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Enhanced separation of antidepressant drugs using a polymerized nonionic surfactant as a transient capillary coating

The separation of seven structurally similar antidepressant drugs (amitriptyline, nortriptyline, imipramine, desipramine, protriptyline, doxepin, and nordoxepin) was achieved in under 15 min using a novel nonionic micelle polymer, poly(*n*-undecyl-α-D-glucopyranoside) (PUG) by use of capillary zone electrophoresis (CZE). Systematic studies with varying polymer concentration, pH, and percent organic modifier were conducted in order to find the optimum conditions for baseline separation of the seven tricyclic antidepressants. In addition, equations for capacity factor were used to estimate the extent of what was initially thought to be micelle analyte interaction. A series of calculations show that a modified CZE system (PUG-CZE) was the actual mode of separation. Thus, our study concluded that PUG functioned in a non-electrokinetic chromatography mode.

1 Introduction

Tricyclic antidepressant drugs have been found to be very beneficial for the treatment of severe depression, attention-deficit disorders in children, bulimia, anorexia nervosa, panic disorder, post-traumatic stress disorder, obsessive-compulsive disorder, and migraines. Since these drugs are so widely prescribed, it is important to be able to separate, detect, and/or monitor the tricyclic antidepressants in order to follow individual differences in drug metabolism, as well as provide proper dosage information. However, the seven tricyclic antidepressants are difficult to separate as well as monitor therapeutically [1] due to their similar structures and molecular weights. As shown in Fig. 1, protriptyline, desipramine, nortriptyline, and nordoxepin are secondary amines and imipramine, amitriptyline, and doxepin are tertiary amines. It has also been found that some of these drugs, *e.g.* amitriptyline and imipramine, can be metabolized to nortriptyline and desipramine, respectively [1]. Such a problem could cause additional difficulties in the separation and detection of these particular drugs. Therefore, the measurement method used should be able to separate and detect all of the tricyclic antidepressants and any of their metabolities.

Prior to the early nineteen-eighties, the analytical methodology used to detect and/or monitor tricyclic antidepressants in serum was seriously deficient. Thus, standard analytical techniques needed to be developed to aid clinicians in assessing dosage needs, as well as to aid chemists in acquiring quality-control data, performance data, and drug measurements in samples such as serum [1]. In 1981, the analytical methods used for such analyses included high performance liquid chromatography (HPLC), gas chromatography with a nitrogen specific detector, and immunoassays which were found to provide better results when coupled to HPLC [1]. It was

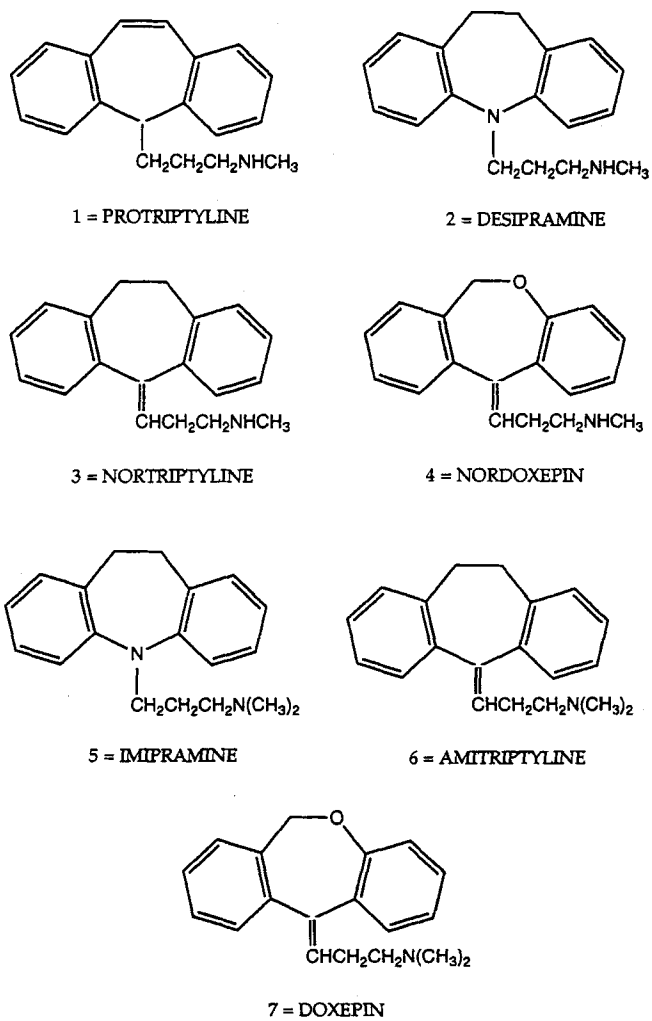

Figure 1. Structure, names, and numeric migration order of the seven tricyclic antidepressants.

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Abbreviations: **CAPSO,** 3-(cyclohexylamino-)-2-hydroxyl-1-propanesulfonic acid; **CZE,** capillary zone electrophoresis; **EKC,** electrokinetic capillary chromatography; **PUG,** poly(*n*-undecyl-α-D-glucopyranoside; **UG,** *n*-undecylenyl-α-D-glucopyranoside

Keywords: Modified capillary zone electrophoresis / Antidepressant drugs / Poly(*n*-undecyl-α-D-glucopyranoside) / Polymerized surfactant

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found that these three techniques were adequate; however, there was concern that the techniques being used were not simplistic and specific enough for the detection and separation of the individual antidepressant drug, its major metabolites, and other coadministered drugs. In addition, the lack of a detailed and practical HPLC procedure for small and large clinical laboratories affected the results of the proficiency studies reported, depending on the procedure used in a given laboratory.

The specificity, simplicity, reproducibility, and rapidity that is desired for the separation and quantification of tricyclic antidepressants can be accomplished by use of capillary electrophoresis (CE). Despite the high efficiency offered by capillary zone electrophoresis (CZE), the separation of organic amines by this technique involves a number of drawbacks. At high pH the silanophilic interactions of amines on bare silica capillaries are unavoidable. Additionally, the increase in the rate of electroosmotic flow (EOF) at alkaline pH usually deteriorates the separation due to faster elution of positively charged compounds [2]. The use of low pH electrolytes reduces the EOF, resulting in longer migration times. In addition, since the organic amines studied in this work have pK_as ranging from 9.8 to 10.7, the separation of such compounds with similar electrophoretic mobilities decreases the separation selectivity at low pH. For these reasons, Salamon and co-workers have used 3-(cyclohexylamino-)-2-hydroxy-1-propanesulfonic acid (CAPSO) as a zwitterionic buffer in combination with methanol to prevent solute adsorption on the capillary wall. The use of 50 mM CAPSO at pH 9.55 (*i.e.*, close to the pK_a values of the amines) provided optimal conditions for the separation of seven antidepressant drugs [3]. In a brief communication, Swedberg reported the use of octyl glucoside for the separation of only desipramine and nortriptyline [4]. To our knowledge, this is the only study that has demonstrated the utility of a nonionic surfactant for the separation of these compounds [4].

Nonionic surfactants are advantagenous because they provide a balance between electrostatic and hydrophobic forces that control separation. Also, nonionic surfactants have the ability to dynamically coat capillaries, which makes the capillary surface less hydrophilic. Therefore, some nonionic surfactants have been found to be more suitable for separation of compounds that are more hydrophobic in character [5]. Moreover, polymeric nonionic surfactants usually have different physical properties from nonionic micellized surfactant monomers, such as selectivity, effect on solution viscosity, and ease of removal prior to mass spectral detection, hence providing additional advantages. The formation of micelles using nonionic surfactants tends to occur at a much lower concentration than ionic surfactants. Note that the critical micelle concentration (CMC) of nonionic surfactants is often on the order of 10^{-4} M [6].

Alkyl glucosides have sparked an interest in being utilized more as nonionic surfactants over the usual polyoxyethylene alkyl ethers which are troublesome to synthesize and purify [7]. Alkyl glucosides are made from naturally occurring resources of fatty alcohols and sugars. Their biodegradability and antimicrobial properties make alkyl glucosides attractive alternatives for biological and pharmaceutical applications [8]. The surfactant used in the study reported here is *n*-undecylenyl-α-D-glucopyranoside (UG). The mnomer, UG, was synthesized using a procedure described by B. Havlinová *et al.* [9]. The complete synthesis, characterization, and solution behavior studies of UG and polymerized surfactant, poly(*n*-undecyl-α-D-glucopyranoside) (PUG) were conducted in our laboratory and reported in detail (C. W. Harrell, *PhD Thesis*, Lousiana State University). At the appropriate CMC, these surfactants form micelles that may be converted into PUG by use of γ-irradiation of the terminal allyl group (Fig. 2).

From previous studies conducted in our laboratory and other research groups, it was found that polymerized surfactants can enhance separation over conventional micelles when used in electrokinetic capillary chromatography (EKC) [5, 10–15]. Polymerized surfactants offer the advantages of being more stable, more rigid, and having

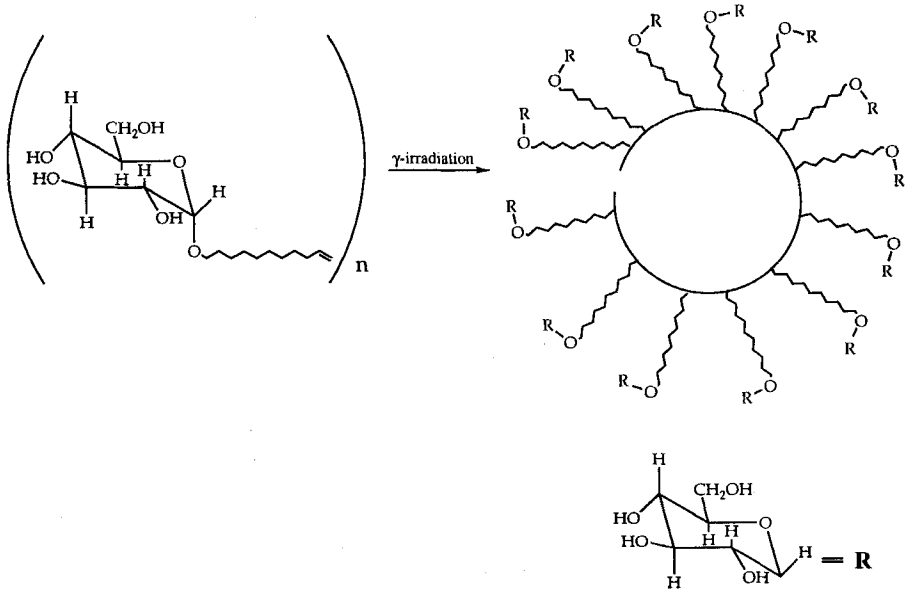

Figure 2. Structure of monomeric *n*-undecylenyl-α-D-glucopyranoside (UG) and schematic representation of poly(*n*-undecyl-α-D-glucopyranoside) (PUG).

zero CMC; further advantages are the tolerance to high volume fractions of organic solvents, and elimination of the dynamic equilibrium that is associated with conventional micelles. These characteristics are achieved by locking the hydrophobic tails of the micelle in position through covalent bonds formed by use of γ-irradiation. The present report describes a CZE buffer system used for the achiral high-resolution separation of all seven tricylic antidepressents using a polymerized nonionic surfactant system. The use of PUG in conjunction with a small amount of organic modifier was explored to provide a highly selective separation of seven tricyclic antidepressants. Development of a systematic method was performed in which the polymer concentration, pH, and the v/v fraction of methanol as an organic modifier were varied to probe the migration behavior and hydrophobic/hydrophilic selectivity.

2 Materials and methods

2.1 Materials and reagents

The starting material, 2,3,4,6-tetra-*O*-acetyl-1-bromo-1-desoxy-β-D-glucopyranose was purchased from Fluka Chemical Company (Ronkonkoma, NY). Mercuric oxide, mercuric bromide, sodium methoxide, ω-undecylenyl alcohol, and alcohol-free chloroform were purchased from Aldrich (Milwaukee, WI). All seven tricyclic antidepressants were purchased from Sigma (St. Louis, MO) and used without further purification. Sodium dibasic phosphate (anhydrous) was obtained from CMS (Houston, TX). The polymeric surfactant was synthesized in our laboratory.

2.2 Synthesis and polymerization of UG

The monomer, UG, was synthesized using a procedure described by B. Havlinová *et al.* [9]. Yellow mercuric oxide, mercuric bromide, and ω-undecylenyl alcohol were added to 2,3,4,6-tetra-*O*-acetyl-1-bromo-1-desoxy-β-D-glucopyranose in the presence of dry alcohol-free chloroform. Under dry conditions, the reaction mixture was stirred overnight at room temperature. After workup, the intermediate was filtered through silica gel. The protective acyl groups were removed from the intermediate, undecylenyl-2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranoside, by reaction with a 1 M sodium methoxide solution. Thin-layer chromatography was used to follow the hydrolysis. Lastly, Dowex 50W (H^+) resin was used to deionize the final product in solution. The CMC was found to be 5.3×10^{-4} M in a 20% methanol: 80% water (v/v) mixture. Polymerization of the monomer was achieved by ^{60}Co γ-irradiation of an 8.7×10^{-4} M surfactant solution in a 20:80 methanol:water mixture. After irradiation for 48 h, the polymer solution was purified by dialysis using a 1000 Da molecular mass cut-off membrane.

2.3 Instrumentation

A Beckman P/ACE 5510 capillary electrophoresis system (Fullerton, CA), controlled by P/ACE Gold software was used to conduct the electrophoretic experiments. The uncoated fused-silica capillary, purchased from Polymicro Technologies (Phoenix, AZ), had an inner diameter of 51 μm and a total length of 57 cm (50 cm effective length). Approximately 0.7 cm of the polyimide coating was burned off the capillary to make a transparent detection window. The temperature of the capillary was controlled by a fluorocarbon coolant at 23°C. A constant voltage of +20 kV was used for the separation. The seven tricyclic antidepressants had a concentration of 0.9–1.0 mM and were injected into the capillary for 2 s by applying 0.5 psi at the anodic end of the capillary. Separation of the drugs was monitored by use of UV absorbance at a wavelength of 214 nm.

2.4 Capillary rinsing procedures

Prior to first use, a new capillary was subjected to a standard wash with 1 M NaOH for 1 h. At the beginning of the experiment, the capillary was rinsed with distilled water for 5 min, 0.1 M NaOH for 30 min, distilled water again for 10 min, and then 15 min with the buffer in use. If more than one buffer was run, the capillary was rinsed for 5 min with water and 10 min with the new buffer.

2.5 Preparation of running buffers and sample solutions

The buffer solutions were prepared from sodium phosphate dibasic (Na_2HPO_4) (anhydrous). The pHs of the buffers were adjusted by use of either 0.1 M phosphoric acid or 0.1 M NaOH. Once the polymer was added, the pH was adjusted, and the appropriate volume of methanol was added after reaching the desired pH. The running buffer solutions were then filtered using a 0.45 μm syringe filter, which is a 25 mm surfactant-free cellulose acetate membrane obtained from Nalgene. Peak identification for each tricyclic antidepressant was determined by spiking with known standards. A multiple injection option on the instrument was used to recognize individual antidepressants in a mixture of the seven drugs by a change (increase) in peak intensity of the individual analyte.

3 Results and discussion

3.1 CZE *versus* EKC separation

Since charged molecules can be separated in free solution, separation of the cationic drugs using a 50 mM sodium phosphate buffer in CZE was attempted. As shown, the seven tricyclic antidepressants comigrated with almost identical electrophoretic mobilities (Fig. 3a). Addition of 2.65 mM (at a concentration five times larger than the CMC) of monomeric UG to the Na_2HPO_4 buffer improved the separation of six of the seven drugs (protriptyline and desipramine baseline-separated) as shown in Fig. 3b. These preliminary results encouraged us to use PUG with the expectation of improving separation (Fig. 3c). The results show that the polymerized nonionic surfactant allowed better discrimination of the cationic drugs in comparison to the CZE system with phosphate buffer or monomeric micelle.

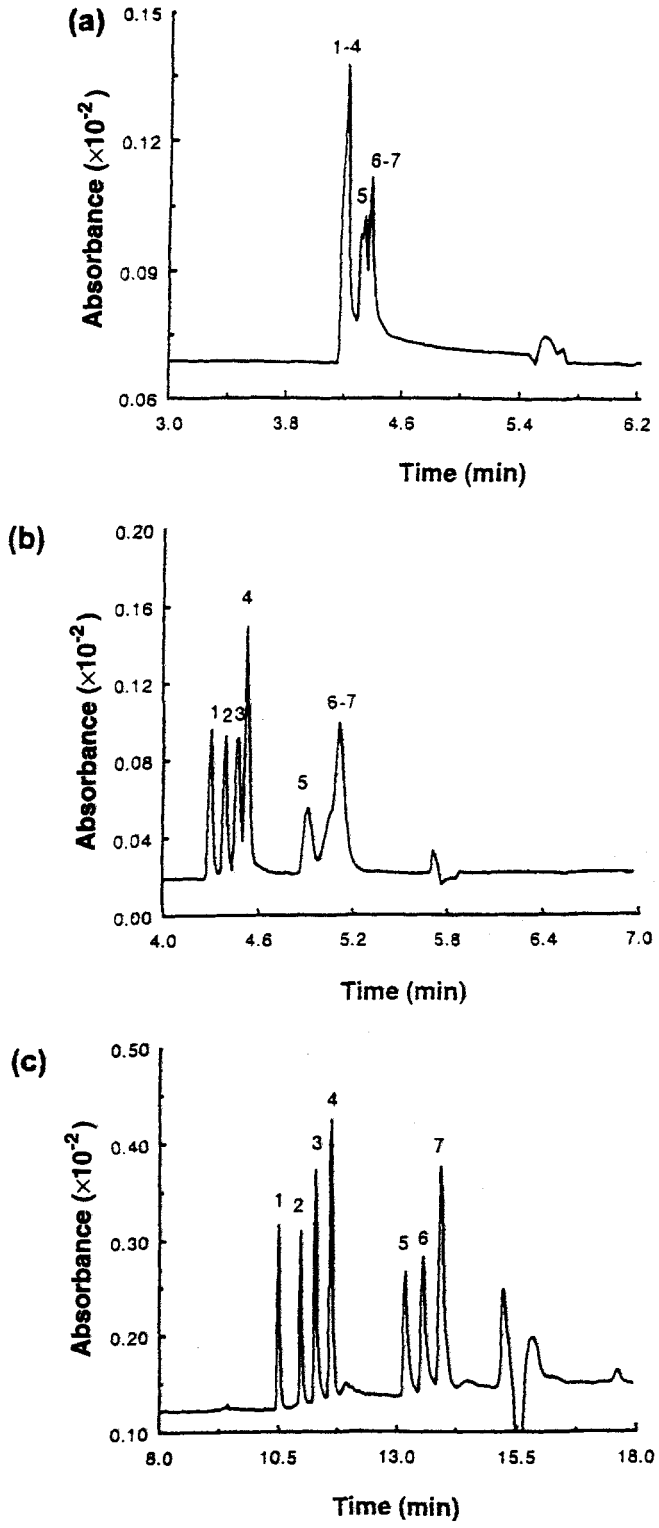

Figure 3. Comparison of drug separation using 50 mM Na_2HPO_4 (a) without monomer and polymer, (b) with 2.65 mM monomer and 20% v/v MeOH, and (c) with 20% v/v PUG and 20% v/v MeOH.

3.2 Variables affecting the separation of the cationic drug

The selectivity of PUG was evaluated in CZE separation of the cationic antidepressant drugs by varying separation conditions, *e.g.*, polymer concentration, pH of the running electrolyte, and percent v/v of methanol and aqueous buffer solution. Using Terabe's rule of thumb [16], the optimum pH to use in CE to separate analytes is below the pK_a of the analyte being separated. The secondary amines (protriptyline, desipramine, nortriptyline, and nordoxepin) have pK_a values of approximately 10.7, whereas the tertiary amines (imipramine, doxepin, and amitriptyline) have pK_a values close to 9.8 [3, 17]. Therefore, 9.55 was the pH initially selected to evaluate the effect of polymer concentration and organic modifier studies on PUG-CZE separation of the drugs. Once the latter conditions were optimized, the pH was varied in order to further optimize the separation.

3.3 Capacity factor calculations

The capacity factor (k) is defined as the moles of solute in the micelle per mole of solute in the bulk solution [18]. Calculations with Eq. (1) derived by Foley were employed, *i.e.* [19],

$$k = \frac{t_R\,(1 + \mu_r) - t_o}{t_o\left(1 - \dfrac{t_R}{t_{mc}}\right)} = \frac{t_R\,(1 + \mu_r) - t_o}{(t_o - t_R)} \qquad (1)$$

where μ_r is the relative electrophoretic mobility of the uncomplexed analytes to electroosmotic flow ($\mu_{ep,A}/\mu_{eo}$); t_R, t_o, and t_{mc} are the observed migration times of the analyte, electroosmotic flow, and polymeric surfactant, respectively. It is important to note that in Eq. (1), μ_r is the quotient of μ_{ep}, and μ_{eo} in the presence of the polymer. However, μ_{ep} (in the presence of the polymer) cannot be measured directly, since it is the weighted average of (i) the apparent mobility of the solute at a given instant when the micelle and solute are not bound (but micelle is present) and (ii) the apparent mobility of the solute at a given instant when the solute is bound to the micelle. Therefore, μ_{ep} (in the presence of polymer) is obtained by measuring μ_{ep} (in the absence of the polymer) and then applying a viscosity correction factor (solution viscosity without polymer/solution viscosity with polymer). For all studies, k was calculated using Eq. (1) and values of zero were obtained. Consequently, the latter results led us to believe that MEKC was not the primary mode of separation.

Since CE and partitioning EKC are orthogonal, having both modes present does not always present the best separation results due to competition of like forces. Also, there is the case where only one mode is contributing to give separation. Therefore, Foley derived two limiting cases to determine if a system is using the CE or EKC mode. If CE is the separation mechanism, then $\mu_{r,1} \neq \mu_{r,2}$ and $k_1 = k_2 = k_{avg} > 0$ (the analytes are identified by the subscript number, *i.e.* 1, 2, ...n). However, separation achieved by EKC has $0 < k_1 < k_2$ and $\mu_{r,1} = \mu_{r,2} = \mu_{r,avg}$. Although k values were not greater than zero, our system followed the limits of CE mode. As stated earlier, $k_1 = k_{2....n} = k_{avg}$ and $\mu_{r,1\ 2....n}$ were not equal. Foley has defined this separation mode as resolution accomplished by differences in electrophoretic mobility and the following equation should be used [19]:

$$t_R = \left(\frac{1 + k}{1 + \mu_r}\right) t_o \quad (2)$$

As a result of k being equal to zero, Eq. 2 reduces to

$$t_R = \left(\frac{1}{1 + \mu_r}\right) t_o \quad (3)$$

Equation (3) is used in CE mode when no second phase is present; therefore, k is equal to zero by definition (Eq. 2). In the latter case, data are reported as a function of retention time instead of k [19]. However, note that a second phase is indeed present in our system. After interpretation of the previous equations and limiting cases it is clear that PUG is not interacting with the analytes in free solution or at the capillary wall. It is evident to us that nonionic PUG is coating the capillary wall, which allows separation to occur by prevention of irreversible binding of the cationic analytes to the capillary wall. Moreover, we determined that a modified PUG-CZE system is consistent with the observed data.

3.4 Concentration of the polymeric nonionic surfactant

The effect of the polymer concentration was investigated with respect to the k using Eq. (1). The k values obtained for all seven tricyclic antidepressants were equal to zero when Eq. (1) was used. Consequently, Eq. (3) was used to determine the effect of migration time, as illustrated in Fig. 4, *versus* the polymer concentration. Polymer concentrations were varied from 0.02% w/v to 0.08% w/v. With the 0.02% w/v fraction of the polymer a sharp increase is observed in the migration time due to the addition of nonionic polymer surfactant to the buffer solution. The nonionic polymer additive caused a reduction in the EOF. Therefore, a decrease in the EOF via an additive which prefers the double layer over the bulk solution can lead to better resolution at the expense of longer analysis times [20]. Similarly, it was observed that the migration times of the cationic drugs and the neutral marker (methanol) increased – however, with coelution

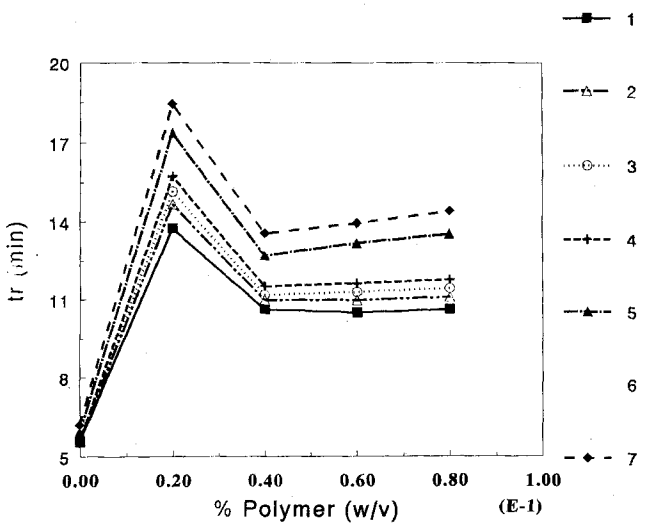

Figure 4. Plot of retention time for the seven tricyclic antidepressant drugs as a function of percent (w/v) of PUG.

of imipramine and amitriptyline. Nevertheless, at 0.04% w/v of the polymer, a gradual decrease in the migration time of all seven drugs is observed. With an increase from 0.04% polymer concentration to 0.08%, a slight and gradual increase in migration time is observed with baseline separation of imipramine and amitriptyline. The best separations are obtained at the 0.04% or 0.06% volume fraction of the polymer. Low (0.02%) and high (0.08%) polymer concentrations provide slightly poorer separations and peak shapes.

3.5 Effect of organic modifier

Organic solvents such as methanol have been used in CZE to increase the elution window. In general, the use of methanol will cause a decrease in the EOF, which in turn increases the migration times of the solutes [2]. The effect of methanol at pH 9.55 using an optimized 0.06% volume fraction of the polymerized surfactant was studied. The concentration of methanol was varied from 0–30% v/v (Fig. 5a–e). At 0 and 5% v/v methanol, six of the seven drugs were partially separated. Complete baseline resolution of all seven drugs was achieved at 20% v/v of methanol as shown in Fig. 5d. Further increases in methanol concentration to 30% v/v deteriorated the resolution and peak splitting was observed (Fig. 5e). Plotting retention time *versus* percent of methanol gave the expected trend in retention time (data not shown). Therefore, the improved selectivity and resolution for the tertiary amines upon the addition of organic modifier resulted from the reduced EOF [2].

3.6 Effect of pH

To achieve further baseline separation of the seven tricyclic antidepressants after optimization of polymer concentration and organic modifier content, the electrophoretic mobility of the analytes was altered by changing the pH from 7 to 9.55. We monitored the effect of pH values on the various antidepressants (Fig. 6). It is observed that the secondary amines are resolved at lower pH values (pH 7 and pH 8). In addition, slight hydrogen bonding between the polymerized surfactant coating and the secondary amines may be occurring, which may supply some selectivity, whereas it has been established that tertiary amines do not hydrogen-bond as strongly as secondary amines [21]. Above pH 8, the secondary and tertiary amines begin to resolve. However, baseline separation is not achieved. Low pH should increase the electrophoretic mobilities. Thus, as the pH is increased, the electrophoretic mobility of the amines decreases, giving better separation. Baseline separation was achieved at pH 9 and improved as the pH was increased to 9.55 (Figs. 6d and 6e). Lastly, when retention time versus pH was plotted, there was an increase in migration time as the pH was increased (data not shown). At pH 8 and pH 8.5, we observe a plateau which suggests that there is not much difference in separation between those pH values. However, when the pH is raised to 9.0 and 9.55, the migration time increases, which is indicative of solutes close to their pK_a and therefore partially ionized and partially neutral in character.

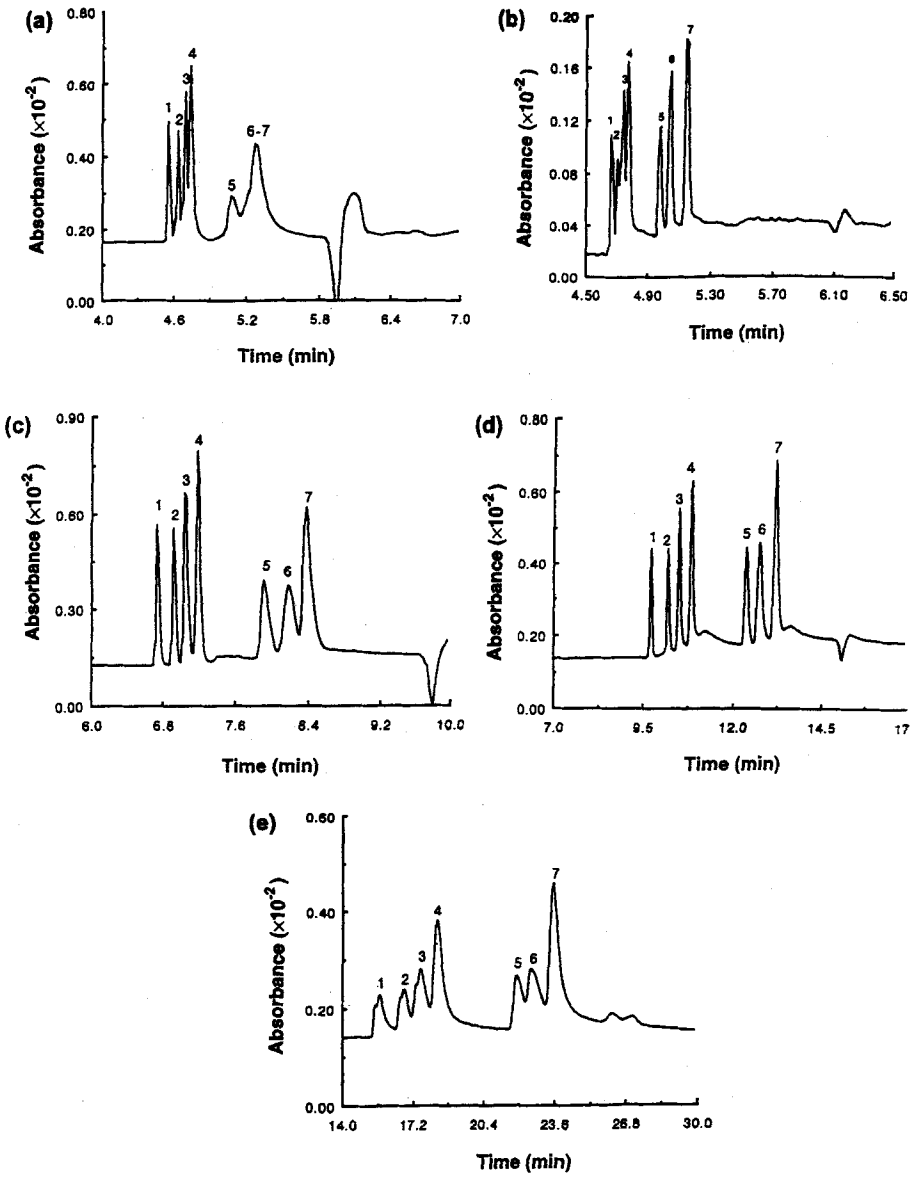

Figure 5. Electropherograms showing the effect of percentage (v/v) of methanol on the separation of the seven tricyclic antidepressant drugs. (a) 0% MeOH, (b) 5% MeOH, (c) 10% MeOH, (d) 20% MeOH, and (e) 30% MeOH. Conditions were 50 mM phosphate buffer, 0.06% w/v PUG, pH 9.55; pressure sample injection for 5 s; applied field strength 400 V/cm; and detection at 214 nm.

4 Concluding remarks

Hydrophobic and hydrogen bonding (hydrophilic) of a polymerized nonionic surfactant, PUG, to the capillary wall allows complete and reproducible baseline resolution of seven closely related tricyclic antidepressant drugs in CE. Optimum selectivity was found using 0.06% w/v fraction of the polymer, containing 20% v/v methanol, at pH 9.55.

The basis of this separation is believed to be hydrogen bonding of PUG to the capillary wall, which makes the capillary wall neutral in character. This in turn keeps the cationic analytes from interacting with what would normally be a mixture of silanol and uncapped silanol groups on the capillary wall. As shown in Fig. 5a, this was observed when partial separation of the seven drugs without the use of organic modifier was achieved by decreasing the irreversible binding of the cationic drugs to the capillary wall. In order to achieve optimum separation, the organic modifier and pH were varied to change the elution window and achieve separation of the tertiary amines. The use of a nonionic polymerized surfactant to coat the capillary walls using a modified CZE mode allowed negligible Joule heating, low molar absorptivity in the low UV region (improves the signal-to-noise ratio for the analytes), and the use of higher organic modifier content, without destroying the polymeric surfactant coating. Consequently, a simplistic, and rapid methodology to separate the seven antidepressant drugs was provided. The possibility of this polymerized surfactant as a transient capillary coating for improved separation of other analytes will be examined.

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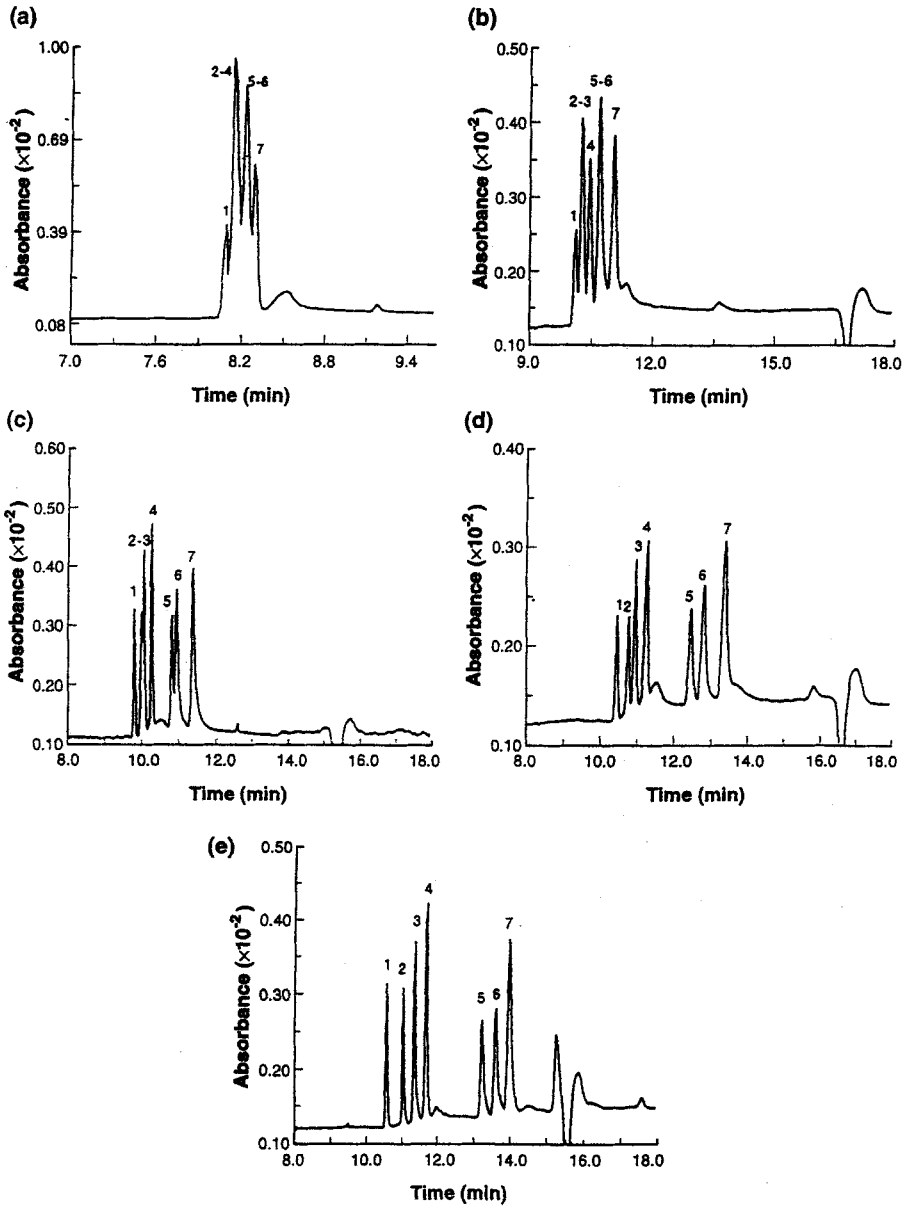

Figure 6. Electropherograms illustrating the effect of pH on the separation of seven tricyclic antidepressants. Conditions same as Fig. 5 with 20% MeOH. (a) pH 7.00, (b) pH 8.00, (c) pH 8.50, (d) pH 9.00, (e) pH 9.55.

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