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Journal of Chromatography A, 716 (1995) 49–55

JOURNAL OF CHROMATOGRAPHY A

Investigation and optimisation of the use of organic modifiers in micellar electrokinetic chromatography

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Abstract

The effect of organic modifiers (methanol, ethanol, propan-2-ol, butan-1-ol, butan-2-ol, acetone, methyl ethyl ketone and acetonitrile) on the micellar electrokinetic chromatographic (MEKC) resolution and migration time of seven model compounds has been investigated. The compounds used were all drugs reported to have cardiovascular antiarrhythmic activity. The organic modifiers have each been investigated at 5, 10 and 15% (v/v) of the electrophoretic buffer (100 m*M* borate buffer pH 8.1 containing 50 m*M* SDS as surfactant), to determine the optimum resolution and peak shape. The elution order under almost every condition corresponded to increasing molecular mass of the analyte drugs. Propan-2-ol at a concentration of 10% (v/v) gave optimum separation of the analytes. Replicate injections under these conditions gave excellent precision data for the migration time and corrected peak area. Other modifiers which gave baseline resolution of the analytes but less precise repeatability data were acetone and methyl ethyl ketone.

1. Introduction

Micellar electrokinetic chromatography (MEKC) is a high-resolution technique using surfactant micelles to provide a charged vehicle to perform an electrophoretic separation [1]. MEKC differs from capillary zone electrophoresis (CZE) in that both ionic [1,2] and neutral charged [3,4] analytes may be separated, whereas CZE only separates ionic analytes. Typically, anionic surfactants are included in the electrophoretic buffer at a concentration above its critical micelle concentration (CMC). The formation of micelles results in a pseudostationary phase created by the hydrophobic interior of the charged micellar structure which itself migrates towards the anode [5,6]. However, as the electroosmotic flow (EOF) is faster than the anodic migration of the micelles, the net movement is in the direction of the EOF, i.e. towards the cathode. A mixture of analytes introduced into the EOF will therefore partition between the micelles and the electrophoretic buffer. The partitioning of each analyte will differ depending on its hydrophobic interaction with the micelle [7]. This is used to effect a separation of a mixture of analytes as the migration time of each component is a function of its partitioning with the micelle. The addition of small amounts of organic solvents to the MEKC buffer has several consequences [8], the most important being a change to the partitioning of the analyte between the micelle and the buffer [7,9–11], resulting in a different migration time for the analyte. The work reported here describes how different organic solvents modify the separation of a series

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0021-9673/95/$09.50
SSDI 0021-9673(95)00374-6

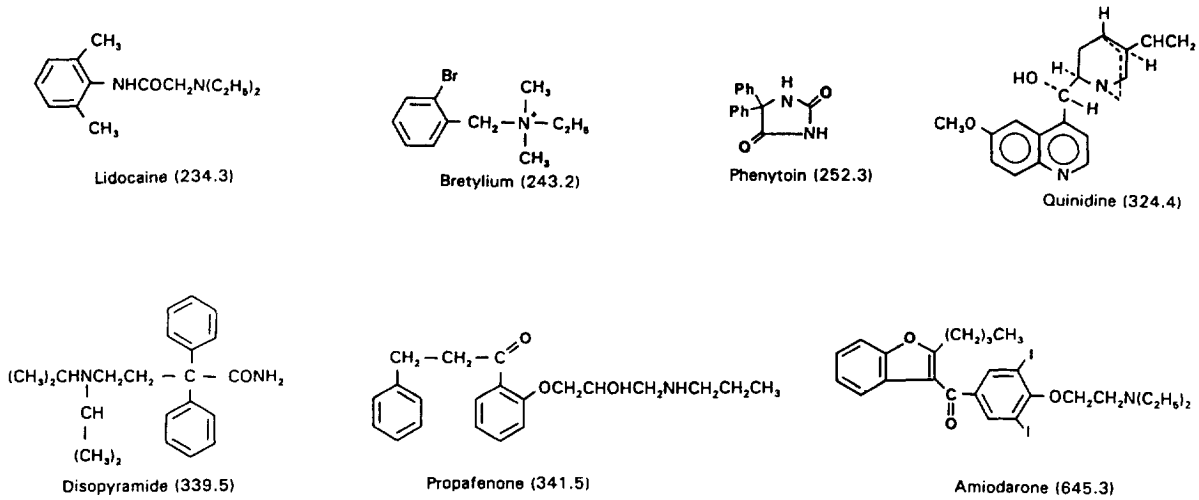

Fig. 1. Structures of drugs used in the electrophoretic optimisation (molecular masses in parentheses).

of seven model drug compounds (Fig. 1) which have cardiovascular antiarrhythmic activity.

2. Experimental

2.1. Reagents

The seven drugs studied were amiodarone hydrochloride, bretylium tosylate, disopyramide, lidocaine, phenytoin, propafenone and quinidine all obtained from Sigma (Poole, Dorset, UK). Purified water was provided by a Milli-Q Plus water purification system (Millipore, Watford, UK). Sodium dodecyl sulphate (SDS), sodium tetraborate, boric acid and sodium hydroxide, acetone, ethanol 96%, butan-1-ol, butan-2-ol, methyl ethyl ketone (Analar grade) and acetonitrile (Hipersolv grade) were obtained from BDH (Poole, Dorset, UK). Methanol and propan-2-ol (high purity grade) were purchased from Fisons Scientific Equipment (Loughborough, Leicestershire, UK)

2.2. Apparatus

A capillary electrophoresis P/ACE system 5510 (Beckman Instruments, High Wycombe, Bucks, UK) equipped with a diode array UV detector, an automatic injector, a fluid-cooled cartridge and a System Gold data station was used in this study. All electrophoresis was carried out at 30°C, with an applied voltage of +25 kV and UV detection wavelength of 200 nm. Sample introduction was performed using the pressure option for 5 s. MEKC was performed in a 57 cm × 75 μm I.D. (50 cm to detector) fused-silica capillary tube (Beckman Instruments), and was rinsed with 0.1 *M* sodium hydroxide and the electrophoresis buffer before each electrophoretic separation was performed. For all buffer systems used, individual solutions of each model analyte were injected to confirm the migration times.

3. Results and discussion

3.1. MEKC conditions

To investigate the effect of different organic solvents on the peak resolution and the migration time of the drug mixture, an electrophoretic buffer was identified to which organic solvents were added up to a level of 15% (v/v) in 5% increments. The electrophoretic buffer consisted of 50 m*M* SDS in 100 m*M* borate buffer, pH 8.1. There are several reported applications of the addition of methanol and acetonitrile to MEKC buffers [8,12,13] and part of this investigation

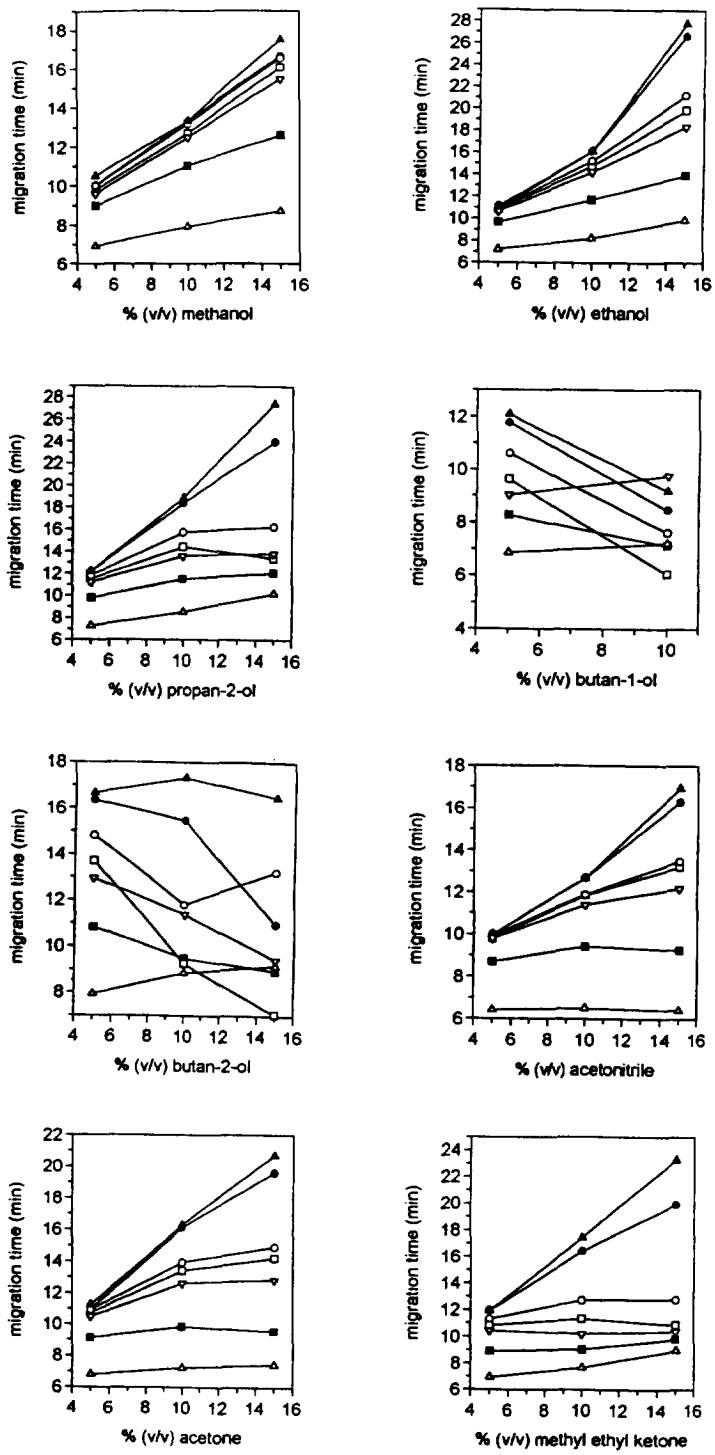

Fig. 2. Migration times of drug analytes in buffer solutions modified with organic solvents. ▲ = Amiodarone; ▽ = bretylium; □ = disopyramide; ■ = lidocaine; △ = phenytoin; ● = propafenone; ○ = quinidine.

focused on the use of other higher-molecular-mass alcohols. There are also reports of the use of acetone in MEKC buffers [12,14,15], and the effects of this solvent, together with another ketone (methyl ethyl ketone) has been investigated.

3.2. Analyte sample preparation

A mixture of the seven drugs was prepared in methanol at a concentration of 2 mg/ml (except amiodarone which was prepared at a concentration of 0.6 mg/ml for reasons of limited solubility). The solution was diluted to a concentration of 0.3 mg/ml (0.1 mg/ml of amiodarone) of each analyte in 50 m*M* SDS. Solutions of each individual analyte were also prepared at a concentration of 0.1 mg/ml in 50 m*M* SDS. These solutions were prepared in methanol at a concentration of 0.5 mg/ml and further diluted to 0.1 mg/ml in 50 m*M* SDS.

3.3. Optimisation of analyte separation

The criteria used for the optimisation of the separation of the seven drugs was the baseline resolution of each individual analyte, together with no peak splitting. The electropherograms generated with each buffer composition show a peak for each drug analyte and also a peak for the tosylate counter-ion of bretylium. Five of the drugs in the mixture remained unresolved when MEKC was performed using electrophoretic buffer containing no organic solvent. The analyte mixture was separated using the electrophoretic buffer containing 5, 10 or 15% (v/v) of the organic solvent, except for the butan-1-ol which could not be used at concentrations greater than 10% (v/v) as it precipitated the buffer salts. The migration time of each analyte in the individual buffer systems generally correlated with an increase in molecular mass. The migration times for each analyte in relation to the organic solvents investigated are shown in Fig. 2. The migration order remained generally unchanged as the organic solvent content of the buffer increased. The analyte migration times also increased as the organic solvent content increased, with the exception of the butan-1-ol, where the migration times decreased with increasing organic content (5 to 10%) and butan-2-ol where little effect was observed as the concentration was changed. Table 1 shows the migration window (difference between the migration times of the first and last analyte) which increases with increasing organic solvent content. The migration window gives a measure of the total time taken to separate the analyte mixture in the electropherogram. It may be used for assessing whether the separation would be

Table 1
Migration window for the MEKC separation using organic modifiers

Organic solvent	Migration window (min) for % organic solvent in electrolyte buffer					
	5% (v/v)		10% (v/v)		15% (v/v)	
Methanol	3.71	(7.95)	5.56	(10.47)	8.89	(13.10)
Ethanol	4.42	(8.72)	7.83	(12.21)	8.96	(18.84)
Propan-2-ol	5.40	(9.49)	10.32	(13.68)	17.25	(18.83)
Butan-1-ol	5.27	(9.46)	4.14	(7.69)	PPT[a]	–
Butan-2-ol	8.96	(12.17)	8.97	(12.85)	11.74	(11.44)
Acetonitrile	4.20	(7.81)	6.22	(9.58)	5.27	(11.69)
Acetone	5.10	(8.72)	9.05	(11.78)	13.28	(14.09)
Methyl ethyl ketone	5.73	(9.08)	10.07	(12.64)	14.32	(16.15)

Migration time corresponding to the centre of the migration window (i.e. the migration time of the first peak plus half the width of the migration window) is shown in parentheses. The migration window for unmodified electrolyte buffer is 2.85 min.
[a] PPT = organic solvent precipitated the buffer.

useful for determining additional analytes to those included in the described mixture. Also included in Table 1 (in parentheses) is the migration time corresponding to the centre of the migration window. This defines the position of the migration window in the electropherogram and indicates the time required for each separation.

Baseline resolution of all the analyte components without splitting of the peaks was only achieved with five organic solvent compositions, these being 10% (v/v) propan-2-ol and butan-2-ol and 15% (v/v) acetone, methyl ethyl ketone and butan-2-ol (Table 2). Under all other conditions, at least two of the peaks were unresolved or one of the peaks was split. The butan-2-ol was not considered a suitable solvent to use as an organic modifier as the background UV absorption was very high at 200 nm which had the effect of making all analyte peaks very small. The 10% (v/v) propan-2-ol and the 15% (v/v) acetone and methyl ethyl ketone all show good resolution (Fig. 3) and the choice of organic solvent to be used as a modifier will depend on the desired width of the migration window. The propan-2-ol gave regularly spaced components in

Table 2
Electrophoretic efficiency of the drug separation for different concentrations of organic modifier

Organic modifier	Concentration (%, v/v)	Peaks unresolved	Split peaks
Methanol	5	Bretylium, disopyramide, quinidine, propafenone and amiodarone.	None
	10	Disopyramide and quinidine; propafenone and amiodarone.	None
	15	Quinidine and propafenone.	None
Ethanol	5	Bretylium, disopyramide, quinidine, propafenone and amiodarone.	None
	10	Propafenone and amiodarone.	None
	15	None	Lidocaine
Propan-2-ol	5	Propafenone and amiodarone.	None
	10	None	None
	15	Disopyramide and bretylium	None
Butan-1-ol	5	Phenytoin and tosylate ion	None
	10	Lidocaine and phenytoin	None
Butan-2-ol	5	Propafenone and amiodarone.	None
	10	None	None
	15	None	None
Acetone	5	Bretylium and disopyramide; propafenone and amiodarone	None
	10	Propafenone and amiodarone	None
	15	None	None
Methyl ethyl ketone	5	Propafenone and amiodarone.	None
	10	None	Disopyramide
	15	None	None
Acetonitrile	5	Bretylium, disopyramide, quinidine, propafenone and amiodarone.	None
	10	Disopyramide and quinidine.	None
	15	Disopyramide and quinidine.	None

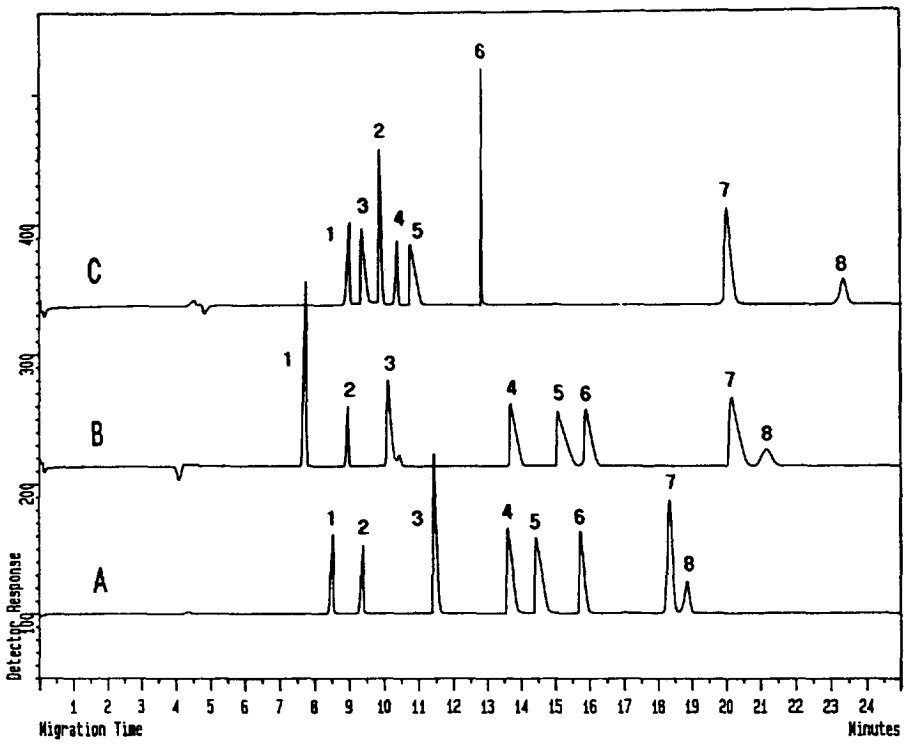

Fig. 3. Electropherograms of the drug analyte mixture using (A) 10% (v/v) propan-2-ol, (B) 15% (v/v) acetone and (C) 15% (v/v) methyl ethyl ketone as organic modifier in electrophoretic buffer. Peaks: 1 = phenytoin; 2 = tosylate ion; 3 = lidocaine; 4 = bretylium; 5 = disopyramide; 6 = quinidine; 7 = propafenone; 8 = amiodarone.

the electropherogram whereas with the ketone solvents, the spacing was irregular. If other analytes required separating from the drugs used in this analysis, the organic modifier could be selected to give optimum separation of the additional component from the existing drugs.

Use of organic modifiers is often avoided because of problems with evaporation of the organic solvent from the electrolyte buffer which leads to poor precision between replicate injections [8,14]. The repeatability (R.S.D.) of both the migration time and the corrected peak area was determined over five replicate injections for the three buffer systems which gave the best separations. The data indicates that the repeatability of the migration time (Table 3) when these modifiers are incorporated into the electrophoretic buffer is excellent, especially propan-2-

Table 3
Repeatability data (% R.S.D.) for migration time for 10% (v/v) propan-2-ol, 15% (v/v) acetone and 15% (v/v) methyl ethyl ketone used as organic modifiers

Organic modifier (%, v/v)	Analyte drug						
	Phenytoin	Lidocaine	Bretylium	Disopyramide	Quinidine	Propafenone	Amiodarone
Propan-2-ol (10%)	0.19	0.13	0.22	0.23	0.20	0.47	0.50
Acetone (15%)	0.14	0.17	0.24	0.31	0.67	0.33	0.61
Methyl ethyl ketone (15%)	0.30	0.35	0.29	0.88	0.24	0.19	0.62

Table 4
Repeatability data (% R.S.D.) for corrected peak area for 10% (v/v) propan-2-ol, 15% (v/v) acetone and 15% (v/v) methyl ethyl ketone used as organic modifiers

Organic modifier (%, v/v)	Analyte drug						
	Phenytoin	Lidocaine	Bretylium	Disopyramide	Quinidine	Propafenone	Amiodarone
Propan-2-ol (10%)	2.53	1.22	0.37	0.32	0.29	0.37	0.33
Acetone (15%)	2.73	1.74	1.88	1.77	1.86	1.21	1.33
Methyl ethyl ketone (15%)	2.51	3.07	1.86	1.46	3.46	2.46	3.27

ol which has a %R.S.D. of 0.5 or less for all analytes in the mixture. The repeatability data for the corrected peak area (Table 4) indicates that propan-2-ol is superior to acetone and methyl ethyl ketone with excellent precision for all but one of the analytes in the mixture.

Other studies investigating the use of organic modifiers have typically used concentrations <10% (v/v) [12]. This investigation demonstrates that levels of organic solvent >10% are also able to effect a high-resolution separation of a mixture of seven analytes which are structurally dissimilar, with good precision for migration time and peak-area measurement.

4. Conclusions

The addition of organic solvents as modifiers to the MEKC electrolyte buffer enhances the resolution of the seven drugs described. As has been previously demonstrated with beta-blockers, [12] the organic modifiers lengthen the migration time and widen the migration window. Although propan-2-ol gives the best resolution for all analytes in the mixture used here, excellent separation has also been demonstrated with low-molecular-mass ketones. Use of such modifiers gives further options in optimising the selectivity of the MEKC separation. No concerns were experienced with the repeatability of the peak migration time or the peak-area quantitation. The use of organic modifiers (including propan-2-ol and various ketones, as well as the more commonly used methanol and acetonitrile) is therefore recommended where it has not been possible to achieve an analytical separation using unmodified MEKC buffers.

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