Journal of Chromatography A, 655 (1993) 317–324
Elsevier Science Publishers B.V., Amsterdam

CHROM. 25 493

Effects of organic mobile phase modifiers on elution and separation of β-blockers in micellar electrokinetic capillary chromatography

P. Lukkari

Department of Chemistry, Analytical Chemistry Division, University of Helsinki, P.O. Box 6, SF-00014, University of Helsinki, Helsinki (Finland)

H. Vuorela

Department of Pharmacy, Pharmacognosy Division, University of Helsinki, P.O. Box 15, SF-00014, University of Helsinki, Helsinki (Finland)

M.-L. Riekkola*

Department of Chemistry, Analytical Chemistry Division, University of Helsinki, P.O. Box 6, SF-00014, University of Helsinki, Helsinki (Finland)

(First received May 28th, 1993; revised manuscript received August 9th, 1993)

ABSTRACT

A study was made of the effect of organic modifiers (acetone, acetonitrile, ethanol, ethylene glycol, methanol and 2-propanol) in phosphate buffer (0.08 *M*) containing 15 m*M* cetyltrimethylammonium bromide as surfactant on the elution and separation of eleven common β-adrenergic blocking agents. The amount of the modifier was varied from 0.1 to 10.0% (v/v). At maximum addition, the organic solvents increased the viscosity of the buffer solution as follows: acetone 16%, acetonitrile 9%, ethanol 26%, ethylene glycol 27%, methanol 20% and 2-propanol 29%. In contrast to the migration time of the other β-blockers, that of labetalol was not increased by the addition of organic solvent to the buffer solution. Rather, labetalol eluted more quickly with increase in the amount of modifier, and thereby effected changes in the elution order of the β-blockers. The addition of modifiers also affected the resolution, and the best resolution values were achieved with the following amounts of organic solvent in MEKC buffer: acetone 0.1%, acetonitrile 0.1–0.5%, ethanol 5.0–7.5%, ethylene glycol 1.0–2.5%, methanol 5.0% and 2-propanol 1.0–2.5% (v/v). No significant relationship was found between the elution order and separation and the structure of the β-blockers in micellar electrokinetic capillary chromatography with an organic modifier in buffer solutions.

INTRODUCTION

Micellar electrokinetic capillary chromatography (MEKC) is a high-resolution separation method that has been in use since 1984 [1,2]. Many types of molecules are amenable to MEKC, including phenols [1,2], amino acids [3], nucleosides and oligonucleotides [4], nucleic acids [5], chiral substances [6,7] and pharmaceuticals [8–14]. In studying the effects of organic modifiers on separation in MEKC we have been using β-adrenergic blocking agents (β-blockers) as model compounds. These are a therapeutically important group of drugs, chemically derived from the adrenergic agonist iso-

* Corresponding author.

0021-9673/93/$06.00

prenaline [15]. β-Blockers are widely used in the treatment of angina pectoris and cardiac arrhythmias and more recently have been administered for the control of blood pressure and in ophthalmic disease (glaucoma). β-Blockers have also been used to improve athletic performance in cases where sympathetic activity causes the heart to race [16]. The structures of the β-blockers studied are shown in Fig. 1.

MEKC, which is a capillary zone electrophoretic (CZE) technique employing a surfactant above its critical micelle concentration (CMC), permits the separation of neutral and of uncharged molecules in an electroosmotically driven system [3,4]. The separation of neutral solutes is based on their differential partition between the electroosmotically pumped aqueous phase and the hydrophobic interior of the charged "pseudo-stationary" phase, or the micelles, which owing to electrophoretic effects are moving more slowly than the mobile phase. Charged compounds are distributed between the micellar and aqueous phases and simultaneously separated according to their electrophoretic mobilities.

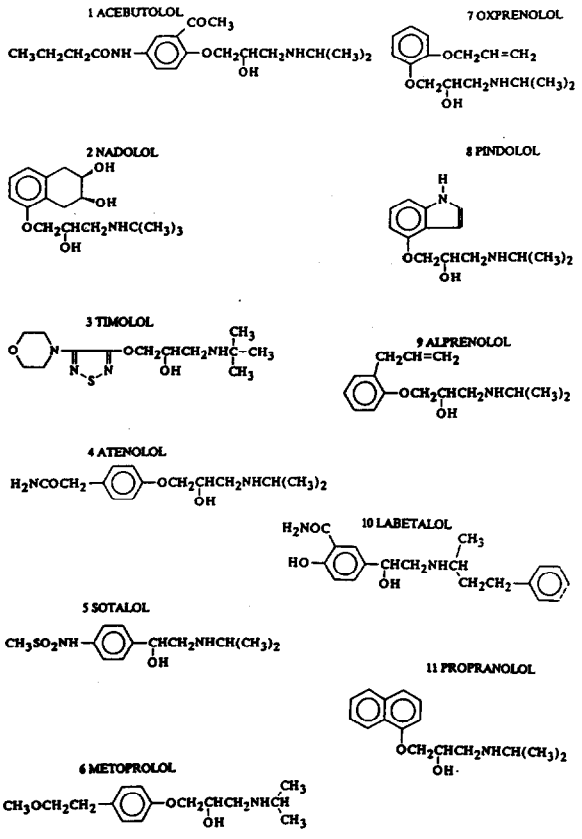

Fig. 1. Structures of β-blockers.

A surfactant exists in the form of micelles when its concentration in solution exceeds the CMC. The CMC and the aggregation number of micelles depend on physico-chemical parameters such as temperature, ionic strength and added electrolytes in a surfactant–water system [17]. Organic modifiers have been found to have several effects in MEKC. Even small concentrations of an organic solvent added to an aqueous micellar system may change the micelle formation and shape by decreasing the CMC [18]. Organic modifiers may also change the size of micelles and the electroosmotic flow (EOF) [19]. As the addition of an organic modifier to the MEKC electrolyte improves the wetting of the capillary wall, the silica surface is modified, with resulting changes in zeta-potential, and consequently in EOF [20]. Organic solvents also cause variations in EOF by changing the viscosity of the buffer solution [19]. Finally, modifiers affect the mass-transfer rates in and out of the micelles [20].

Molecular connectivity indices, introduced by Randic [21] and further developed by Kier and Hall [22], are numerical values describing the molecular structure, and are used to explain quantitatively the behaviour of an analyte in a chromatographic system. These indices have been applied to separations in both HPLC [23] and MEKC [24].

In this work, six organic modifiers (acetone, acetonitrile, ethanol, ethylene glycol, methanol and 2-propanol) of different selectivity [25], potentially useful in MEKC buffer solutions, were studied for their effects on the elution and separation of eleven β-blockers. Of interest were their effects on the corrected migration times ($t'_r = t_r - t_0$) of the β-blockers and on the electroosmotic breakthrough time (t_0). Resolution (R_s) values for each peak pair were calculated by the half-width method. In addition, an attempt was made to explain the migration behaviour of the β-blockers through reference to their structures as described by molecular and molecular connectivity indices.

EXPERIMENTAL

Apparatus

MEKC was performed in 580 × 0.050 mm I.D. fused-silica capillary tubes (Polymicro Technologies, White Associates, Pittsburgh, PA, USA), where the distance from the injector to the detector (L_d) was 500 mm. A Waters Quanta 4000 capillary electrophoresis system (Millipore, Waters Chromatography Division, Milford, MA, USA) was employed. The detection wavelength was 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, Avondale, PA, USA).

The pH of the buffer solution was adjusted to 6.8 with a Jenway (Felsted, UK) Model 3030 pH meter connected to a Jenway electrode containing 4 *M* KCl in saturated AgCl. The electrode system was calibrated with potassium hydrogenphthalate (0.05 *M*, pH 4.00) and sodium tetraborate (0.01 *M*, pH 9.81) solutions.

The viscosities of the buffer solutions were measured with a Model 0.003 SIL viscometer (Gallenkamp, London, UK) at 20, 25, 30, 35 and 40°C. The kinematic viscosities in 10^{-2} m^2 s^{-1} were calculated according to the equation $V = 0.00282t - (7.1/t)$, where t is the measured time in seconds. The amounts of organic solvents added to buffer solutions in the viscosity studies were 0.1, 1.0, 2.5, 5.0 and 10.0% (v/v).

The Molconn X 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 statistical programs. All of these programs were run on a MacIntosh IIsi computer.

Materials

The β-blockers studied 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). The reagents used included sodium dihydrogenphosphate monohydrate, disodium hydrogenphosphate dihydrate, N-cetyl-N,N,N-trimethylammonium bromide (CTAB), acetone, acetonitrile, ethylene glycol and methanol from Merck (Darmstadt, Germany). Ethanol was obtained from Alko (Helsinki, Finland) and 2-propanol from Rathburn Chemicals (Walkerburn, UK). Other reagents were of analytical-reagent grade. A Water-I system from Gelman Sciences (Ann Arbor, MI, USA) was used for ion-exchange of distilled water. All the micellar buffer solutions were filtered through 0.45-μm membrane filters (Millipore, Molsheim, France).

MEKC buffer was prepared from sodium dihydrogenphosphate and disodium hydrogenphosphate solutions containing CTAB so that, after addition of organic modifier, the concentrations were 0.08 *M* for phosphate and 15 m*M* for CTAB. The pH was adjusted to 6.8. The organic modifiers were added to achieve concentrations of 0.1, 0.5, 1.0, 2.5, 5.0, 7.5 and 10.0% (v/v). All solutions were degassed before use. Before each injection, the separation capillary was purged for 2 min with the buffer solution.

RESULTS AND DISCUSSION

The magnitude of the EOF is determined according to the equation

$$\mu_{eo} = (L_d L_t / t_0) V \quad (1)$$

where μ_{eo} is electroosmotic mobility, L_d distance from injector to detector, L_t total capillary length, t_0 migration time of the electroosmotic flow marker and V applied voltage [26]. In this study EOF can be monitored with t_0 values, as the other factors are constant as is evident from eqn. 1; t_0 values were measured with methanol.

The repeatability of the migration of the β-blockers was confirmed by measuring corrected migration times (t_r') and t_0 values as six replicates at concentrations of organic modifier of 0.1, 0.5, 1.0, 2.5, 5.0, 7.5 and 10.0% (v/v). The results for buffer solution when 10.0% (v/v) of acetone was added as modifier are not available because the separation conditions were too unstable for reliable analysis. The relative standard deviation

(R.S.D.) of the t_r' values of β-blockers varied from 0.5 to 9.9% ($n = 6$), and the R.S.D. of t_0 values varied from 0 to 4.3% ($n = 6$). The measurements can be regarded as reliable. However, the day-to-day repeatability was not satisfactory, perhaps because of the lack of temperature control in the apparatus, or because of the precipitation that occurs at the electrodes when phosphate-based buffers are used in MEKC. The changes in migration of β-blockers due to the organic modifiers were therefore measured on the same day.

The consequent changes of EOF, as measured by t_0 values, are shown in Table I. EOF showed an inverse correlation with the viscosity of the organic modifiers, which increased in the order acetonitrile, acetone, methanol, ethanol, ethylene glycol and 2-propanol (Table II). The addition of organic modifier increased the viscosity of the buffer solution. When the amount of acetonitrile was increased from 0.1 to 10.0% (v/v) the viscosity increased by 9.0%. Likewise, the other modifiers increased the viscosity of the buffer solution: acetone 16.0%, ethanol 25.9%, ethylene glycol 26.7%, methanol 19.6% and 2-propanol 28.9% (Table III). It is worth noting that acetonitrile changed the viscosity of the buffer solution much less than did the other organic solvents, and also effected the smallest changes in the migration times of the β-blockers (Fig. 2). The changes in EOF and in the elution behaviour of the β-blockers are probably due to the changes in interactions between the silica wall and the buffer solutions containing different organic modifiers.

TABLE I

THE ELECTROOSMOTIC BREAKTHROUGH TIMES (t_0) IN BUFFER SOLUTION MODIFIED WITH SIX ORGANIC SOLVENTS

The volumes of organic modifier are 0.1% and 10.0% (v/v).

Organic solvent	EOF (min)	
	0.1%	10.0%
Acetone	5.9	6.7
Acetonitrile	5.8	5.7
Ethanol	5.3	6.8
Ethylene glycol	5.8	8.7
Methanol	5.4	7.1
2-Propanol	5.8	8.3

TABLE II

KINEMATIC VISCOSITIES (10^{-2} m^2 s^{-1}) OF THE PHOSPHATE BUFFER SOLUTION AND PURE ORGANIC MODIFIERS AT TEMPERATURES FROM 20 TO 40°C

Solvent	20°C	25°C	30°C	35°C	40°C
Buffer	1.12	0.91	0.81	0.73	0.64
Acetone	0.41	0.36	0.33	0.31	0.29
Acetonitrile	0.40	0.39	0.37	0.35	0.34
Ethanol	1.85	1.36	1.26	1.07	1.09
Ethyl glycol	2.23	2.09	1.82	1.64	1.47
Methanol	0.97	0.66	0.62	0.59	0.54
2-Propanol	2.89	2.38	2.12	1.89	1.64

Except for acetonitrile and labetalol, when the amount of organic modifier was increased from 0.1 to 10.0% (v/v) the migration times (t_r') of the β-blockers increased (Fig. 2). With acetonitrile the migration times decreased or did not change. For labetalol the t_r' values were reduced in all modifiers. Acetonitrile decreased the t_r' of labetalol by 3.3 min, 2-propanol reduced it by 1.5 min and the other solvents reduced it by a few seconds (Fig. 2).

The migration window (difference between the first and last compounds) was decreased by 30% when the addition of acetonitrile was increased from 0.1 to 10.0% (v/v), but it was widened by at least 30% when the other organic solvents were added in increased amounts. The best separations of the β-blockers achieved with the following amounts of organic modifiers: 2-propanol 2.5%, ethylene glycol 1.0% and methanol 5.0% (v/v).

As the migration of labetalol was less affected than that of the other analytes, the elution order of labetalol relative to the other β-blockers changed markedly, but as the amount of modifier increased it co-eluted successively with alprenolol, pindolol, oxprenolol, metoprolol, sotalol, atenolol, timolol and nadolol (Fig. 2). Timolol and atenolol eluted together when the volume of methanol was below 2.5% (v/v) and when acetonitrile was the buffer modifier. Ox-

TABLE III

KINEMATIC VISCOSITIES OF THE PHOSPHATE BUFFER SOLUTION SUPPLEMENTED WITH ORGANIC MODIFIERS AT TEMPERATURES FROM 20 TO 40°C

Temperature (°C)	Kinematic viscosity (10^{-2} m^2 s^{-1})											
	Acetone						Ethylene glycol					
	0.1%	1.0%	2.5%	5.0%	10.0%	Δ (%)	0.1%	1.0%	2.5%	5.0%	10.0%	Δ (%)
20	1.04	1.07	1.10	1.16	1.27	18.1	1.04	1.10	1.15	1.22	1.46	28.8
25	0.93	0.93	0.96	1.05	1.11	16.2	0.92	0.94	0.99	1.08	1.26	27.0
30	0.86	0.88	0.90	0.92	1.04	17.3	0.80	0.83	0.88	0.94	1.11	27.9
35	0.73	0.74	0.77	0.80	0.86	15.1	0.73	0.75	0.79	0.85	0.99	26.3
40	0.65	0.65	0.67	0.71	0.75	13.3	0.66	0.67	0.70	0.75	0.86	23.3
	Acetonitrile						Methanol					
	0.1%	1.0%	2.5%	5.0%	10.0%	Δ (%)	0.1%	1.0%	2.5%	5.0%	10.0%	Δ (%)
20	1.04	1.06	1.08	1.11	1.20	13.3	1.04	1.06	1.10	1.18	1.32	21.2
25	0.93	0.93	0.96	1.00	1.02	8.8	0.92	0.95	0.98	1.04	1.14	19.3
30	0.82	0.83	0.85	0.88	0.91	9.9	0.81	0.82	0.87	0.91	0.99	18.2
35	0.75	0.74	0.75	0.77	0.80	6.2	0.72	0.76	0.77	0.80	0.89	19.1
40	0.66	0.66	0.67	0.69	0.71	7.0	0.63	0.65	0.69	0.71	0.79	20.3
	Ethanol						2-Propanol					
	0.1%	1.0%	2.5%	5.0%	10.0%	Δ (%)	0.1%	1.0%	2.5%	5.0%	10.0%	Δ (%)
20	1.03	1.08	1.12	1.22	1.45	29.0	1.04	1.12	1.17	1.30	1.57	33.8
25	0.93	0.95	0.99	1.08	1.25	25.6	0.93	0.96	1.01	1.10	1.32	29.5
30	0.80	0.85	0.88	0.94	1.09	26.6	0.81	0.85	0.87	0.95	1.13	28.3
35	0.73	0.75	0.77	0.84	0.98	25.5	0.72	0.74	0.79	0.85	0.99	27.3
40	0.65	0.66	0.69	0.75	0.84	22.6	0.64	0.68	0.69	0.75	0.86	25.6

prenolol and pindolol co-eluted when acetonitrile or ethanol was the organic solvent, and also when the volume of ethylene glycol exceeded 5.0% (v/v). Atenolol and sotalol could not be separated from each other in acetone-modified buffers, and they also eluted together when the volume of ethylene glycol exceeded 5.0% (v/v). With 10.0% (v/v) of 2-propanol, atenolol and sotalol co-eluted, as also did oxprenolol and pindolol. In MEKC buffer without an organic modifier labetalol eluted as the tenth compound between alprenolol and propranolol (Fig. 3).

Compounds were considered to elute together if the R_s value between them was less than 0.4. As can be seen in Fig. 4, when acetone or acetonitrile was used, no good separation was obtained for all eleven β-blockers. With the other organic solvents as buffer modifier the best R_s values were achieved in the following ranges: ethanol 5.0–7.5, ethylene glycol 1.0–2.5, methanol 5.0 and 2-propanol 1.0–2.5% (v/v). The separation of the β-blockers can therefore be improved through the addition of a suitable amount of these modifiers to the buffer solution [*e.g.*, 2-propanol 2.5% (v/v)] (Fig. 3).

In order to explain the overall migration behaviour of the solutes in terms of their structures, the molecular and molecular connectivity

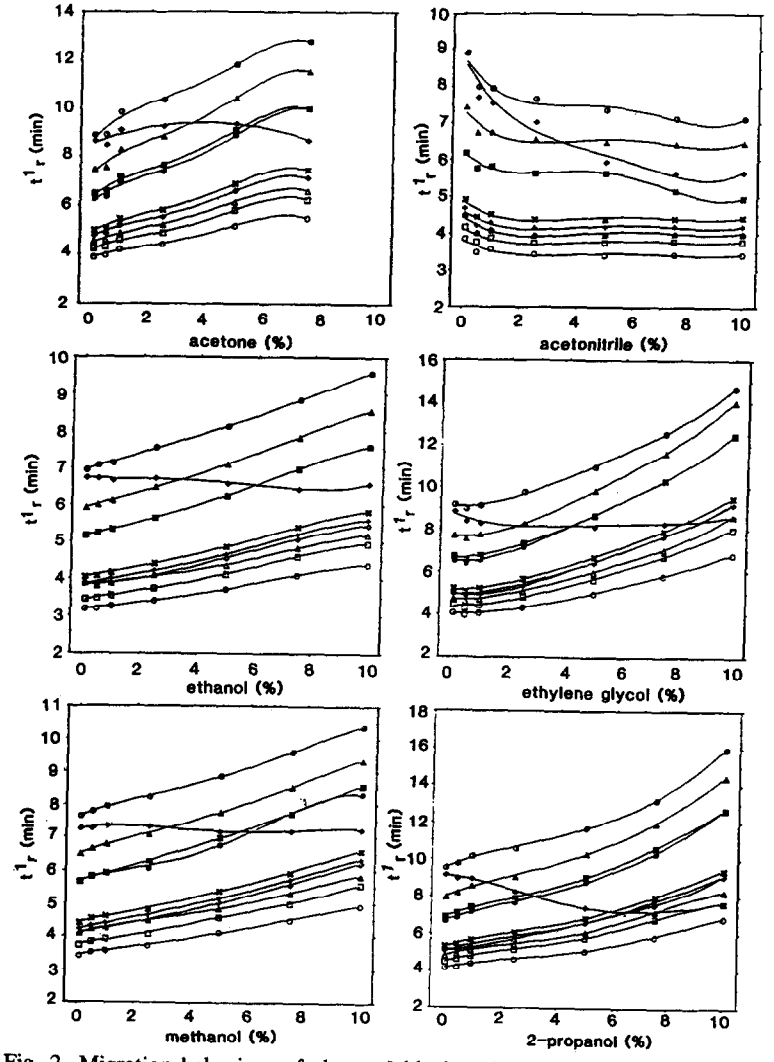

Fig. 2. Migration behaviour of eleven β-blockers in buffer solutions modified with six organic solvents. ○ = 1; □ = 2; △ = 3; ◇ = 4; + = 5; × = 6; ● = 7; ■ = 8; ▲ = 9; ◆ = 10; ◑ = 11 (for compound numbers, see Fig. 1).

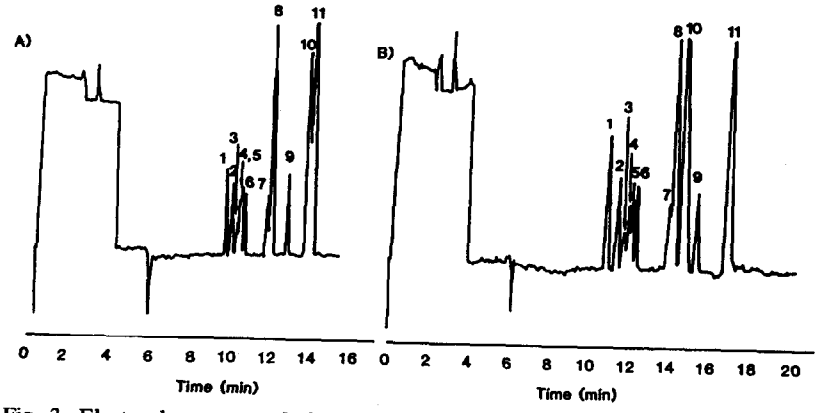

Fig. 3. Electropherograms of eleven β-blockers (A) in phosphate buffer and (B) in 2.5% (v/v) 2-propanol-modified buffer. Separation conditions are given under Experimental and compound numbers in Fig. 1.

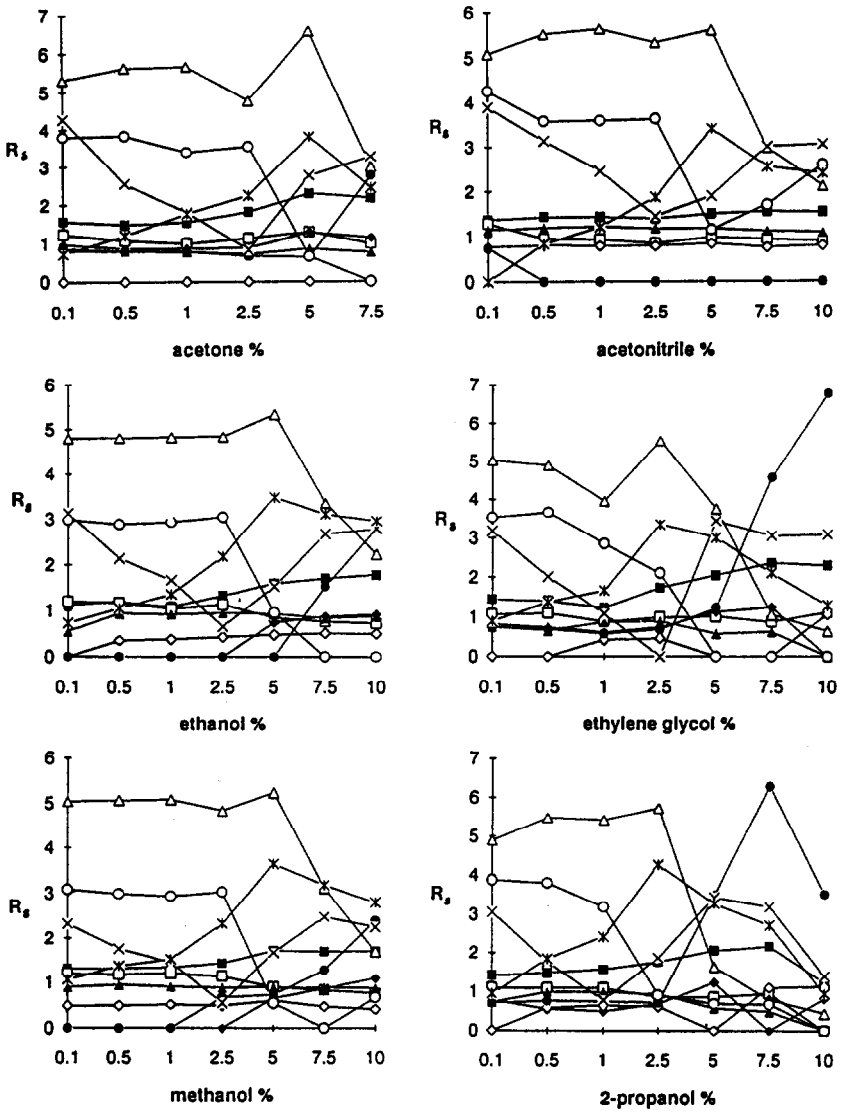

Fig. 4. Plotted resolution values for the β-blockers in different organic-modified buffers. For the identification of R_s values, see elution order from Fig. 2 and compound numbers from Fig. 1. Rx is the resolution value between the compounds x and $x+1$. The analysis conditions are reported under Experimental. ■ = R1; □ = R2; ◆ = R3; ◇ = R4; ▲ = R5; △ = R6; ● = R7; ○ = R8; × = R9; * = R10.

indices of the β-blockers were related to migration, as in our earlier study [24]. The migration changes were described as migration differences between concentrations of 0.1 and 10.0% (v/v): $\Delta t'_r = t'_{r\ org\ 10.0\%} - t'_{r\ org\ 0.1\%}$. The structural descriptors of the β-blockers did not correlate with the migration changes under the conditions studied. Only 2-propanol produced changes in migration that could be related to structure. The atom indices for atoms 1 and 2 showed some correlation, *i.e.*, $r = 0.92$ and 0.91, respectively. Fig. 5 shows the atom numbering for the carbon skeleton of β-blockers.

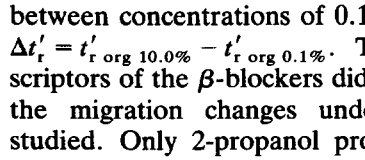

Fig. 5. Atom numbering of the skeleton common to all the β-blockers studied.

Because the molecular indices did not give satisfactory results, a non-numerical approach was used to explain the correlation between the migration and the structures of β-blockers. The exceptional behaviour of labetalol is evidently due to its carboxyl amide and hydroxy groups (Fig. 1). Normally, in dilute acidic or basic solutions, the carboxyl amide group hydrolyses to the corresponding acid, but with labetalol, the carboxyl amide and hydroxy groups form a six-membered ring via either intramolecular or intermolecular hydrogen bonding, so that competition for the ring-forming reaction occurs. These reactions are not possible for the other β-blockers studied as they do not contain carboxyl amide groups (Fig. 1). Formation of the six-membered ring decreases the interactions between labetalol and slowly eluting micelles, and probably explains why unlike the t_r' values of the other β-blockers, that of labetalol was not increased when the amount of organic modifier in MEKC buffers was increased.

CONCLUSIONS

The organic modifiers affected the separation of β-blockers by MEKC in several ways. Except for acetonitrile they lengthened the migration times and widened the migration window. In suitable amounts they improved the resolution values. 2-Propanol seems to be the best choice for the organic modifier in the separation of these eleven β-blockers by MEKC. The most likely explanation for the changes effected by the modifiers is a change in the viscosity of the buffer and consequently in the EOF. The organic modifiers studied increased the viscosity of the buffer solution in the order acetonitrile < acetone < methanol < ethanol < ethylene glycol < 2-propanol, and the magnitude of EOF decreased in approximately the same order. When using organic solvents as buffer modifiers in MEKC, one must also be aware of the exceptions in the migration behaviour. With fewer β-blockers the use of organic modifiers may not be necessary, as adequate separations can sometimes be achieved without them.

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