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Capillary electrophoresis of methylderivatives of quinolines. I

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Abstract

Migration behavior of quinoline, isoquinoline and related methylderivatives has been investigated with respect to the influence of running buffer acidity and to the presence of polyethylene glycol (PEG) 2000 as additive. Dissociation constants and ionic mobilities were determined by capillary electrophoresis (CE). Mobility and viscosity measurements in PEG containing buffers show that analyte transport is not in accordance with Walden's rule and microviscosity plays the role in analyte retardation. Variation of pH and PEG concentration provides the optimal conditions for the CE separation of methylquinolines (0.0176 *M* acetate–Tris buffer, pH 5.5, 10% PEG 2000). Analysis of industrial mixture (isoquinoline fraction from distillation of coal tar) was performed and good agreement with gas chromatographic results was found.
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Keywords: Dissociation constants; Viscosity; Coal tar; Quinolines; Poly(ethylene glycol)

1. Introduction

Quinoline and its alkyl derivatives are substances of industrial importance. They originated during high-temperature carbonization of black coal, which is one of their sources [1–3]. Analysis of tobacco smoke demonstrated that some of methyl derivatives of quinoline appear among fouling [4]. Hepatocarcinogenicity of such compounds has been tested as well [5].

Analysis of a mixture of position isomers requires highly efficient separation techniques in general way. Thus, capillary gas chromatography (often after derivatization) and high-performance liquid chromatography has been utilized to the analysis of quinolines [5–7]. Retention behavior of some methylquinolines on different reversed-phases has also been studied with respect to their pK_a values [8].

Capillary electrophoresis seems to be efficient and relatively cheap alternative to methods mentioned above. pH of the running electrolyte is considered as an extremely important parameter for the optimization at the separation of weak acids or bases [9]. The effective electrophoretic mobility of a weak base u_{eff} is given as weighted average of mobilities of its equilibrium forms. For a monovalent weak base holds [10,11]:

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$$u_{\rm eff} = \frac{[\rm BH^+]}{[\rm BH^+]+[\rm B]} \cdot u_{\rm BH^+} = \frac{[\rm H^+]}{[\rm H^+]+K_a} \cdot u_{\rm BH^+} \tag{1}$$

where $[BH^+]$ and [B] are equilibrium concentrations of protonized and deprotonized form of weak base, respectively, $[H^+]$ is the concentration of protons, u_{BH^+} is the ionic mobility and K_a is concentration dissociation constant (in given ionic strength).

Dissociation constant K_a, usually given as negative logarithm (pK_a), is the main parameter describing acid base equilibrium. In order to determine the pK_a value, two different views of the Eq. (1) are usually considered:

(1) Well known Henderson–Hasselbalch equation was concluded by the simple rearrangement of Eq. (1) [11]:

$$pK_a^{\rm th} = {\rm pH} + \log\frac{u_{\rm eff}}{u_{\rm BH^+} - u_{\rm eff}} + \log \gamma_{\rm BH^+} \tag{2}$$

where pK_a^{th} is a negative logarithm of thermodynamic dissociation constant and γ_{BH^+} is activity coefficient of protonized form (it can be approximated with mean activity coefficient).

(2) Eq. (1), reformulated into its exponential form, is suitable for nonlinear fitting of the experimental dependence u_{eff} versus pH [12]:

$$u_{\rm eff} = \frac{u_{\rm BH^+}}{1 + 10^{({\rm pH} - pK_a^{\rm th} + \log \gamma_{\rm BH^+})}} \tag{3}$$

Use of various macromolecular additives is an auspicious choice in many cases to solve the separation problems. Effectivity of polyethylene glycol (PEG) when used as running electrolyte constituent is widely discussed in the literature nowadays. PEG and related non-ionic surfactants are used for the size-dependent separations of biomacromolecules [13]. Besides, the co-ordination reaction between PEG chain and metal cation was employed as an analogy to crownethers [14]. Migration behavior of small organic acids (i.e., carboxylic acids, phenol derivatives) was studied considering pK_a-dependent PEG pseudophase/water (buffer) phase partitioning [15] and the effect of hydrogen bonds [16] as main discrimination phenomena. Recently we showed that high amount of PEG 2000 (tens of mass percent) added to acid running buffer enables the separation of alkylpyridine bases [17].

In this contribution, the influence of the buffer acidity as well as the presence of PEG 2000 on migration behavior of methylquinolines is studied and employed for the separation of common methylquinoline derivatives.

2. Experimental

2.1. Chemicals

Standards of quinoline derivatives were dissolved with 0.012 *M* HCl to obtain stock solutions of each standard ($c = 0.02$ *M*). Real sample (about 0.1 g of mass) was dissolved in 100 ml of 0.012 *M* HCl. Model samples were prepared by mixing of stock solutions and adding 10 times diluted running buffers to obtain final concentration of each compound 2×10^{-5} *M*. Real sample was diluted thousand times in the same way as the model ones. Standards were purchased from Sigma (St. Louis, MO, USA). Sample of isoquinoline fraction from coal tar as well as the standard of 2,4-dimethylquinoline was kindly provided by DEZA company (Valašské Meziříčí, Czech Republic). Mesityloxide (Fluka, Buchs, Switzerland) served as electroosmotic flow marker. Tris (Trishydroxymethylaminomethane) (Fluka) was used as common cation of all buffers. Phosphoric acid, acetic acid (both from Merck, Darmstadt, Germany), 2-morpholinoethanesulfonic acid (MES) and 3-morpholinopropanesulfonic acid (MOPS) (both from Sigma) served subsequently as buffering components in the pH range 2–7.5. The concentration of buffering acid was chosen in order to obtain final ionic strength of the buffer 0.015 *M* (Table 1). PEG-containing buffers were prepared by the appropriate dilution of 50% (w/w) water solution of PEG 2000 with 0.2 *M* phosphate–Tris buffer (pH 2.5) or 0.0352 *M* acetate–Tris (pH 5.5), respectively, so that the final solutions were 0.05 *M* phosphate and 0.0176 *M* acetate, respectively. The concentration series 0, 3, 6.25, 10, 12.5, 20, 25, 33 and 37.5% PEG were prepared in phosphate buffer and 0, 5, 10, 15% PEG in acetate buffer. 50% PEG was deionized prior to use as described previously [17].

Table 1
Composition of buffers used for pK_a measurements[a]

pH	Buffering acid	c (M)
2.0	Phosphoric acid	0.036
2.5	Phosphoric acid	0.021
3.0	Phosphoric acid	0.017
3.5	Acetic acid	0.276
4.0	Acetic acid	0.097
4.5	Acetic acid	0.041
5.0	Acetic acid	0.023
5.5	Acetic acid	0.018
6.0	MES	0.034
6.5	MES	0.021
7.0	MOPS	0.039
7.5	MOPS	0.023

[a] Common cation of the buffers: Tris, ionic strength $I=0.015$ M.

2.2. Equipments and procedures

A SpectraPhoresis 100 system with a fast scan UV–Vis detector (Thermo Separation Products, USA) and PrinCE-C 600 instrument (Prince Technologies, Emmen, The Netherlands) equipped with a UVD 340S DA detector (Dionex Softron, Germering, Germany) were used for experiments. Uncoated fused-silica capillaries [75 cm (effective length 45 cm)×75 μm I.D. and 95 cm (effective length 50 cm)×75 μm I.D.] were used. Viscosity measurements were performed using Ubbelhode viscosimeters (0.5–3, 1–10, 4–60 cSt) placed in home made glass shell enabling to maintain constant temperature.

The capillary was washed with 0.1 M NaOH, water and running buffer (each 10 min) daily before experiments. The conditioning under separation voltage followed for 15 min. Between experiments, capillary was washed with additiveless buffer and conditioned briefly (about 5 min) under separation voltage, which seems to stabilize electroosmosis. All experiments were performed in scanning or diode-array mode and the wavelength 225 nm was selected for data processing. Mobility measurements were performed on PrinCE-C 600 instrument and temperature was maintained at 25°C.

GC–MS experiments were done on HP 6890 series GC System with HP 7683 Series Injector equipped with Agilent 5973 N mass selective detector (column HP–5MS 30 m×0.25 mm, 0.25 μm, 0.9 ml/min He). Analysis was performed with programmed temperature: 50°C, 2 min; 5°C/min, 200°C, 10 min. Sample of isoquinoline fraction was dissolved in acetone (5.8 mg/5 ml) and the solution was 10 times diluted with acetone (injection: 1 μl, 250°C).

Computer evaluation of sigmoidal model was performed using software CurveExpert 1.3 and MathCad 8.0.

3. Results and discussion

3.1. Effect of pH

Quinolines are fully protonated in strongly acidic buffers so the mobility differences are due to different shapes of solvated ions of position isomers. However, only small differences were found at pH 2.5 and the separation is poor (Fig. 1, electropherogram 1).

In order to optimize pH, effective mobilities of all quinolines were measured in the pH range 2.0–7.5. Acquired data were consequently employed for the calculation of the pK_a and u_{BH^+}.

Mobility changes measured in dependence on pH bring information about changes in concentration ratio of both forms of a weak electrolyte. Thus, the Henderson–Hasselbalch equation in the form of Eq. (2) may be used for the point-to-point calculation of the pK_a if ionic mobility is measurable (the mobility of the free base is zero) [11,12,18–20]. Ionic mobilities were determined as an average of two or three experimental points laying in the upper plateau of 'titration curve'. The ionic mobility and effective mobility at given pH was used for the calculation of pK_a values. The average of dissociation constants obtained in pH within p$K_a \pm 1$ (4 or 5 points) are given in Table 2.

In the opposite to the point-to-point calculation, nonlinear fitting of the experimental points by theoretical curve (Eq.3) was used [12,18,21]. If the quality and the amount of experimental data are sufficient, the parameters of the curve can be easily found by computer data handling. In this manner, both pK_a and u_{BH^+} are determined simultaneously. Advantageously, the procedure evaluates all effective mobilities in one run, which brings better accuracy

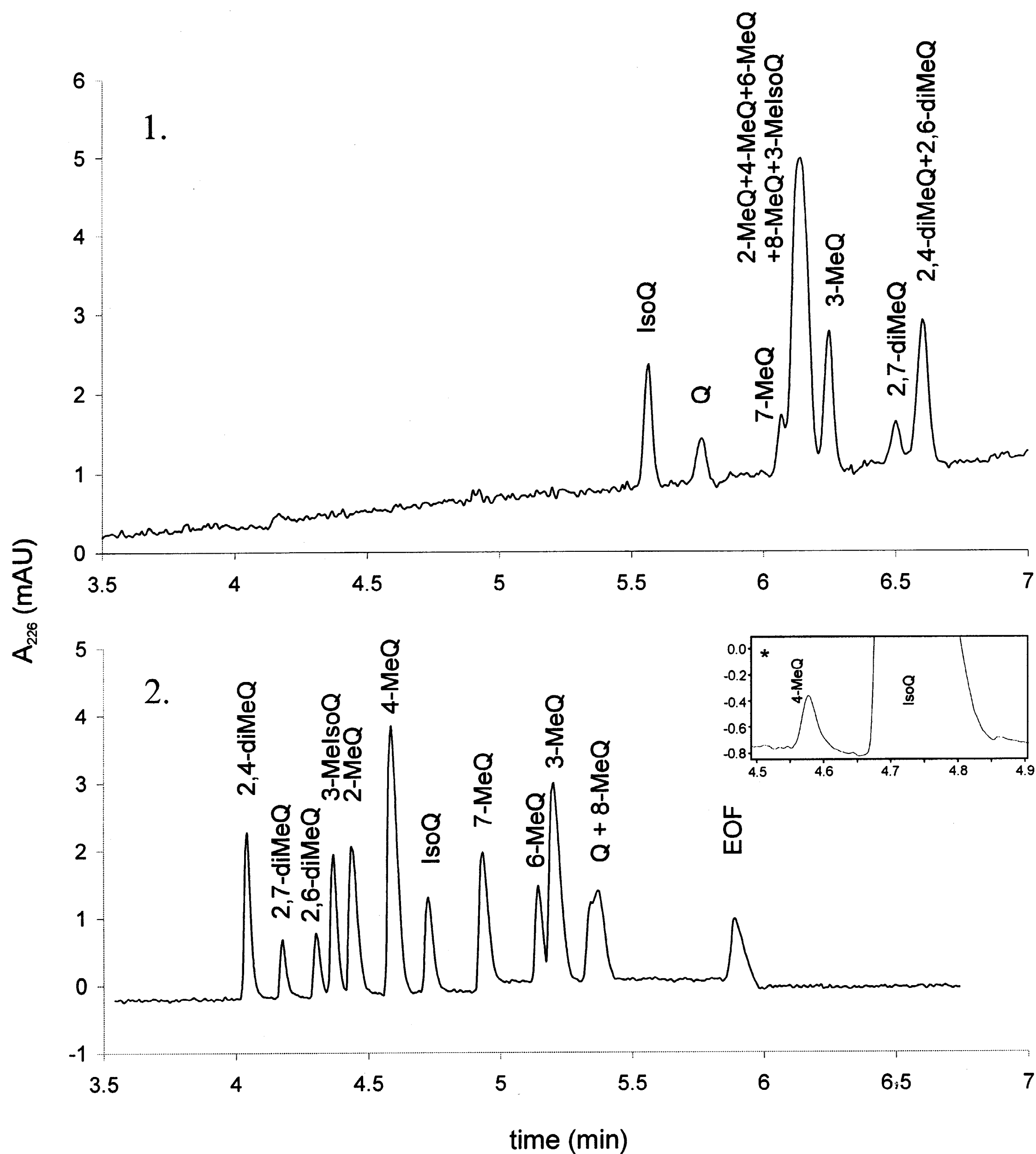

Fig. 1. Separation of model mixture of quinolines. (1) BGE: 0.05 M phosphate–Tris, pH 2.5, $U=30$ kV, $I=50$ μA; (2) BGE: 0.0176 M acetate–Tris, pH 5.5, $U=30$ kV, $I=12$ μA. *Inset: separation of 4-MeQ from the major peak of IsoQ.

of output parameters (lower standard deviation). The results as well as the comparison with the earlier published data [22] are given in Table 2. pK_a values of investigated quinolines range from 4.5 to 6.5 in accordance with influence of positive induction effect of the methyl groups. Electropherogram 2 in Fig. 1 shows the separation at optimized pH 5.5. The mixture is separated except of the pair Q–8-MeQ.

Table 2
pK_a and ionic mobility values

Compound		Dissociation constant (pK_a)			Mobility (m^2 V^{-1} s^{-1}; average values)
Name	Abbreviation	Eq. (2)	Eq. (3)	Ref. [22]	
Quinoline	Q	4.79	4.76	4.80	41.8
2-Methylquinoline	2-MeQ	5.63	5.65	5.42	37.8
3-Methylquinoline	3-MeQ	5.01	4.98	5.14	37.9
4-Methylquinoline	4-MeQ	5.49	5.52	5.20	38.9
6-Methylquinoline	6-MeQ	5.01	5.03	4.92	38.9
7-Methylquinoline	7-MeQ	5.22	5.25	5.08	39.1
8-Methylquinoline	8-MeQ	4.86	4.91	4.60	38.4
2,4-Dimethylquinoline	2,4-diMeQ	6.30	6.26	5.12	35.3
2,6-Dimethylquinoline	2,6-diMeQ	5.85	5.90	6.10	35.5
2,7-Dimethylquinoline	2,7-diMeQ	6.00	6.01	5.02	36.0
Isoquinoline	IsoQ	5.32	5.33	5.36	43.0
3-Methylisoquinoline	3-MeIsoQ	5.78	5.73	5.64	38.2

All constants and mobility data were corrected for ionic strength by standard procedure based on Debye–Hückel theory [20].

3.2. Effect of PEG 2000 in background electrolyte

Generally, seven separation principles are considered in PEG containing electrolytes: (1) sieving effect (size-dependent separations) [13]; (2) cationization of PEG and complexation with inorganic or organic cations [14,17]; (3) distribution between bulk buffer phase and PEG pseudophase [15]; (4) hydrogen bonding of PEG with H-donors [16]; (5) interactions based on hydrophilic/lipophilic (hydro-

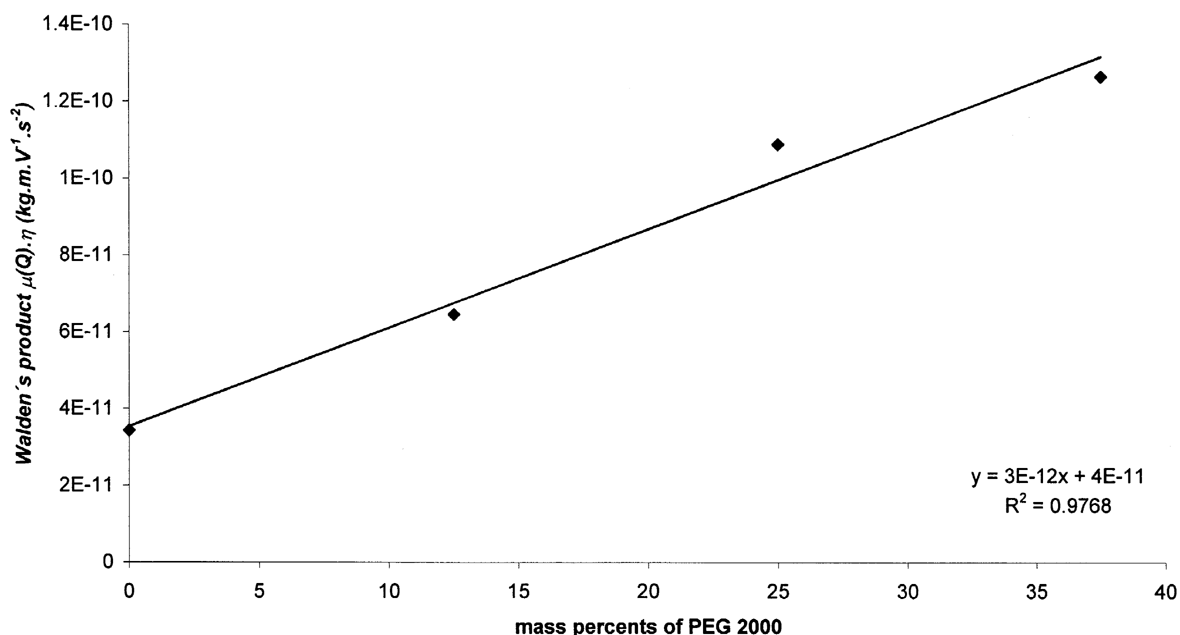

Fig. 2. Dependence of Walden's product on PEG 2000 content.

phobic) balance (HLB); (6) viscosity or *micro*viscosity (as defined in [23], for instance) [24]; (7) modification of electrokinetic potential [25].

It can be seen from the mobility and viscosity measurements (Fig. 2) that Walden's product is not constant over the PEG concentration range. The system behaves as the solution of macromolecule and *micro*viscosity plays role in analyte transport process. The dependence between effective mobility and specific conductivity of the background electrolyte (BGE) (or separation current) is linear (Fig. 3) when measured in wide PEG concentration range (0–37.5%, w/w). The fact indicates that the main retardation process is similar for both analytes and running electrolyte constituents. The small differences in regression parameters among quinoline derivatives suggest, however, that some minor (selective) interactions should not be precluded. However, we did not study exactly the difference between PEG complexation of quinoline and Tris or whether Tris can come into a selective interaction with PEG at all.

Fig. 4 shows the dependence of relative mobility of two methylquinolines and corresponding dimethylderivatives (relative to quinoline) on the concentration of PEG added to the acidic buffer (pH 2.5). It can be seen that migration order of 7-MeQ and 4-MeQ is changing. Moreover, decreasing of the mobility differences between quinoline and its methylderivatives is observed at higher PEG concentrations and migration order is even reversed at the concentration 37.5% PEG. An analogous trend can be seen for dimethylderivatives. Fig. 5 describes the dependence of relative mobility on pH. It can be clearly seen from the comparison of both experiments that the changes of migration order are

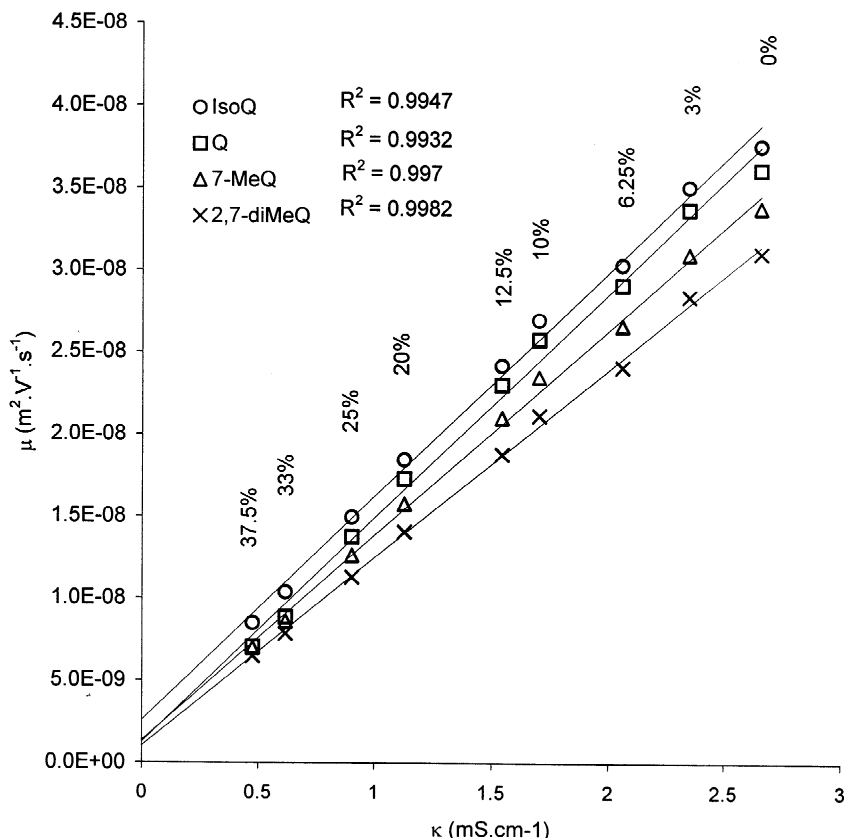

Fig. 3. Dependence of effective mobility on specific conductivity of BGE (corresponding amount of PEG 2000 is given in mass percent).

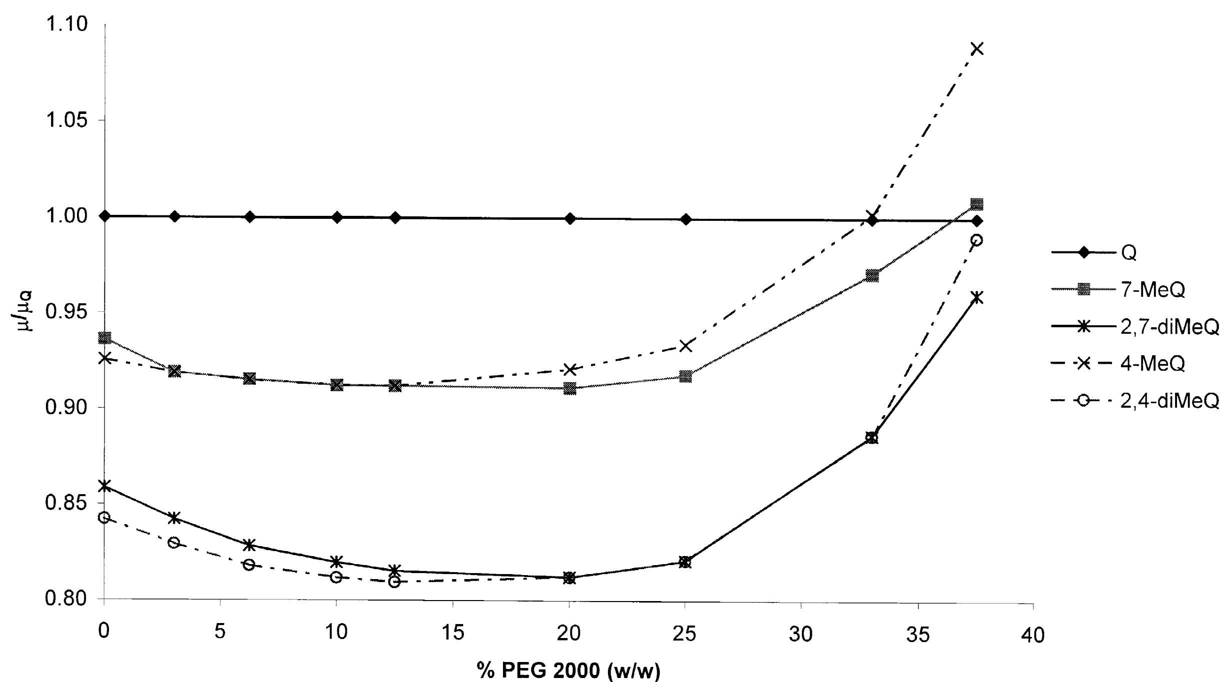

Fig. 4. Effect of PEG 2000 concentration on migration of quinolines (BGE: 0.05 *M* phosphate–Tris buffer, pH 2.5).

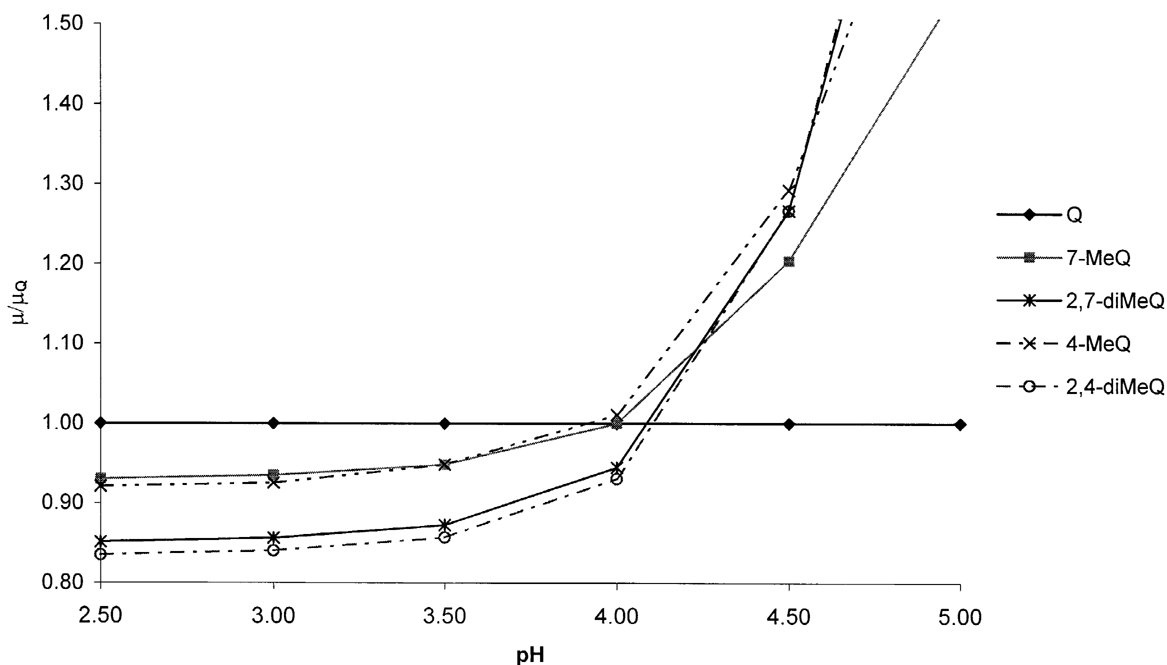

Fig. 5. Effect of buffer pH on migration of quinolines (for composition of buffers see Table 1).

similar. Hence, authors suppose that there are several important factors which influence the effective mobility of quinolines:

(1) modification of buffer acidity (with increasing concentration of PEG as nonaqueous medium, the influence of the transfer activity coefficient grows, consequently, the acidity changes [26]);

(2) modification of the charge of analyte (PEG as nonaqueous medium influences pK_a of analyte [26,27]);

(3) weak-specific interaction between PEG and protonized form of analyte (electron-donor and proton-acceptor interactions, namely [15,16]).

An initial decrease of relative mobility in Fig. 4 can be explained with two effects — increasing of *micro*viscosity (and thus slightly higher retardation of bigger molecule compared to smaller one, quinoline) and/or hydrophobic interaction (order of hydrophobicity is dimethyl derivatives > monomethyl derivatives > quinoline). At higher additive concentrations the above-mentioned factors prevail.

3.3. Optimization of the separation

At first, discrimination capacity of PEG was tested on the model mixture of common methylquinolines in acidic buffer (pH 2.5). The system does not allow total separation even using very high additive concentration. It can be mentioned, however, that in studied concentration series the highest content (37.5%, w/w) gives the best results — the mixture is separated except for the pair 2-MeQ–4-MeQ (data not shown). Higher concentrations are not convenient for practical use because of extremely long migration times and impendence of capillary obstruction.

When pH optimization is performed as first step, the complete separation is achieved except of the pair Q–8-MeQ at pH 5.5 (Fig. 1). The analysis is short (finished at 6 min) due to the high electroosmotic flow. The addition of PEG was tested at optimal pH 5.5. It was found out that relatively low concentration of additive (10% of PEG) is needed for the total separation of all the studied compounds (Fig. 6). The analysis time reaches about 25 min. Here, the reduction of electroosmotic flow also contributes to the separation.

An analysis of isoquinoline fraction from coal tar is shown as an example of the control of industrial mixture (Fig. 7). Large excess of IsoQ caused the loosing of its separation from 4-MeQ. The problem was solved with reduction of injection pressure (inset in Fig. 7). Although incomplete, the separation offers sufficient information for industrial control. If a

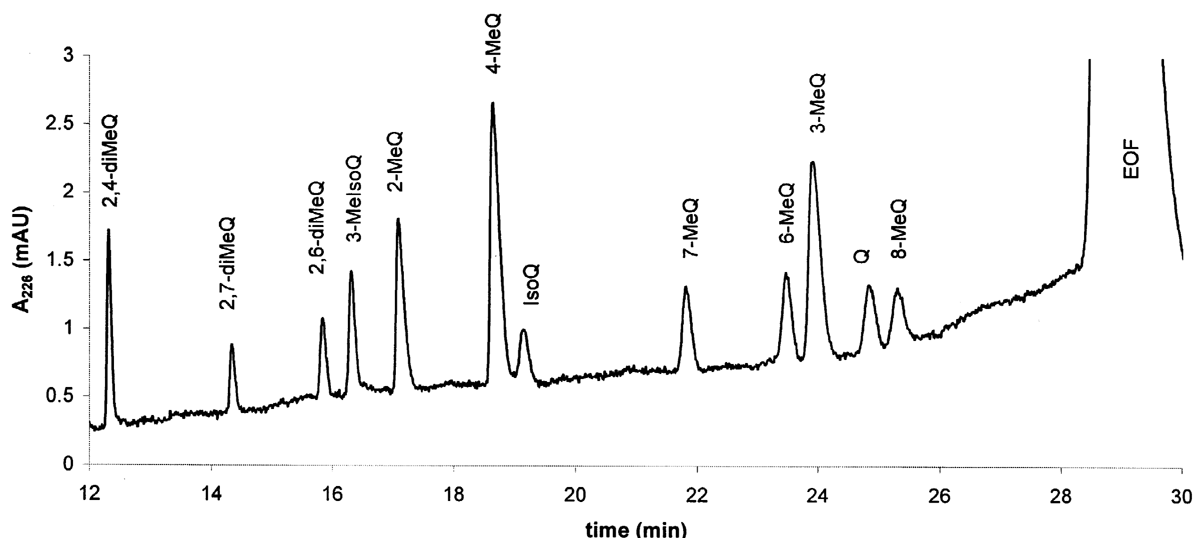

Fig. 6. Optimized separation of the model mixture (BGE: 0.0176 *M* acetate–Tris, pH 5.5, 10%, w/w, PEG 2000, U=30 kV, I=10 μA).

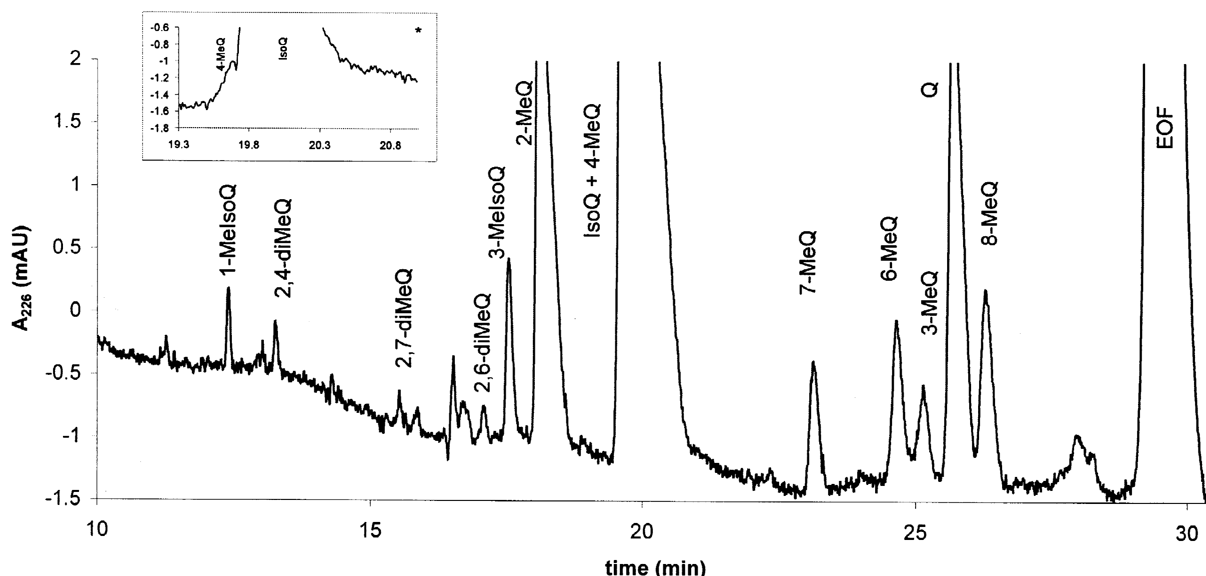

Fig. 7. Analysis of isoquinoline fraction from coal tar (0.0176 *M* acetate–Tris, pH 5.5, 10% PEG 2000, $U=30$ kV, $I=10$ μA, injection 0.6 s, 50 mbar; *inset: injection 0.6 s, 50 mbar).

higher precision of 4-MeQ determination is required, a subsequent analysis in additiveless buffer pH 5.5 can be performed where the resolution of the critical pair IsoQ–4-MeQ is better (inset in Fig. 1). It should be mentioned that each kind of industrial fraction needs its own optimization in virtue of possible co-migration of large excess of one derivative over the others, but the optimized composition of running electrolyte can be found quickly by the small variations in pH value and PEG concentration. Internal normalization method was used for quantitative evaluation (peak areas were corrected on migration time and response factor).

The results of CE analysis are compared with GC analyses (Table 3). Fig. 8 shows the GC–MS analysis of the sample (for conditions see Section 2). The procedure used does not allow the separation of some derivatives. The using of the selected monitoring (SIM) mode solved the problem with quantification of 4-MeQ. In general, MS detection (as routinely used detection for GC nowadays) makes easy the identification and GC offers the possibility to spread the set of analytes to non-ionizable compounds in one run. When compared, CE showed a higher flexibility for the optimization of the separation of position isomers and it is also a cheaper alternative.

Table 3
Analysis of isoquinoline fraction from coal tar

Compound	CE-PEG (%)	GC (%)[a]	GC–MS
Q	9.83	8.67	8.64
2-MeQ	17.74	15.70	19.54
4-MeQ	~1.79	1.34	1.41
3-MeQ	1.10		1.56
6-MeQ	2.21	5.27[b]	3.55[c]
7-MeQ	1.96		
8-MeQ	3.06	4.35	4.89
2,4-diMeQ	0.54	0.72	0.57
2,6-diMeQ	0.52	0.86	0.79[d]
2,7-diMeQ	0.27	0.74	
IsoQ	56.19	56.72	51.43
1-MeIsoQ	0.83	0.61	0.64
3-MeIsoQ	2.20	1.61	1.56
Unidentified compounds	4.26	3.46	5.29

[a] Column 2 shows the values of supplier certificate.
[b] Sum of 3-MeQ, 6-MeQ and 7-MeQ.
[c] Sum of 6-MeQ and 7-MeQ.
[d] Sum of 2,6-diMeQ and 2,7-diMeQ.

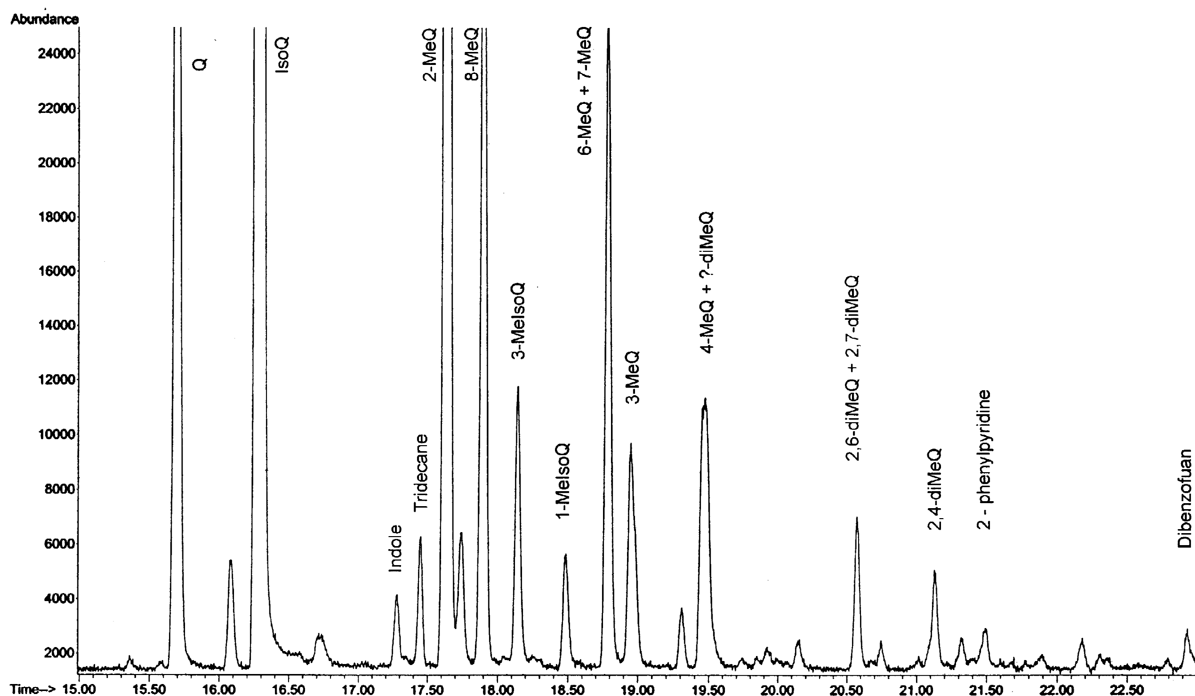

Fig. 8. GC–MS analysis of isoquinoline fraction from coal tar.

Acknowledgements

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