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Effect of buffer solution pH on the elution and separation of β-blockers by micellar electrokinetic capillary chromatography

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ABSTRACT

Study was made of the effect of the pH of phosphate buffer (0.08 *M*) containing 15 m*M* cetyltrimethylammonium bromide as surfactant on the elution order of eleven widely used β-adrenergic blocking agents. In the pH range 6.0–7.8 the elution order of six of the β-blockers remained the same, while the order of five of them changed. Sotalol eluted as the sixth compound at pH 6.8 and migrated more quickly with increasing pH. Below pH 7.0 labetalol eluted before propranolol and above pH 7.0 afterwards. Likewise, the order of elution of atenolol and timolol was reversed at pH 7.0. The pH also affected the resolution; the best resolution values were achieved between pH 6.6 and 7.0 and between pH 7.4 and 7.8. The relationship between the structure of the β-blockers described by molecular and molecular connectivity indices and the elution order and separation of the β-blockers in micellar electrokinetic capillary chromatography at varying pH of the buffer solution is discussed.

INTRODUCTION

Adrenergic β-receptor blocking agents, commonly known as β-blockers, are a relatively new group of drugs. β-Blockers are widely used to treat angina pectoris, cardiac arrhythmias and hypertension [1]. They are also used as doping agents by athletes in order to reduce sympathetic activity in cases where high psychcological pressure may impair performance [2]. Propranolol, in 1965, was the first β-blocker to be formally approved for clinical use, and many more β-blockers have been introduced during the last 20 years. The structures of the studied β-blockers are shown in Fig. 1.

Micellar electrokinetic capillary chromatography (MECC) is a recently developed form of capillary zone electrophoresis (CZE) used for the analysis of electrically neutral species [3]. MECC utilizes as a pseudo-stationary phase a surfactant system at concentrations above the critical micelle concentration (CMC) of the surfactant. Separation of neutral particles is accomplished by partitioning of the solute between the pseudo and aqueous phases. Migration times of solutes in MECC systems are affected by the

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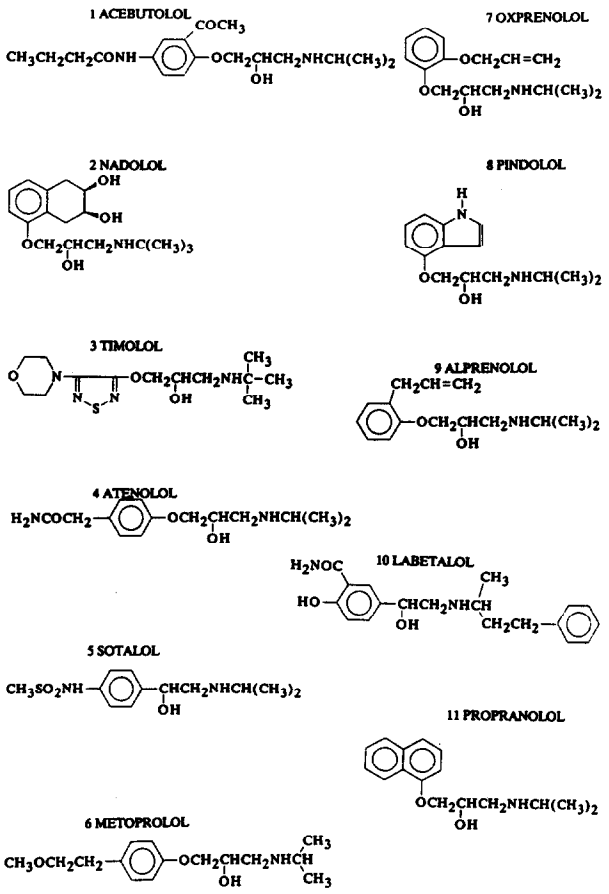

Fig. 1. Structures of the β-blockers.

electrophoretic mobility of the solute, the reactions between the solute molecules and the micelles and the magnitude of electro-osmotic flow (EOF) [4].

Proper selection of the pH of the buffer solution is often critical in MECC because the migration times of solutes, as well as the resolution and separation, tend to change rapidly with even small changes in the pH [5–7]. Moreover, EOF decreases inside the capillary at more acidic pH, owing to the decrease in the ζ-potential and the suppressed dissociation of SiOH groups of the capillary wall [8].

The addition of N-cetyl-N,N,N-trimethylammonium bromide (CTAB) to the buffer reverses the EOF towards the anodic end of the silica capillary by changing the negative charge of the wall to positive. With the decrease in the pH the molecular interactions between CTAB and the capillary wall increase. That is, the electroosmotic flow towards the anodic end of the capillary increases as the pH decreases, leading to changes in the migration times of solutes [9]. In addition, micellar partitioning of solutes around their pK_a values is very sensitive to the changes in pH [10].

In order to estimate quantitatively the relationship between the structure of analytes and their chromatographic behaviour, molecular connectivity indices, introduced by Randic [11] and developed further by Kier and Hall [12], have often been used [13]. These are numerical values that define and quantitatively describe the adjacency relationships in a molecular structure. When the nature of the atom is not taken into consideration, the index is referred to as the connectivity level index, χ, and when it is the index is described as the valence level, χ^v. Kier and Hall [12] extended the connectivity indices to a higher order, classifying the subgraphs into four types —path, cluster, path/cluster and chain— described by the subscripts p, c, pc and ch, respectively.

We studied the effect of the pH of phosphate buffer solution on the elution and separation of eleven β-blockers. The pH range was from 6.0 to 7.8. In addition, the effect of changing pH on the corrected migration times ($t'_r = t_r - t_0$) of the β-blockers and on the electroosmotic breakthrough time (t_0) was studied. Resolution (R) values for each peak pair were calculated by the half-width method. An aim of the study was also to explain the migration behaviour of the β-blockers with reference to the structure of solutes.

EXPERIMENTAL

MECC was performed in 580 × 0.050 mm I.D. fused-silica capillary tubes (Polymicro Technologies, White Associates, Pittsburgh, PA, USA); 500 mm was the effective length for separation. A Waters Quanta 4000 capillary electrophoresis system (Millipore Corporation, Waters Chromatography Division, Milford, MA, USA) was employed. Detection was at wavelength 214 nm. All experiments were carried out

at ambient temperature. Samples were injected hydrostatically for 15 s and the running voltage was −20 kV. The data (peak height) were collected with an HP 3392A integrator (Hewlett-Packard, Anondale, PA, USA).

The MolconnX 1.0 program (Lowell H. Hall, Hall Associates Consulting, Eastern Nazarene College, Quincy, MA, USA) was used to calculate the molecular and molecular connectivity indices of the β-blockers up to ten order indices. The Stat View II 1.03 (Abacus Concepts, Berkeley, CA, USA) and Systat 5.1 (Systat, Evanston, IL, USA) procedures were used as the statistics programs. All of these programs were run on a Macintosh IIsi computer.

The pH of the buffers was adjusted with a Jenway 3030 pH meter connected to a Jenway electrode (Jenway, Felsted, UK) containing 4 *M* potassium chloride in saturated silver chloride. Calibration of the electrode system was done with potassium hydrogenphthalate (0.05 *M*, pH 4.00) and sodium tetraborate (0.01 *M*, pH 9.81) solutions.

Materials

The reagents used in analysing the β-blockers were acebutolol hydrochloride, alprenolol hydrochloride, atenolol, labetalol hydrochloride, (±)-metoprolol (+)-tartrate, nadolol, oxprenolol hydrochloride, pindolol, (*S*)-(−)-propranolol hydrochloride, sotalol hydrochloride and timolol maleate, all from Sigma (St. Louis, MO, USA). Sodium dihydrogenphosphate monohydrate, disodium hydrogenphosphate dihydrate and CTAB were from Merck (Darmstadt, Germany). Other reagents used were of analytical grade. Distilled water was ion-exchanged through a Water-I system from Gelman Sciences (Ann Arbor, MI, USA). All the micellar buffer solutions were filtered through 0.45-μm membrane filters (Millipore, Molsheim, France).

The buffers were prepared from 0.08 *M* sodium dihydrogenphosphate and 0.08 *M* disodium hydrogenphosphate solutions. Both solutions contained 15 m*M* CTAB. The accurate pH of buffer solutions was increased from 6.0 to 7.8 in steps of 0.2 or 0.4 units, with degassing after each step.

MECC procedure

Between injections the capillary was purged for 2 min with the buffer solution before each injection.

RESULTS AND DISCUSSION

We confirmed the repeatability of migration of the β-blockers by measuring t'_r and t_0 values (measured with methanol) as six replicates at pH 6.2, 6.6, 7.0, 7.4 and 7.8. The relative standard derivations (R.S.D.) of t'_r of the β-blockers varied from 0.3% to 3.4% ($n = 6$), and the R.S.D.% of the t_0 values from 0% to 1.5% ($n = 6$). The R.S.D. of the R values varied from 0.6 to 10.1% ($n = 6$). The measurements can be regarded as quite reliable. However, day-to-day repeatability of t'_r values of β-blockers described as R.S.D.% varied from 33.4% to 42.6% ($n = 6$) and can be considered to be low. One of the reasons could be the lack of temperature control in our apparatus. Therefore the changes in migration of β-blockers caused by changes in pH were measured on the same day.

The effect of pH change on migration was studied by increasing pH from 6.0 to 7.8. The elution order of the eleven β-blockers changed with pH, as shown in Fig. 2 (for numbering of the compounds, see Fig. 1). At pH 6.0 sotalol (**5**) migrated as the sixth solute. With increasing pH it migrated more quickly until above pH 7.0 it migrated as the first compound. Propranolol (**11**) migrated after labetalol (**10**) below pH 7.0 and before it above pH 7.0. Likewise, the elution order of atenolol (**4**) and timolol (**3**) changed at pH 7.0. If the R value between two β-blockers was less than 0.45, the compounds were considered to elute together.

Increased pH caused some changes in the t'_r of the β-blockers (Fig. 3). The migration time of sotalol was significantly reduced, while the migration times of alprenolol, labetalol and propranolol were increased with the increase in pH. Sotalol migrated 1.6 times faster at the basic end of the pH range, and the other three compounds migrated 1.4, 1.9 and 1.6 times slower under basic conditions. The t_r' of the other β-blockers was affected only very little by the change in pH. The migration window (differ-

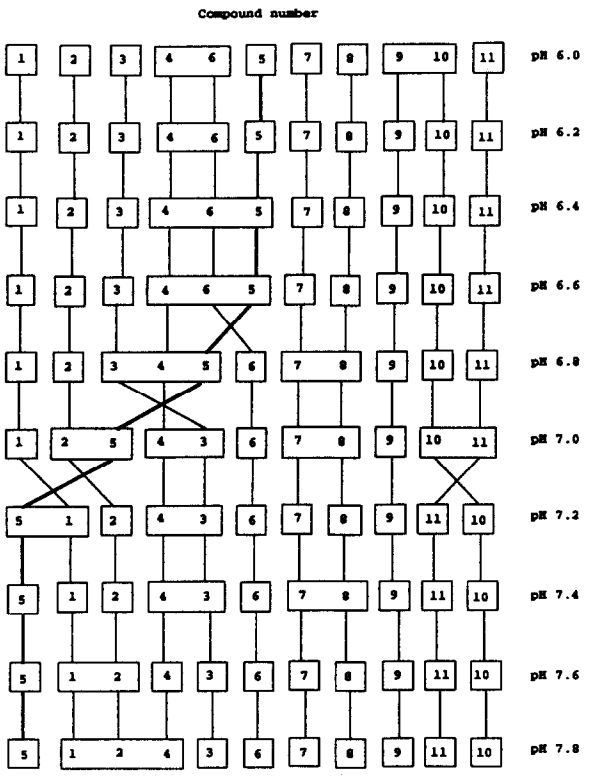

Fig. 2. Elution order of the β-blockers at ten different pH values of the phosphate buffer (0.08 *M*) solution containing 15 m*M* CTAB (for compound numbers, see Fig. 1).

ence between the first and last compound) was three times as wide at pH 7.8 as at pH 6.2 (Fig. 3). Even small variations in pH can cause unexpected changes in migration times of solutes and, especially in the analysis of complex mixtures, changes in migration order relative to other analytes.

The pH of the buffer solution also strongly affected the resolution of the β-blockers (Fig. 4). The major cause of the change in resolution was the change in migration behaviour of sotalol. As sotalol migrated more quickly it eluted, in turns, together with metoprolol, atenolol, timolol, nadolol and acebutolol. At pH 7.0 labetalol and propranolol eluted together, but otherwise separately. Oxprenolol and pindolol eluted together in the middle of the pH range. Likewise, atenolol and timolol eluted together at pH 7.0 and pH 7.4, and acebutolol and nadolol were not separated from each other at the highest pH values. The best resolution between the eleven β-blockers appeared to be between pH 6.6 and pH 7.0 as well as between pH 7.4 and pH 7.8. The migration window was wider at the basic end of the pH range but the total analysis time was much shorter at lower pH values. The t'_r for the last-migrating β-blocker was 10.4 min and 12.9 min at pH 7.4–7.8, whereas at pH 6.6 and pH 7.0 the times were 8.4 min and 8.9 min, respectively. Further optimization of analytical conditions is therefore more attractive at lower pH.

In order to explain the exceptional migration behaviour of some analytes, the structure was related to migration by the molecular and molecular connectivity indices. The effect of the pH of the buffer solution on the migration was also tested by factor analysis (Table I). Factor 1 can be related to pH, *i.e.* it is a pH-dependent factor, and factor 2 can be regarded as the non-pH-dependent factor. The results show that the effect of the pH of the buffer solution on the migration behaviour was significant only for sotalol, alprenolol, labetalol and propranolol.

To diminish the intercorrelations later in regressions, 183 molecular structure descriptors were calculated by the MolconnX program and the indices were divided into nine groups by cluster analysis using one Pearson-correlated coefficient single-linkage method (nearest neighbour). From nine groups, 1–3 best indices were selected for the multiple variable and stepwise regression model. The indices were tested against the migration changes of the studied β-blockers during the pH change. These migration changes were described as migration differences between pH 6.6 and pH 6.2 as well as between pH 7.8 and pH 6.2: $\Delta t'_{r(pH\ 6.6-6.2)}$ and $\Delta t'_{r(pH\ 7.8-6.2)}$, respectively. These regression analyses showed that three indices, SI3 (electrotopological atom state index for atom number 3), K^3 (the third-order kappa index) and KA^3 (the third-order kappa-alpha index), are related to substitution (SI^3) and shape of the molecule (K^3, KA^3). Fig. 5 shows the atom numbering of the skeleton common to all the studied β-blockers which were used in the calculation of SI indices. The indices can describe relatively well the migration changes due to pH (eqn. 1):

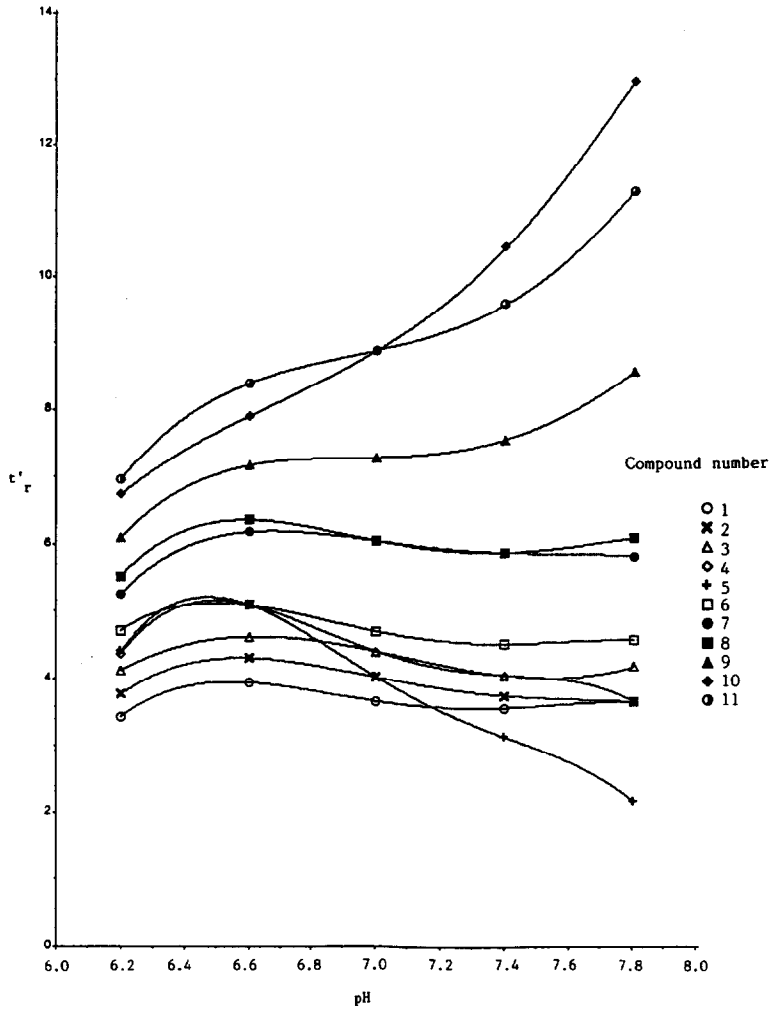

Fig. 3. Migration behaviour of eleven β-blockers at five different pH values of the buffer solution (buffer solutions and compound numbers as in Fig. 2).

$$\Delta t'_{r(pH\ 6.6-6.2)} = 2.62\ SI3 + 0.13\ K^3 - 0.21\ KA^3 - 7.44\ (r = 0.80, n = 11) \quad (1a)$$

$$\Delta t'_{r(pH\ 7.8-6.2)} = 7.75\ SI3 + 1.11\ K^3 - 1.26\ KA^3 - 26.35\ (r = 0.90, n = 11) \quad (1b)$$

As eqn. 1a shows, the data do not correlate very well, because the pH change from 6.2 to 6.6 was too small. However, with the larger change in pH (from 6.2 to 7.8) the correlation between the migration data and molecular structure of the β-blockers can be considered satisfactory (eqn. 1b). The SI3 index of the nitrogen atom (Fig. 5) as well as the K^3 and KA^3 indices, which describe the shape of molecule, were found to be significant when the effect of the pH of the buffer solution on the elution of β-blockers was studied. Also, the deductions made about the structures of β-blockers clearly support the conclusion based on these calculations.

The β-blockers can be divided into two groups according to their migration behaviour: I, acebutolol, atenolol, metoprolol, oxprenolol, alprenolol and propranolol; and II, nadolol, timolol, sotalol, pindolol and labetalol. The

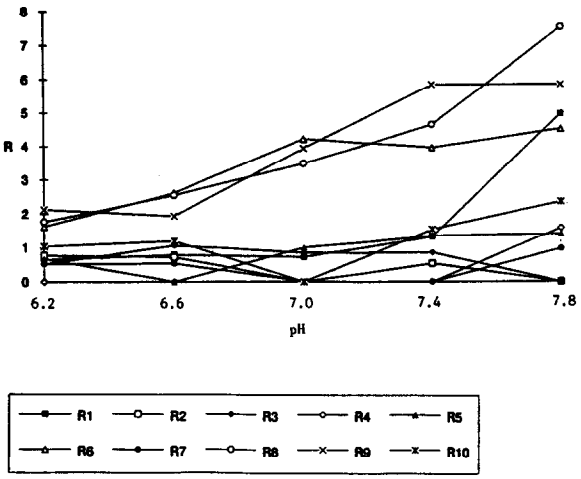

Fig. 4. Plotted resolution values for the β-blockers at different pH values. For the identification of *R* values, see the elution order (Fig. 2; for analysis conditions, see the Experimental section).

compounds always eluted in the same order within the groups in the studied pH range. In group I *p*-substituted β-blockers eluted before *o*-substituted compounds. Also, the molecules with smaller and less polar substituents eluted more slowly than the molecules with larger and more polar substituents because of their more

TABLE I

FACTOR ANALYSIS OF THE pH OF THE BUFFER SOLUTION ON THE MIGRATION BEHAVIOUR OF ELEVEN β-BLOCKERS

Factor 1 is the pH-dependent factor and factor 2 is the non-pH-dependent factor

Variable	Factor 1	Factor 2
pH	0.9942	−0.0162
Acebutolol	0.0717	0.9857
Nadolol	−0.4737	0.8731
Timolol	−0.2846	0.9323
Atenolol	−0.736	0.6656
Sotalol	−0.8981	0.435
Metoprolol	−0.5846	0.7615
Oxprenolol	0.3284	0.8813
Pindolol	0.3378	0.9402
Alprenolol	0.9689	0.2353
Labetalol	0.9939	−0.057
Propranolol	0.9928	0.1055

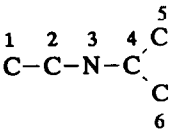

Fig. 5. The atom numbering of the skeleton common to all the studied β-blockers.

intense interactions with the micelles. In group II nadolol and timolol have the same propanol amine side chain and nadolol eluted before timolol because in timolol the ring structures probably interact more intensely with the micelles. For the same reason pindolol elutes before labetalol. The exceptional behaviour of sotalol is also due to its structure (Fig. 1): the sulphonamide group obtains a negative charge under neutral and basic conditions, causing sotalol to migrate more quickly towards the positive electrode (detector end of the capillary). Because the pK_a values of β-blockers are between 9.2 and 9.8 they are ionized at the studied pH range, and thus further ionization of β-blockers should cause only minor if any changes in their electrophoretic mobilities. The increased migration times of alprenolol, labetalol and propranolol are probably due to the lower EOF, which gives them more time to react with the slowly eluted micelles.

CONCLUSIONS

The pH of the buffer solution needs to be carefully controlled and optimized when ionic compounds are analysed by MECC because at different pH values the compounds elute in dissimilar order and at different rates. The migration window can be much wider at the basic end of the pH range. Likewise, pH greatly affects the resolution of the compounds. The migration behaviour of compounds can be related to the structure.

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