France). Sodium hydroxide (purity ≥98%) came from VWR (VWR France, Fontenay sous Bois, France). All solutions were prepared by using the 18 MΩ water produced by means of a Direct-Q Millipore system (Millipore, Bedford, MA, USA).

Standard solutions of all phenothiazines and those of N-demethyl derivatives were prepared by dissolving analytes in aqueous solution at concentration of 10^{-3} mol/L. The analyzed solutions were freshly prepared before use by diluting 10 folds standard solutions with deionised water.

2.2. Apparatus

All analyses were carried out by using a P/ACE 2100 capillary electrophoresis system (Beckman-Coulter, Fullerton, CA, USA) equipped with a UV detector. Acquisitions were performed by means of P/ACE 2000 software version 2.0 (Beckman-Coulter). The samples were systematically injected in hydrodynamic mode (injection pressure 0.5 p.s.i., i.e. 3.4 kPa) and their analysis was achieved on fused silica capillaries of 57 cm (50 cm effective length) × 50 μm I.D. × 375 μm O.D. purchased from Thermo Electron (Thermo Electron France, Les Ulis, France). The analyses were carried out at 25 °C and the pH of running electrolytes was measured before utilization, at this temperature, with a model IQ 240 pH meter (I.Q. Scientific Instruments, San Diego, CA, USA). The electrolytes were systematically degassed by sonification by means of an Ultrasonic Cleaner model 2510 (Branson Ultrasonic, Danbury, USA). The detection was performed at 254 nm.

2.3. Electrophoretic procedure

New capillaries were activated by 1.0 mol/L NaOH for 60 min and then they were flushed with deionised water for 10 min. To assure reproducibility, between 2 injections, the separation capillary was washed for 1 min with water, 3 min with 0.1 mol/L NaOH, 2 min with water and finally for 2 min with the running electrolyte.

3. Results and discussion

3.1. CZE separations

We focused our attention not only on the CZE separation of the mixture including the 12 phenothiazines but also that of the mixture of 3 phenothiazines (levomepromazine, chlorpromazine and cyamemazine) and of their N-demethyl derivative (DM derivative). Previously, Lin et al. [5] have shown that a pH range of 2.5–3.5 was optimal to separate a mixture of 13 phenothiazines using phosphate buffer. Eleven out of these 13 phenothiazines being studied in this work, we therefore decided to carry out our experiment at pH 2.5 by using H_3PO_4 as background electrolyte. At this pH, the electroosmotic flow was suppressed and the phenothiazines and their N-demethyl derivatives migrated at their own mobility. We studied the influence of H_3PO_4 concentration towards resolution in the 2.5×10^{-3} to 50×10^{-3} mol/L range.

Up to 3×10^{-2} mol/L, the resolution increases with H_3PO_4 concentration whatever the studied mixture. Rising up higher concentration, the improvement of resolution was not significant and the applied voltage had to be decreased because of Joule heating. Consequently, we have fixed H_3PO_4 concentration at 3×10^{-2} mol/L. The obtained electropherograms are presented in Fig. 2.

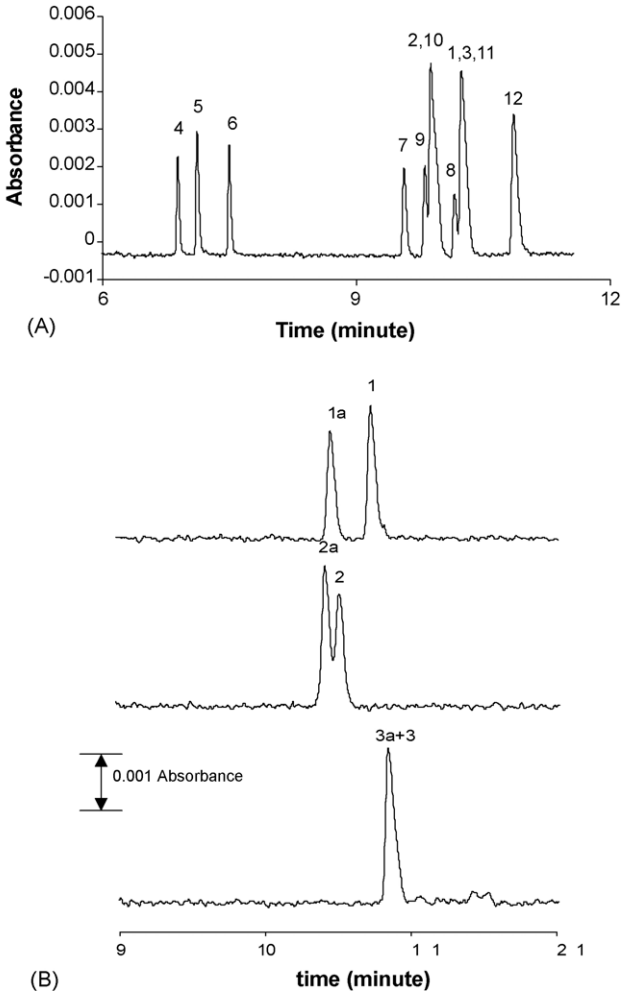

Fig. 2. CZE separations of phenothiazines and N-demethyl derivatives using phosphoric acid as running electrolyte. Operating conditions: fused silica capillary 57 cm total length (50 cm effective length) × 50 μm I.D. × 375 μm O.D.; electrolyte: 3×10^{-2} mol/L H_3PO_4 (pH 2.5); temperature: 25 °C; hydrodynamic injection for 4 s; applied voltage: +20 kV; cathodic detection at λ = 254 nm. (A) CZE separation of a mixture of 12 phenothiazines. Sample: levomepromazine (1), chlorpromazine (2), cyamemazine (3), prochlorperazine (4), trifluoperazine (5), fluphenazine (6), promethazine (7), acepromazine (8), alimemazine (9), methopromazine (10), triflupromazine (11) and thioridazine (12). (B) CZE separation of 3 phenothiazines and their N-demethyl (DM) derivative. Sample: levomepromazine (1), N-demethyl levomepromazine (1a), chlorpromazine (2), N-demethyl chlorpromazine (2a), cyamemazine (3) and N-demethyl cyamemazine (3a).

These results are consistent with those obtained previously [6]. First, the substituent bonded at 10-position of phenothiazine ring appeared critical with regard to separation. Secondly, the obtained resolutions being poor, the addition of modifier within the running electrolyte was required to increase the separation of the studied solutes.

Owing to the fact that the introduction of organic solvent, as propan-2-ol or acetonitrile, did not appear to improve significantly the resolution, we tried to perform the previous separations by MEKC using octaethylene glycol monododecyl ether ($C_{12}E_8$) as neutral surfactant.

3.2. MEKC separations

We studied the influence of $C_{12}E_8$ concentration on separation by introducing this surfactant into 3×10^{-2} mol/L H_3PO_4 (pH 2.5) at higher concentration than its critical micellar concentration (cmc) that is equal to 6.9×10^{-5} mol/L [15]. First, with regard to the separation of the 12 phenothiazines, we have studied the evolution of electrophoretic selectivity (defined as the ratio of the electrophoretic mobility of neighbour peaks) as a function of $C_{12}E_8$ concentration. This surfactant concentration ranged from 2.5×10^{-4} mol/L to 5×10^{-3} mol/L. The electrophoretic mobility of each phenothiazine markedly decreased with increasing surfactant concentration, especially in the range 0–10^{-3} mol/L. This showed that the solutes interact strongly with $C_{12}E_8$. For example, when the concentration of $C_{12}E_8$ varied from 0 to 10^{-3} mol/L in the electrolyte, the electrophoretic mobility of thioridazine and that of triflupromazine was approximately divided by 2. Nevertheless, the influence of surfactant concentration on the electrophoretic mobilities appeared lower for the less hydrophobic phenothiazines.

Moreover, as shown in Fig. 3, the selectivity roughly rises with $C_{12}E_8$ concentration, up to a 2×10^{-3} mol/L concentration except for some analytes. This is particularly noticeable for the trifluoperazine/fluphenazine pair for which the selectivity remains almost constant with increasing surfactant concentration. It should be noted that these two phenothiazines have relatively poor interactions with the micelles as evidenced by the low modification of their electrophoretic mobility when $C_{12}E_8$ is added in the electrolyte.

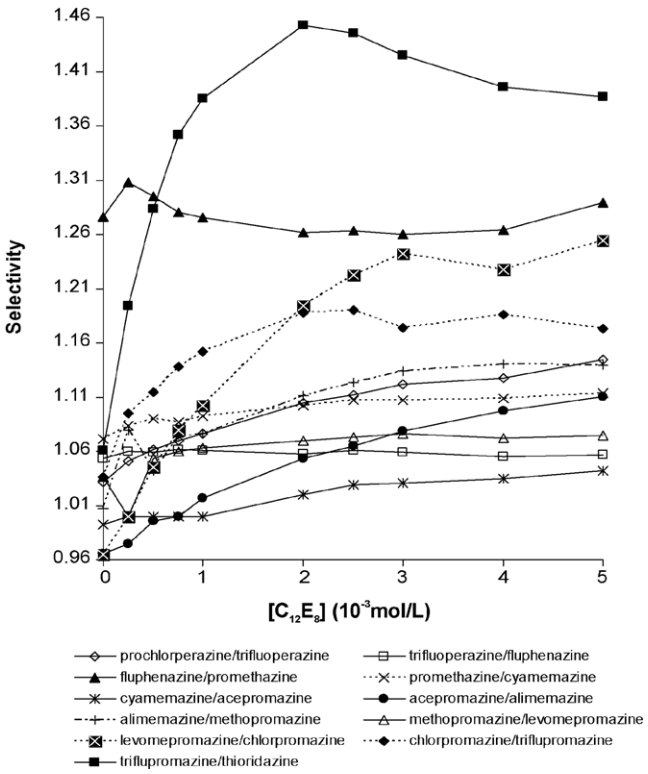

Fig. 3. Evolution of electrophoretic selectivity (α) as a function of octaethylene glycol monododecyl ether ($C_{12}E_8$) concentration in the running electrolyte. Operating conditions: fused silica capillary 57 cm total length (50 cm effective length) × 50 μm I.D. × 375 μm O.D.; electrolyte: 3×10^{-2} mol/L H_3PO_4 (pH 2.5) + variable $C_{12}E_8$ concentration; temperature: 25 °C; sample: mixture of the 12 studied phenothiazines; hydrodynamic injection for 4 s; applied voltage: +20 kV; cathodic detection at λ = 254 nm.

Nevertheless, while acepromazine, cyamemazine and alimemazine co-migrated when 7.5×10^{-4} mol/L $C_{12}E_8$ was used. Increasing surfactant concentration up to 2×10^{-3} mol/L enabled us to separate alimemazine from acepromazine/cyamemazine pair (Fig. 4).

For upper surfactant concentrations than 2×10^{-3} mol/L, the selectivity remained roughly constant. Nevertheless, the analysis time increased with the surfactant concentration because of interaction between neutral micelles and the solutes. For example, increasing surfactant from 7.5×10^{-4} mol/L to 2×10^{-3} mol/L, the analysis time increased from 30 to 45 min and peak broadenings were observed.

At last, as shown in Fig. 3, the calculated selectivity with regard to three pairs of phenothiazines (cyamemazine/acepromazine, levomepromazine/chlorpromazine and acepromazine/alimemazine pair) was initially lower than one then it became higher than this value by increasing $C_{12}E_8$ concentration. This evidenced a reversal of the migration order for these pairs of phenothiazines.

With regard to the separation of phenothiazines and their DM derivatives, the six studied analytes were baseline separated when $C_{12}E_8$ concentration was equal to 7.5×10^{-4} mol/L as shown in Fig. 5.

Comparing this Fig. 5 to Fig. 2, we can point out that the separations of chlorpromazine–DM chlorpromazine and cyamemazine–DM cyamemazine pairs were drastically improved by using only 7.5×10^{-4} mol/L $C_{12}E_8$. Moreover, we can note that in the case of the levomepromazine–DM levomepromazine pair, octaethylene glycol monododecyl ether has caused a reversal in migration order. With 3×10^{-2} mol/L

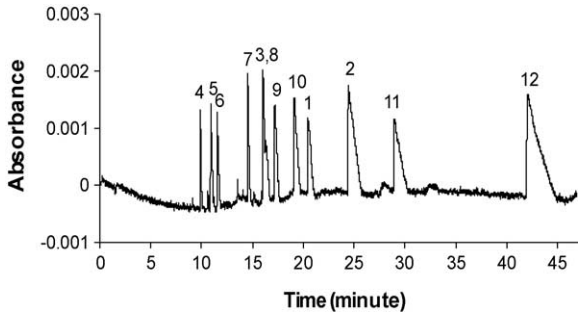

Fig. 4. MEKC separation of a mixture of 12 phenothiazines using $C_{12}E_8$ as neutral surfactant. Operating conditions: fused silica capillary 57 cm total length (50 cm effective length) × 50 μm I.D. × 375 μm O.D.; electrolyte: 3×10^{-2} mol/L H_3PO_4 (pH 2.5) + 2×10^{-3} mol/L $C_{12}E_8$; temperature: 25 °C; sample: levomepromazine (1), chlorpromazine (2), cyamemazine (3), prochlorperazine (4), trifluoperazine (5), fluphenazine (6), promethazine (7), acepromazine (8), alimemazine (9), methopromazine (10), triflupromazine (11) and thioridazine (12); hydrodynamic injection for 4 s; applied voltage: +20 kV; cathodic detection at λ = 254 nm.

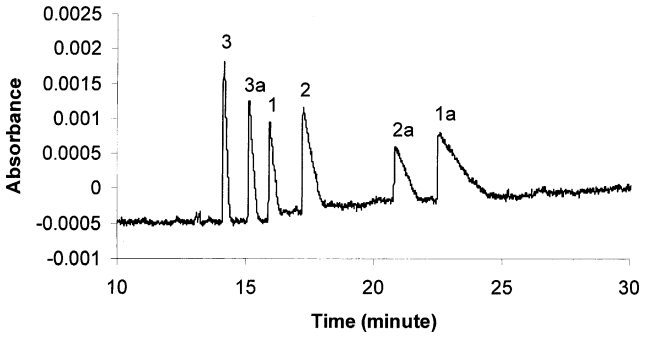

Fig. 5. MEKC separation of 3 phenothiazines and their N-demethyl (DM) derivatives using $C_{12}E_8$ as neutral surfactant. Operating conditions: fused silica capillary 57 cm total length (50 cm effective length) × 50 μm I.D. × 375 μm O.D.; electrolyte: 3×10^{-2} mol/L H_3PO_4 (pH 2.5) + 7.5×10^{-4} mol/L $C_{12}E_8$; temperature: 25 °C; sample: levomepromazine (1), N-demethyl levomepromazine (1a), chlorpromazine (2), N-demethyl chlorpromazine (2a), cyamemazine (3) and N-demethyl cyamemazine (3a); hydrodynamic injection for 4 s; applied voltage: +20 kV; cathodic detection at λ = 254 nm.

H_3PO_4, DM levomepromazine migrated faster than levomepromazine, but in presence of octaethylene glycol monododecyl ether it was the opposite. So, even at low concentration, interactions between micelles and phenothiazines are strong enough to be critical for the resolution. Because excellent resolution was obtained for all analytes, it is useless to increase over 7.5×10^{-4} mol/L the concentration of $C_{12}E_8$.

4. Conclusion

After studying the limitations of CZE for the separation of phenothiazines and their N-demethyl derivatives, we have undertaken MEKC separations by using octaethylene glycol monododecyl ether ($C_{12}E_8$) as neutral surfactant. This strategy enabled us to obtain baseline separations, on one hand, of eleven phenothiazines and, on the other hand, of three phenothiazines and their N-demethyl derivatives. Although the utilization of $C_{12}E_8$ as surfactant allows us to use it at very high concentration, without increasing the ionic strength, the optimal conditions were obtained by using relatively low $C_{12}E_8$ concentrations. This results from the very low critical micellar concentration of $C_{12}E_8$ and from the strong interactions between micelles and phenothiazines. Finally, we proved that our approach appeared to be an interesting analytical tool to analyze phenothiazines and to understand their metabolism often leading to N-demethyl derivatives. Consequently, we hope to apply our method to the actual clinical analysis soon. Indeed, this study should enable us to quantify the phenothiazines in biological fluids. Besides, these good results obtained by using $C_{12}E_8$ lead us to carry out additional investigations for studying the effect of the neutral surfactant structure on resolution.

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