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Migration behavior and selectivity of β-blockers in micellar electrokinetic chromatography
Influence of micelle concentration of cationic surfactants

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Abstract

The influence of micelle concentration of cationic surfactants on the migration behavior and selectivity of ten β-adrenergic blocking agents in micellar electrokinetic chromatography (MEKC) were systematically investigated at pH 6.5 and 7.0. Tetradecyl- and hexadecyltrimethylammonium bromides (TTAB and CTAB) were selected as cationic surfactants. The results indicate that, in addition to buffer pH, micelle concentration is an important separation parameter that influences the migration and selectivity of β-blockers in MEKC. The migration behavior and selectivity of labetalol and propranolol are most markedly affected. The resolution of peaks between atenolol, metoprolol and levobunolol greatly enhances on increasing the micelle concentration. In contrast, the peaks between acebutolol and nadolol and those between timolol and atenolol become unresolvable at concentrations near 30 m*M* at pH 7.0. Complete separation of these β-blockers was achieved either with CTAB and TTAB at a concentration in the range 15–20 m*M* and 12–15 m*M*, respectively, at pH 7.0 or with CTAB at a concentration in the range 27–30 m*M* at pH 6.5. Moreover, partition coefficients of β-blockers between the aqueous and micellar phases at pH 7.0 were evaluated. The plot of the logarithm of migration factor (log k') versus the logarithm of octanol–water partition coefficient (log P_{ow}) reveals that, the migration of β-blockers possessing small hydrogen bond strength depends on the extent of micellar solubilization based on hydrophobic interactions, whereas the migration and selectivity of β-blockers with hydrogen bond donor characteristics are influenced considerably by hydrogen bonding interactions, in addition to hydrophobic interactions, in MEKC. © 1997 Elsevier Science B.V.

Keywords: Buffer composition; β-Blockers

1. Introduction

β-Adrenergic blocking agents (β-blockers) are a group of therapeutically important drugs widely used in the treatment of angina, cardiac arrhythmias hypertension, myocardial infarction, and glaucoma [1]. They are also used as doping agents in sports [2]. The structures of β-blockers studied are shown in Fig. 1. These compounds possess two structural features: an alkanolamine side-chain terminating in a secondary amino group and an aromatic group with various substituents. Depending on the structural features, some β-blockers are hydrophilic and others lipophilic. Thus the difference in polarity is so large that difficulties to separate them may occasionally be encountered.

Various chromatographic methods [3–10] and capillary electrophoresis [11–18] have been de-

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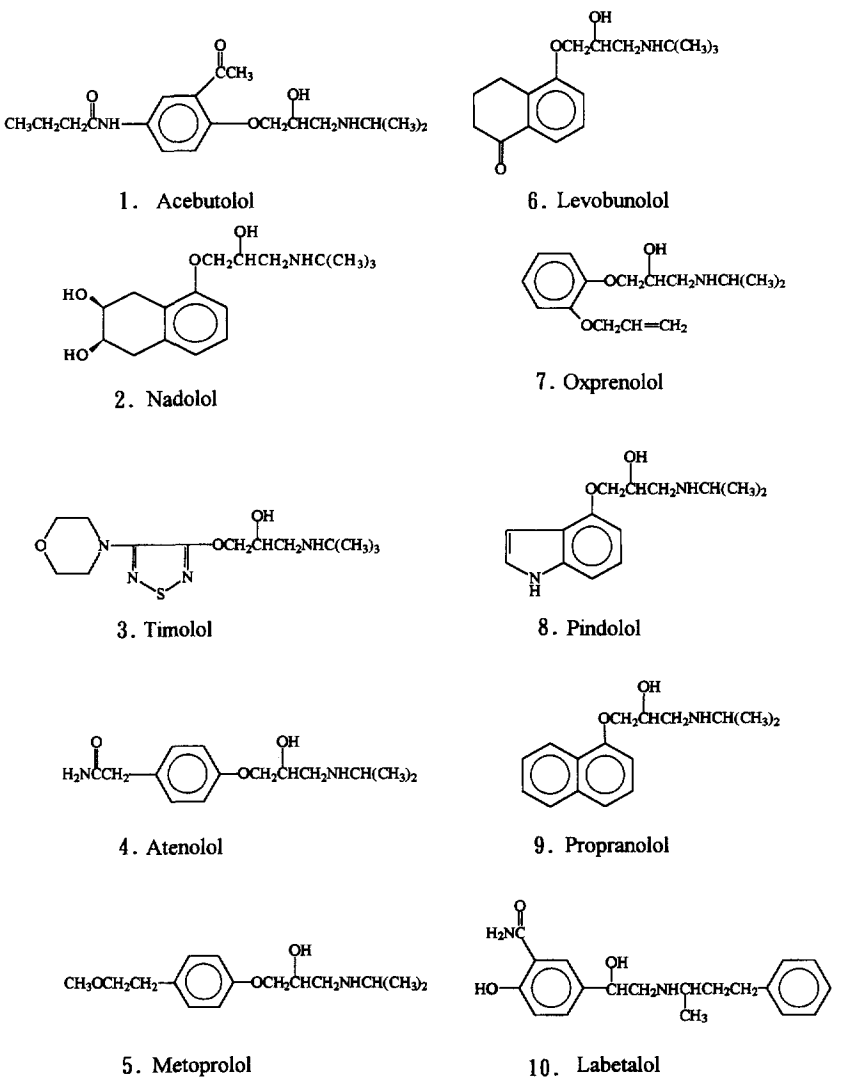

Fig. 1. Structures of the β-blockers studied.

veloped to determine and identify β-blockers. The separation of ten β-blockers by capillary zone electrophoresis was successfully achieved using citrate buffer at high concentration and at low pH [18]. The separation of the enantiomers of β-blockers by cyclodextrin-mediated capillary zone electrophoresis was conducted with the use of 70 m*M* tetramethylammonium phosphate containing 20 m*M* derivatized cyclodextrin at pH 2.50 [16]. On the other hand, the separation and determination of β-blockers with MEKC was attempted by Lukkari et al. [11–15] using phosphate buffer (80 m*M*) containing 10–15 m*M* cetyltrimethylammonium bromide (CTAB) as a cationic surfactant in the pH range 6.0–7.8. The effects of buffer pH [11] and organic modifier [13] on the migration behavior and separation of β-blockers were examined. However, the influence of micelle concentration on the migration behavior and separation was neglected. As micelle concentration is expected to play an important role in the separation of β-blockers in MEKC, a systematic investigation of the effect of this separation parameter on the migration behavior and separation of β-blockers is desirable.

It has been generally accepted that the separation of neutral and charged solutes in MEKC is based on the differential partitioning of solutes between aqueous phase and micellar phase in which hydrophobic interaction is the sole underlying force that influences the migration behavior of solutes. However, the large differences in migration behavior in MEKC with different types of surfactants indicates that this general belief is not accurate for all MEKC systems [19,20]. Depending on the chemical nature of both solutes and micelles, various chemical interactions other than hydrophobic interactions, such as dipolar or hydrogen bonding interactions between solute and micelle, may be involved in the partitioning process [19–21]. These interactions influence the migration behavior of solutes with various functional groups to a different extent, thus resulting in the differentiation of the selectivity and the alteration of the migration order of solutes.

In order to shed light on the migration order of β-blockers, partition coefficients of β-blockers between the aqueous and micellar phases are evaluated; the correlation between migration factor and octanol–water partition coefficient which is an index for hydrophobicity are examined so that underlying chemical interactions that influence the migration and selectivity of β-blockers can be better understood.

2. Experimental

2.1. Chemicals and reagents

Ten β-blockers were used in this study. The structures of these β-blockers are shown in Fig. 1. Among them, acebutolol hydrochloride, nadolol and (±)-metoprolol (+)-tartrate were purchased from Aldrich (USA); oxprenolol hydrochloride, pindolol, propranolol hydrochloride, timolol maleate, atenolol, labetalol hydrochloride and levobunolol were supplied by National Laboratories of Foods and Drugs, Department of Health, Taiwan, ROC. Hexadecyltrimethylammonium bromide (cetyltrimethylammonium bromide, CTAB) and tetradecyltrimethylammonium bromide (TTAB) were obtained from Tokyo Kasei Kogyo (TCI, Japan). Sudan III, used as the marker for the micelle, was purchased from Sigma (St. Louis, MO, USA). All other chemicals were of analytical-reagent grade. Deionized water was prepared with a Milli-Q system (Millipore, Bedford, MA, USA).

Standard solutions of a mixture of ten β-blockers in aqueous solution containing various concentrations of each individual β-blocker ranging from 0.10 m*M* for acebutolol to 0.80 m*M* for timolol were prepared. The pH of the buffer was adjusted on mixing various proportions of sodium dihydrogenphosphate (70 m*M*) buffer solution with disodium hydrogenphosphate solution (70 m*M*) to reach the desired value in the range 6.5–7.0. All solutions were filtered through a membrane filter (0.22 μm) before use.

2.2. Apparatus

Separations were made with a capillary electrophoresis system described previously [22]. The capillary dimensions were 67 cm×50 μm, I.D. The UV detection position is 7.0 cm from the cathodic end. Sample injection was done in a hydrodynamic mode during 1 s. The CE system was interfaced with a microcomputer and printer with software CE 500 1.05A. For pH measurements, a pH meter (Suntex Model SP-701, Taipei, Taiwan) was employed with precision of ±0.01 pH unit.

2.3. Electrophoretic procedure

When a new capillary was used, the capillary was washed using a standard sequence described previously [18]. To ensure reproducibility, all experiments were performed at 25°C and measurements were run at least in triplicate. The capillary was pre-washed with running buffer for 5 min before each injection and postwashed with sodium hydroxide solution (1.0 *M*) at 60°C for 5 min, followed with sodium hydroxide solution (0.1 *M*) at 60°C for 10 min, and then with deionized water at 25°C for 5 min to maintain proper reproducibility for run-to-run injections. The detection wavelength was set at 220 nm.

2.4. Calculations

2.4.1. Electrophoretic mobility

The electrophoretic mobility of analytes was calculated from the observed migration times with the equation:

$$\mu_{ep} = \mu - \mu_{eo} = \frac{L_d L_t}{V}\left(\frac{1}{t_m} - \frac{1}{t_{eo}}\right) \quad (1)$$

where μ_{ep} is the electrophoretic mobility of the analyte tested, μ is the apparent mobility, μ_{eo} is the electroosmotic mobility, t_m is the migration time measured directly from the electropherogram, t_{eo} is the migration time for an uncharged solute (methanol as neutral marker), L_t is the total length of capillary, L_d is the length of capillary between injection and detection, and V is the applied voltage.

2.4.2. Migration factor

The migration factor of analytes (k') was calculated from the observed migration times with the equation [23]:

$$k' = \frac{t_m - t_o}{t_o\left(1 - \frac{t_m}{t_{mc}}\right)} \quad (2)$$

where t_o is the migration time in the absence of micelles, and t_{mc} is the migration time of micelles, or

$$k' = \frac{(\mu_{ep} - \mu_o)}{(\mu_{mc} - \mu_{ep})} \quad (3)$$

where μ_{ep}, μ_o,and μ_{mc} are the electrophoretic mobility of a solute, the electrophoretic mobility of a solute in the absence of micelles, and the electrophoretic mobility of micelle, respectively, calculated from the corresponding migration times.

2.4.3. Partition coefficient

The migration factor in MEKC is directly proportional to the micelle concentration through the following equation [23,24]:

$$k' = P_{mw}\nu\{[S] - cmc\} \quad (4)$$

where P_{mw} is the partition coefficient of solutes between the aqueous and micellar phases, ν is the partial molal volume of the surfactant, and [S] is the total surfactant concentration.

3. Results and discussion

The β-blockers studied have an alkanolamine side-chain terminating in a secondary amino group with pK_a values in the range 8.7–9.1 [24–27], and are positively charged over a wide range of pH, including the physiological pH. In order to prevent basic (cationic) solutes from interacting with the capillary wall, the adsorption of cationic surfactants on the capillary wall is effective [16,17]. This may lead to a change of the polarity of capillary surface, thus resulting in a reversal of the electroosmotic flow [16,17]. The electroosmotic mobility (μ_{eo}) of the buffer electrolyte was varied from $-3.87\cdot10^{-4}$ to $-4.10\cdot10^{-4}$ $cm^2\,V^{-1}\,s^{-1}$ with CTAB and from $-3.65\cdot10^{-4}$ to $-4.28\cdot10^{-4}$ $cm^2\,V^{-1}\,s^{-1}$ with increasing CTAB and TTAB concentrations, respectively, in the concentration range 5–30 mM at pH 7.0.

3.1. Effect of surfactant concentration

3.1.1. CTAB at pH 7.0

Fig. 2 shows the electrophoretic mobility of ten

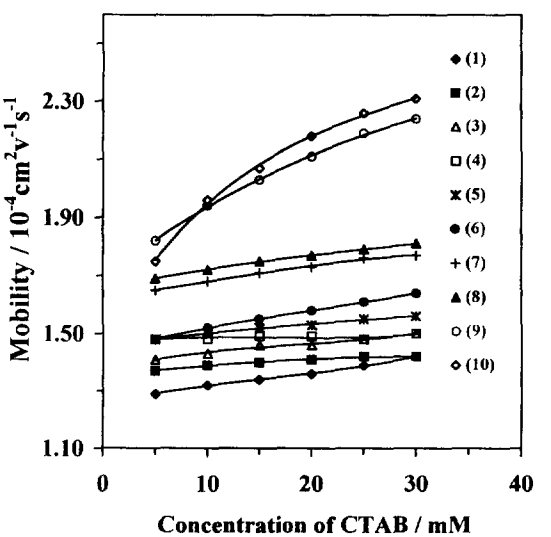

Fig. 2. Variatrion of the electrophoretic mobility of β-blockers as a function of CTAB concentration in a phosphate buffer (70 mM) at pH 7.0. Other operating conditions: −20 kV, 25°C. Curve identification: (♦) 1; (■) 2; (△) 3; (□)4 ; (*) 5; (●) 6; (+) 7; (▲) 8; (○) 9; (◇) 10; the numbers denote the analytes shown in Fig. 1.

β-blockers studied as a function of CTAB concentration in the range 5–30 m*M* at pH 7.0. As illustrated, depending on the solute–micellar interactions, the electrophoretic mobilities of β-blockers are affected to various extents as the concentration of CTAB increases. The electrophoretic mobility of propranolol (9) and labetalol (10) increases markedly with increasing the concentration of CTAB, whereas that of levobunolol (6) and acebutolol (1) also increases, but to a much less extent. The electrophoretic mobility of pindolol (8), oxprenolol (7), metoprolol (5), and timolol (3) increases only slightly, whereas that of atenolol (4) and nadolol (2) remains almost unchanged.

The separation and selectivity of β-blockers were affected on varying surfactant concentration. Propranolol (9) and labetalol (10) co-migrate with CTAB at concentration 9 m*M*. These two compounds are well-resolved when the concentration of CTAB is either greater than 11 m*M* or less than 8 m*M*. Propranolol (9) migrates after labetalol (10) when the concentration of CTAB is less than 9 m*M*, but elutes before labetalol when the concentration exceeds 9 m*M*. Oxprenolol (7) and pindolol (8) are well separated throughout the entire concentration range (5–30 m*M*) with oxprenolol migrating before pindolol. Levobunolol (6), metoprolol (5) and atenolol (4) co-migrate at concentration 5 m*M*, but levobunolol is completely separated from the other two analytes and migrates after metoprolol and atenolol at concentrations greater than 8 m*M*. Likewise, metoprolol (5) is completely separated from atenolol (4) and migrates after atenolol at concentrations greater than 14 m*M*. The results indicate that levobunolol (6), metoprolol (5) and atenolol (4) can be resolved at concentrations greater than 14 m*M*. Moreover, atenolol (4) and timolol (3) are well separated at a CTAB concentration less than 25 m*M*; acebutolol (1) migrates faster than nadolol (2) when the CTAB concentration is less than 30 m*M*, but co-migrates with nadolol (2) at 30 m*M*. Hence, complete separation of these β-blockers was achieved with CTAB at a concentration in the range 15–20 m*M*. Fig. 3 shows a typical electropherogram of β-blockers obtained with CTAB concentration at 15 m*M* and at pH 7.0.

The resolution of these β-blockers obtained in this work is better than that reported previously [11].

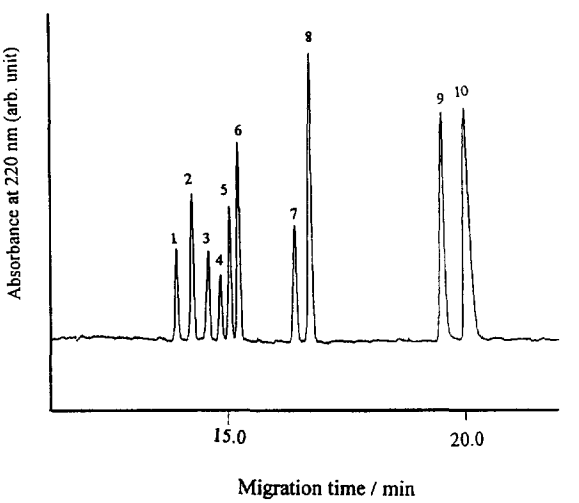

Fig. 3. Electropherogram of β-blockers obtained with phosphate buffer (70 m*M*) containing CTAB (15 m*M*) at pH 7.0. Other operating conditions and peak identification are the same as for Fig. 2.

Probably due to different surface conditions of the capillary column, Lukkari et al. [11] were unable to completely separate the following three pairs of consecutively migrating analytes: (9, 10), (7, 8) and (4, 5).

3.1.2. TTAB at pH 7.0

Fig. 4 shows the effect of surfactant concentration of TTAB on the migration behavior and separation of ten β-blockers at pH 7.0. As illustrated, the trends in the variation of electrophoreric mobility of β-blockers with TTAB are quite similar to those obtained with CTAB, except that the electrophoretic mobility of each individual β-blockers is smaller and that the extent of the variation in the electrophoretic mobility of each individual β-blockers in the same concentration range with TTAB is greater than that with CTAB.

Labetalol (10) migrates after propranolol (9) with TTAB at concentrations greater than 24 m*M*, but migrates before propranolol (9) at concentrations less than 18 m*M*. These two analytes were better separated with TTAB at concentrations less than 15 m*M*, because the extent of the decrease in the electrophoretic mobility of labetalol is relatively greater than that of propranolol with TTAB than that with CTAB. At pH 7.0, complete separation of the

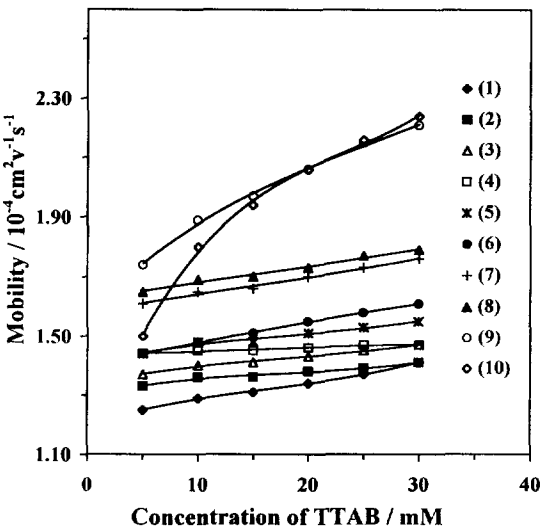

Fig. 4. Variation of the electrophoretic mobility of β-blockers as a function of TTAB concentration in a phosphate buffer (70 m*M*). Other operating conditions and peak identification are the same as for Fig. 2.

ten test β-blockers can be achieved with TTAB at a concentration in the range 12–15 m*M*. Fig. 5 shows the electropherograms of ten β-blockers obtained with TTAB at concentrations 5, 15, and 30 m*M*.

3.1.3. CTAB and TTAB at pH 6.5

The effects of surfactant concentration on the migration behavior and separation of ten β-blockers with CTAB and TTAB at pH 6.5 are shown in Fig. 6A,B, respectively. The mobility curves of labetalol and propranolol cross over at a smaller concentration (5 m*M*) with CTAB, but at about the same concentration with TTAB. The electrophoretic mobility of each individual β-blockers decreases considerably on decreasing the pH of the buffer from 7.0 to 6.5. This is probably due to the stronger electrostatic repulsion between solutes and cationic surfactants, thus resulting in less micellar solubilization and smaller electrophoretic mobility. The trends in the variation of electrophoretic mobility of these β-blockers at pH 6.5 are similar to those obtained at pH 7.0, except that the resolution of peaks between metoprolol and atenolol becomes unresolvable with CTAB or TTAB at a concentration less than 25 m*M* and that the migration window becomes wider when the concentration of CTAB exceeds 20 m*M*.

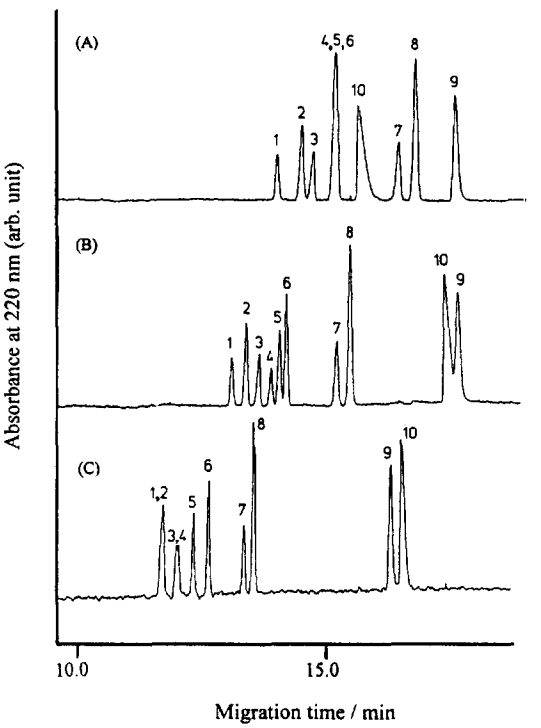

Fig. 5. Electropherogram of β-blockers obtained with phosphate buffer (70 m*M*) containing TTAB at concentrations, (A) 5 m*M*, (B) 15 m*M*, (C) 30 m*M* at pH 7.0. Other operating conditions and peak identification are the same as for Fig. 2.

The migration order of these β-blockers at pH 6.5 remains the same as that at pH 7.0. Complete separation of these β-blockers can only be achieved with CTAB at a concentration in the range 27–30 m*M*. At pH 6.5, peaks between labetalol and propranolol can not be completely separated with TTAB at a concentration of 30 m*M*. However, baseline separation can be achieved on addition of either acetonitrile (7.5%, v/v) or methanol (10%, v/v) to the phosphate buffer containing TTAB (30 m*M*) at pH 6.5 [28].

3.2. Partition coefficient vs. migration order

In order to shed light on chemical interactions involved in partitioning process, partition coefficients of β-blockers between the aqueous and micellar phases are evaluated so that the correlation between the logarithm of micelle–water partition coefficient (log P_{mw}) and the logarithm of octanol–water parti-

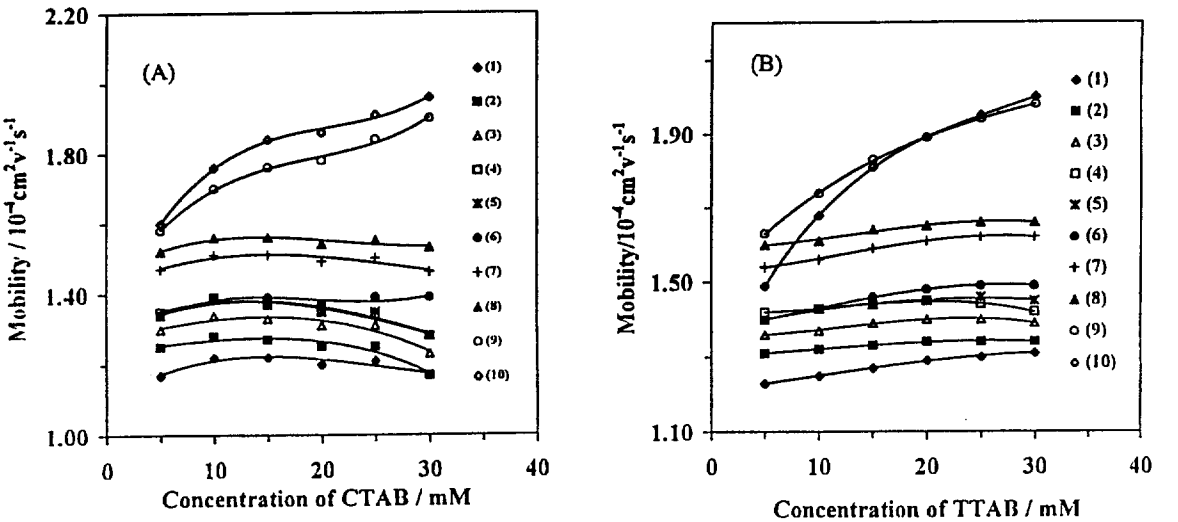

Fig. 6. Variation of the electrophoretic mobility of β-blockers as a function of (A) CTAB and (B) TTAB concentrations in a phosphate buffer at pH 6.5. Other operating conditions and peak identification are the same as for Fig. 2.

tion coefficient (log P_{ow}) can be examined. The migration factors (k') of β-blockers were calculated according to Eq. (2) or Eq. (3). Since β-blockers are not electrically neutral solutes at pH 7.0 or 6.5, the correction to the migration time of analytes in the absence of micelle must be taken into consideration. In this work, the mobility data with TTAB at 0.3 m*M* were selected as μ_o, because 0.3 m*M* is the minimal concentration of TTAB that could be measured with reversed electroosmotic flow. Fig. 7 shows the plots of migration factor (k') of β-blockers vs. total surfactant concentration of TTAB in the range 5–30 m*M* at pH 7.0. Excellent correlations with correlation coefficients (r^2) greater than 0.964 are obtained. The cmc value of TTAB determined by MEKC is about 1.1 m*M* [28]. This value is smaller than the literature value of 3.5 m*M* (in pure water at 25°C) as expected, owing to the presence of buffer electrolyte. As the slope of the line is directly proportional to P_{mw}, the larger the slope, the greater the value of partition coefficient. The P_{mw} values were calculated according to Eq. (4), with the partial molal volume (ν) of TTAB being equal to 0.328 L/mol [29]. Table 1 lists the P_{mw} and k' values evaluated, and the mobility data obtained at pH 7.0, together with the P_{ow} values reported in the literature.

As shown in Table 1, the migration factor of β-blockers increases in the order atenolol (4)≤ nadolol (2)<timolol (3)<acebutolol (1)≤ metoprolol (5)<oxprenolol (7)<levobunolol (6)< pindolol (8)<propranolol (9)<labetalol (10). It should be noted that this elution order (in the order of increasing k') corresponds to the migration order (in the order of increasing μ) observed in Fig. 5B,

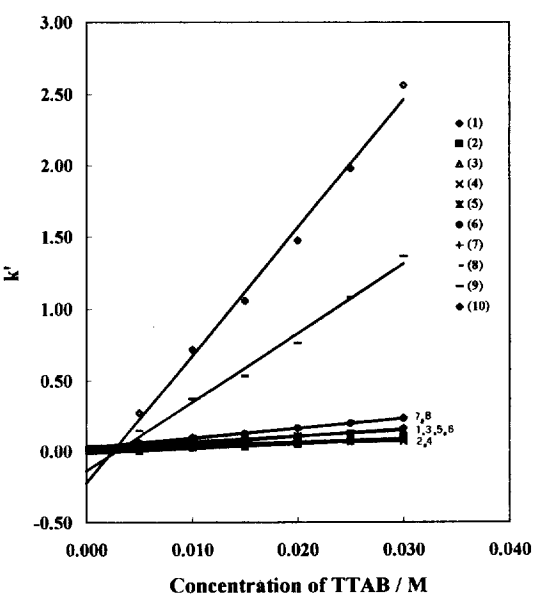

Fig. 7. Plots of migration factor (k') of β-blockers as a function of TTAB concentrations at pH 7.0.

Table 1
Partition coefficients (P_{mw}), migration factor (k') and mobility data of β-blockers measured with a phosphate–TTAB buffer at pH 7.0[a]

Peak no.	β-blocker	μ_{ep}[b]	μ_0[c]	k'	P_{mw}	P_{ow}[d]
1	Acebutolol	1.31	1.20	0.08	15.5	0.68
2	Nadolol	1.36	1.29	0.05	7.4	0.07
3	Timolol	1.41	1.32	0.07	10.3	1.16
4	Atenolol	1.45	1.39	0.05	3.4	0.02
5	Metoprolol	1.48	1.39	0.08	12.1	0.98
6	Levobunolol	1.51	1.37	0.12	21.1	
7	Oxprenolol	1.66	1.55	0.11	21.3	2.28
8	Pindolol	1.70	1.57	0.14	21.3	0.82
9	Propranolol	1.97	1.60	0.54	148.8	20.20
10	Labetalol	1.94	1.16	1.10	278.4	11.50

[a] Mobility in unit of 10^{-4} $cm^2 V^{-1} s^{-1}$; $\mu_{mc} = 2.65 \times 10^{-4}$ $cm^2 V^{-1} s^{-1}$.
[b] TTAB at concentration 15 m*M*.
[c] TTAB at concentration 0.3 m*M*.
[d] From Refs. [30,31].

despite that atenolol (4) and oxprenolol (7) elutes before nadolol (2) and levobunolol (6), respectively, whereas timolol (3) and propranolol (9) are followed by acebutolol (1) and labetalol (10), respectively.

The correlation between P_{mw} and P_{ow} was attempted for β-blockers. When plotting log P_{mw} versus log P_{ow}, a good correlation is difficult to obtain for all of the β-blockers. However, by dividing β-blockers into two groups, good correlations between log P_{mw} and log P_{ow} can be obtained. The correlation coefficients (r^2) for the group with relatively less hydrogen bond characteristics, including propranolol, oxprenolol, metoprolol, timolol and acebutolol, and for the other group with hydrogen bond donor characteristics, including labetalol, pindolol, nadolol and atenolol are 0.918 (with slope = 0.768 and intercept = 1.119) and 0.960 (with slope = 0.645 and intercept = 1.619), respectively. Fig. 8 shows the plots of such correlations.

Similar phenomenon was observed in the plot of log k' vs. log P_{ow}. As shown in Fig. 9, two different correlations can be obtained for β-blockers with a relatively small hydrogen bond characteristic and for those possessing various hydrogen bond donor strength. Based on the findings obtained by Yang et al. [20] on the investigation of quantitative structure–retention relationship (QSRR) which describes the correlation between log k' and log P_{ow} using TTAB as a cationic surfactant. The plot of log k' vs. log P_{ow} thus reveals that the migration of β-blockers which possess relatively small hydrogen bond strength is influenced primarily by hydrophobic interactions. However, hydrogen bonding interactions between TTAB (with the hydrogen bond acceptor characteristic) and β-blockers with strong hydrogen bond donor characteristics should play an important role in influencing the migration and selectivity of β-blockers. As a result, the migration order may be altered. This is particularly true for labetalol.

4. Conclusion

In separating β-blockers with MEKC, micellar

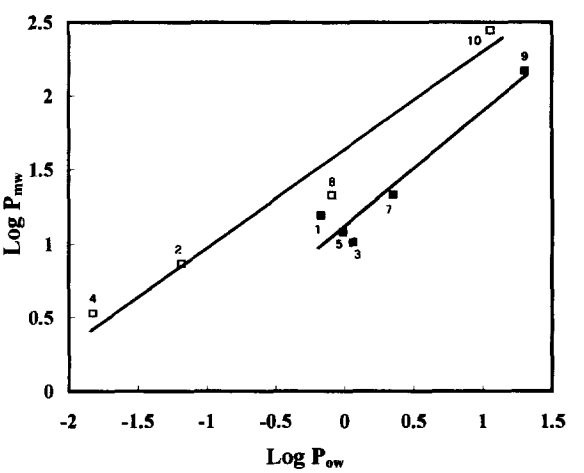

Fig. 8. Plots of log P_{mw} versus log P_{ow} for β-blockers with TTAB concentration at 15 m*M* and at pH 7.0.

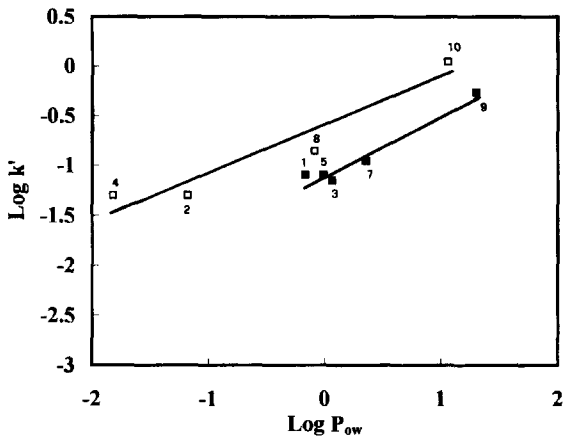

Fig. 9. Plots of log k' versus log P_{ow} with TTAB concentration at 15 mM and at pH 7.0.

concentration is an important separation parameter that influences the migration and selectivity of β-blockers. Complete separation of β-blockers is achievable with a phosphate buffer containing an appropriate concentration of TTAB or CTAB at a pH in the range 6.5–7.0. The plot of log k' and log P_{ow} reveals that hydrophobic interaction is not the sole underlying force that influences the migration and selectivity of β-blockers with hydrogen bond donor characteristics in which hydrogen bonding interactions play an important role.

Acknowledgments

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