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Polyethylene glycol as a separation medium for capillary zone electrophoretic analysis of pyridine derivatives

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Abstract

The influence of the addition of polyethylene glycol (PEG) to the background electrolyte on the capillary zone electrophoretic (CZE) separation of alkylpyridines was studied. It was found out that interactions between polyethylene glycol chains and protonized pyridine bases cause the increasing of the resolution. The separation of a model mixture of pyridine, all isomers of methylpyridine, and some other alkyl and aryl derivatives was performed. The separation was complete except for some dimethylpyridines. The influence of the co-ion was discussed, too. Polyethylene glycol solution as a separation medium was used for the capillary zone electrophoretic determination of pyridine bases in an industrial mixture.

Keywords: Background electrolyte composition; Pyridines; Alkylpyridines; Polyethylene glycol

1. Introduction

It has been known a long time, that inorganic cations cationize oxyethylene chains and create ternary complexes with anions (ionic association) [1]. This phenomenon was used for the determination of oxyethylated products by creating these complexes. Cationization has been used later in chromatography and recently some electromigration applications have appeared in the literature. The complete isotachophoretic (ITP) [2] and capillary zone electrophoretic (CZE) [3] separation of alkali and alkaline earth metal cations was achieved. The effect has been used for the separation of some other inorganic cations, too [4,5].

No proper attention has been given to the fact that the polyethylene glycol (PEG) chain can be cationized also by organic cations-protonized bases. In this paper the application of interactions between PEG 2000 and alkylpyridines for CZE separations of their mixtures is investigated.

The studied alkylpyridine mixtures contained homologues and positional isomers, so the main problem was, in contrast to the inorganic samples, the separation of ions with the same molecular mass and charge. Pyridines are protonized in acid buffers, so the cationization reactions can be considered. Moreover, hydrophobic interactions can occur. So the main discrimination factors are differences in friction, cationization reactions and hydrophobic interactions.

2. Experimental

2.1. Chemicals

Phosphoric acid (analytical-reagent grade, E. Merck, Darmstadt, Germany), sodium hydroxide, potassium hydroxide (both analytical-reagent grade,

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Lachema, Brno, Czech Republic), tetramethylammonium hydroxide (puriss., 1 *M* solution in water), tetraethylammonium hydroxide (purum, 40% solution in water), tetrapropylammonium hydroxide (purum, 20% solution in water), tetrabutylammonium hydroxide (puriss., 40% solution in water), all Fluka, Buchs, Switzerland, benzene (analytical-reagent grade, Lachema, Brno, Czech Republic), polyethylene glycol 2000 (M_r=1900–2100), (analytical-reagent grade, E. Merck), pyridine (analytical-reagent grade, dehydrated and distilled; Lachema), 2-, 3-, 4-methylpyridine, 2,3-, 2,4-, 2,5-, 2,6-, 3,4-, 3,5-dimethylpyridine, 2-, 3-, 4-ethylpyridine, 2,3,4-, 2,4,5-, 2,4,6-trimethylpyridine, 2-propylpyridine, 5-ethyl-2-methylpyridine, 4-*tert*-butylpyridine, 2,6-dimethyl-4-ethylpyridine, 2-, 3-, 4-phenylpyridine (all analytical-reagent grade, Fluka).

Background electrolyte was prepared with 0.05 *M* phosphate buffer pH=2.5 (adjusted by hydroxide of given co-ion). Background electrolytes with additions of 12.5, 25 and 37.5% (w/w) of PEG 2000 were prepared by the dilution of deionized 50% aqueous solution of PEG 2000 with 0.1 and 0.2 *M* phosphate buffer (pH=2.5), respectively, in order to achieve resulting concentration of phosphate 0.05 mol l^{-1}. 50% PEG solution was deionized batchwise using mixed ionex, which was prepared from swelled anion and cation-exchanger (Ostion, Spolchem, Ústí nad Labem, Czech Republic) in the ratio 3:2. This process was repeated three times until the specific conductance dropped under 2 μS cm^{-1}. Pyridine solutions were prepared by the defined dilution to the concentration 0.02 mol l^{-1}. The resulting concentration of them was $8.7 \cdot 10^{-4}$ mol l^{-1} in model mixture.

2.2. Instrumentation

SpectraPHORESIS 100 system with fast scan UV–Vis detector was used for all CZE experiments. For quantitative evaluation of electrophoregrams was used wavelength 260 nm. The uncoated silica fused capillary was used [75 cm (effective length 45 cm)× 75 μm].

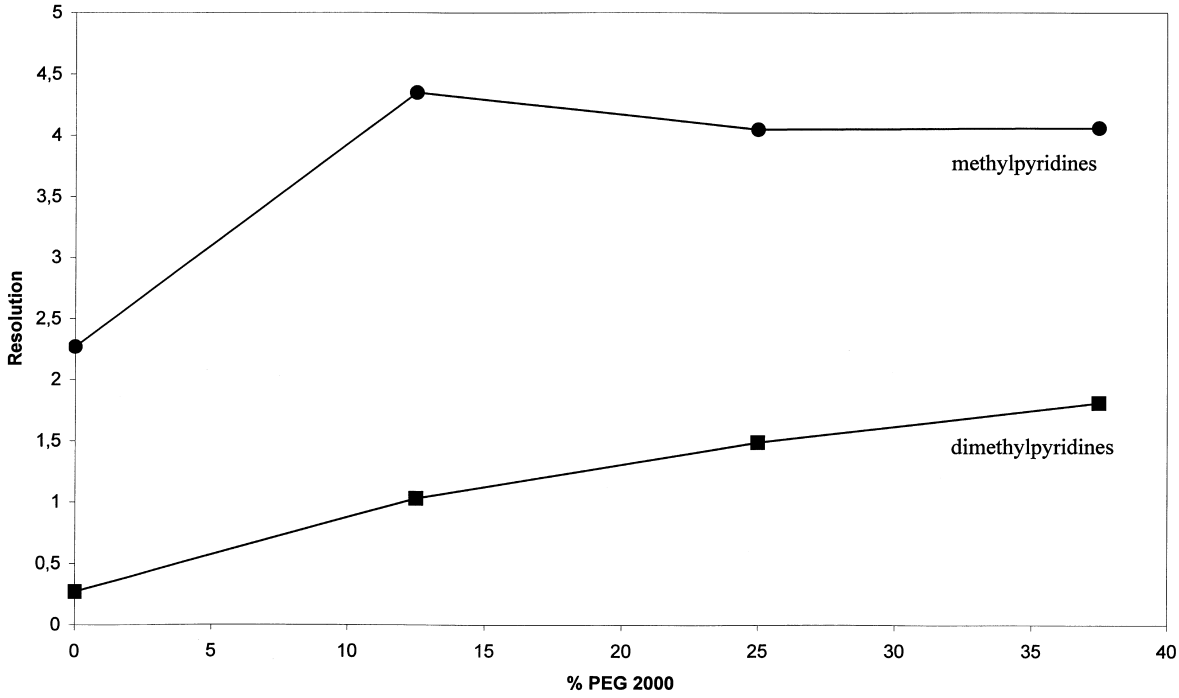

Fig. 1. Relationship between concentration of PEG 2000 and resolution.

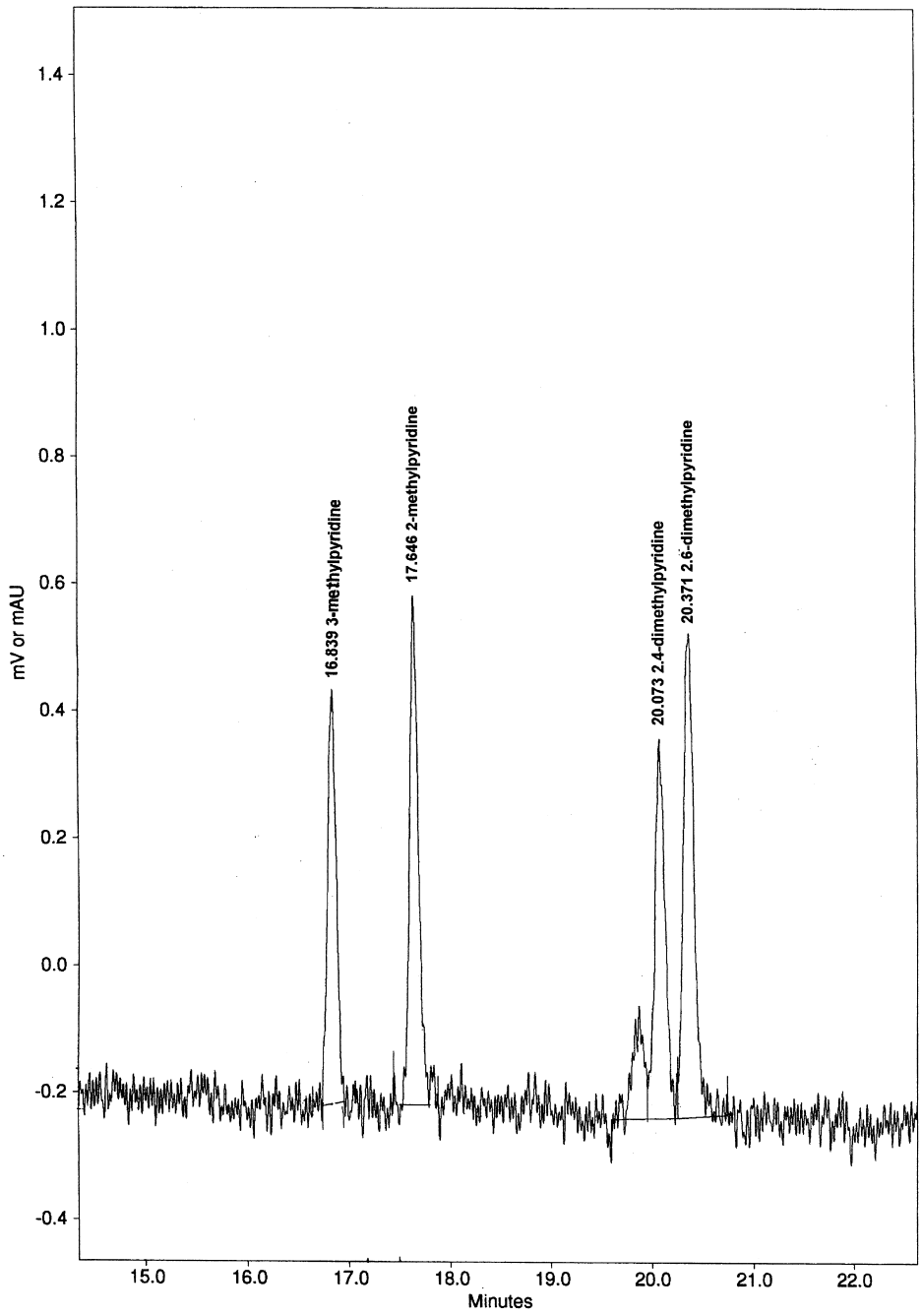

Fig. 2. Separation of 3-methylpyridine, 2-methylpyridine, 2,4-dimethylpyridine and 2,6-dimethylpyridine. BGE: 50 m*M* phosphate buffer (Na^+) pH=2.5+25% PEG 2000, vacuum injection 0.5 s, U=25 kV.

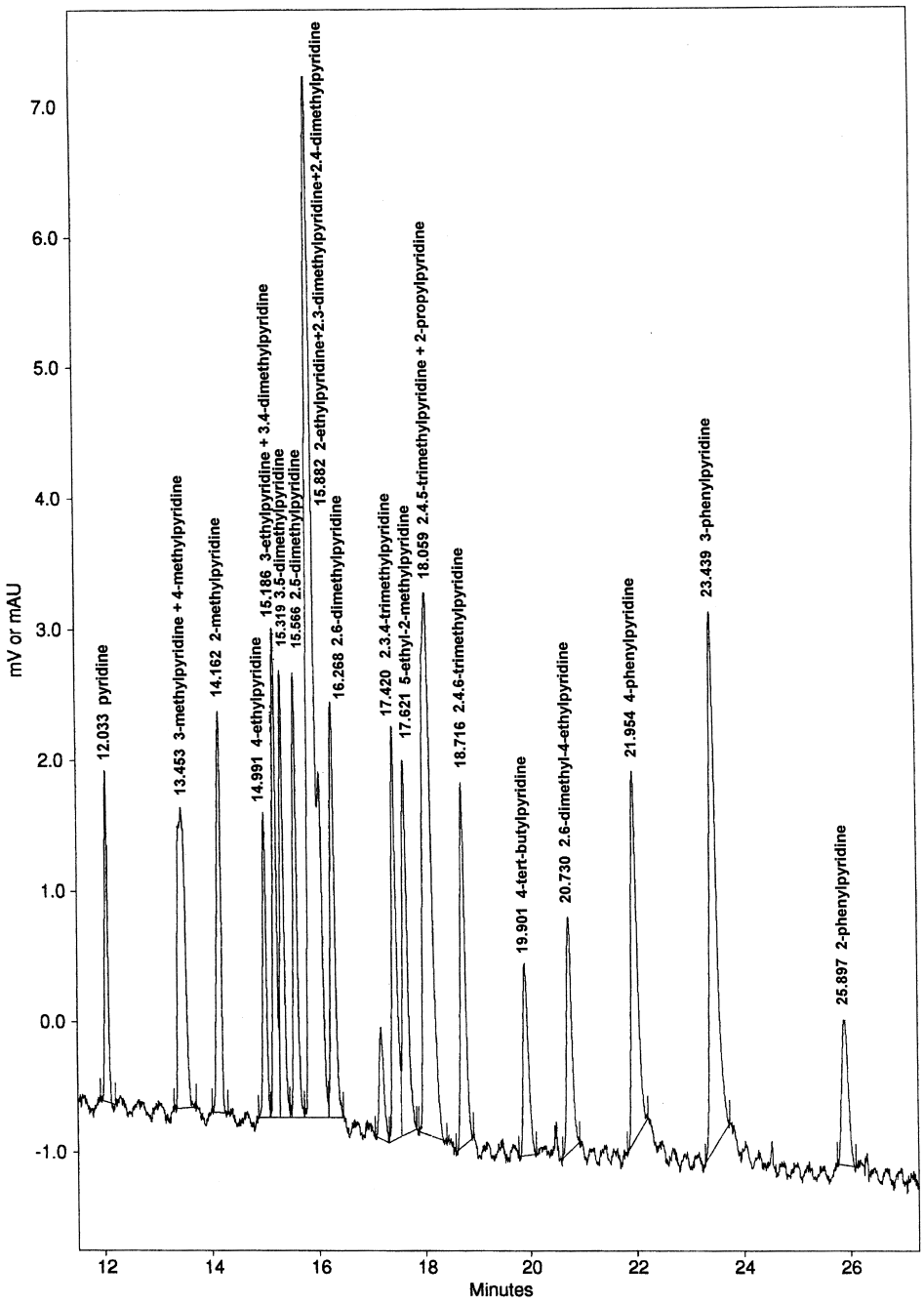

Fig. 3. Model mixture separation of alkyl and aryl pyridine derivatives. BGE: 50 m*M* phosphate buffer (Na^+) pH=2.5+25% PEG 2000, vacuum injection 0.5 s, U=25 kV.

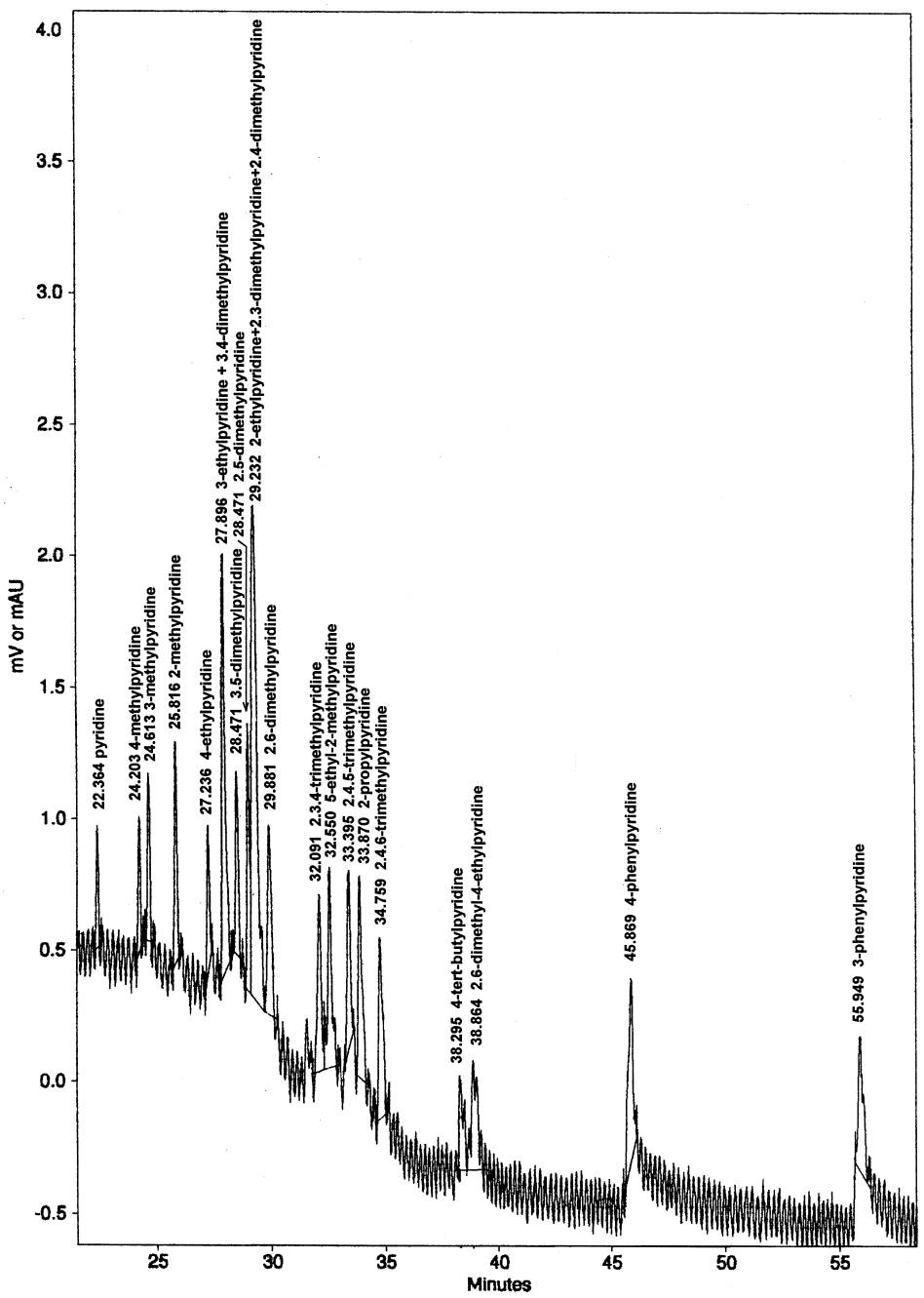

Fig. 4. Model mixture separation of alkyl and aryl pyridine derivatives. BGE: 50 m*M* phosphate buffer (Na^+) pH=2.5+37.5% PEG 2000, vacuum injection 0.5 s, U=25 kV.

2.3. Electrophoretic procedure

The capillary was washed with deionized water, 0.1 *M* sodium hydroxide and background electrolyte (each 5 min). The capillary was conditioned for 10 min with background electrolyte (BGE) under sepa-

ration voltage before the analysis. Vacuum injection 0.5 s was used. All experiments were carried out with constant voltage (20, 25 and 30 kV, respectively). Peaks were identified by the spiking a solution and comparing UV spectra with standards.

3. Results and discussion

Fig. 1 describes the influence of increasing concentration of PEG on the resolution of methylpyridine and dimethylpyridine pairs. While methylpyridines can be separated directly (without the addition of PEG), dimethylpyridines require at least 25% PEG in BGE (Fig. 2). Higher concentration of PEG (37.5%) gives better resolution, however migration times are too long.

Figs. 3 and 4 shows electrophoregrams of model mixture of 23 pyridine derivatives, which was not possible to separate without PEG at all, 25% PEG allowed to separate for example 2,5-, 2,6- and 3,5-dimethylpyridines (Fig. 3), while 2,3- and 2,4-dimethylpyridines were not separated even in 37,5% PEG (Fig. 4). The highest studied concentration allows separation of all isomers of methylpyridine and 2,4,5-trimethylpyridine from 2-propylpyridine. The resolution increase with PEG content take place not only between positional isomers but also in homologous series. It shows that in addition to cationization and friction (viscosity effects) also hydrophobic interactions influence separations (see Introduction).

The influence of co-ion was also tested for improving the separation. K^+, Li^+, tetramethylammonium (TMA^+), tetraethylammonium (TEA^+), tetrapropylammonium (TPA^+), tris(hydroxymethyl)aminomethane ($Tris^+$) and tetrabutylammonium (TBA^+) were tested beside Na^+. As can be seen from Figs. 5 and 6, separation of 3- and 4-methylpyridine is suitable using Na^+. The resolution increase can be observed with ionic diameter of quaternary ammonium co-ions. The pair 3-ethylpyridine and 3,4-dimethylpyridine is not separated with inorganic co-ions and TMA^+. The positive effect of increased ionic diameter of quaternary ammonium ions can be used here, however, total

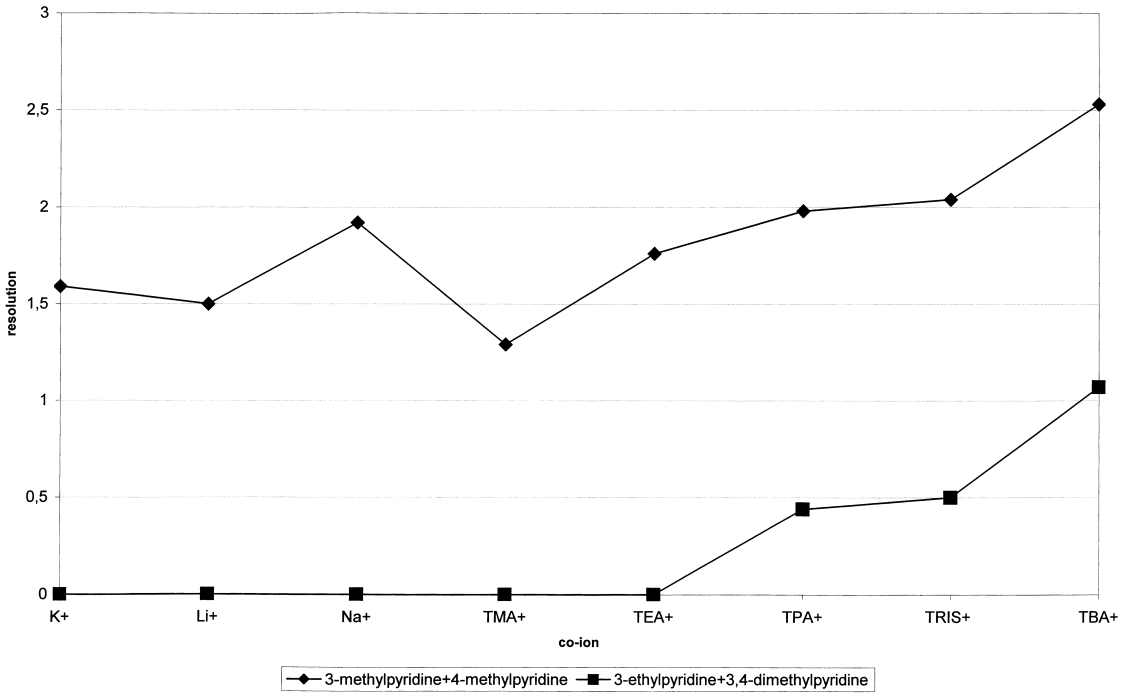

Fig. 5. Influence of co-ion on the separation I. (37.5% PEG 2000, U=25 kV,vacuum injection 0.5 s).

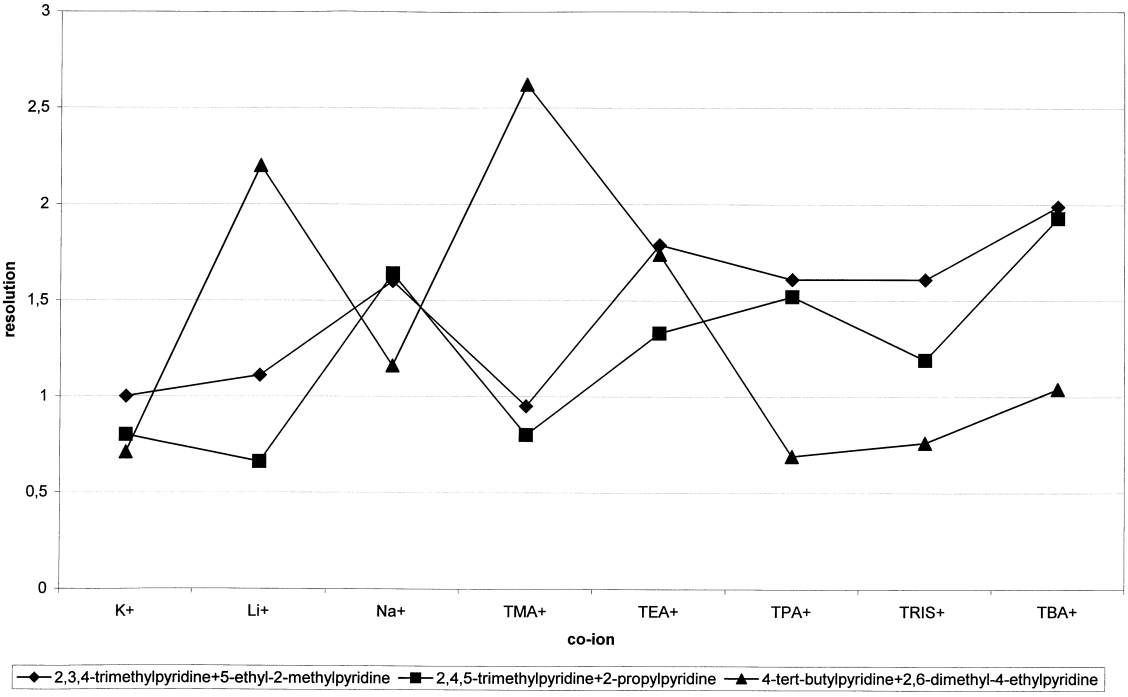

Fig. 6. Influence of co-ion on the separation II. (37.5% PEG 2000, U=25 kV,vacuum injection 0.5 s).

separation was not achieved even with TBA^+ (R_s= 1.07). The whole separation of the pair 2,3,4-trimethylpyridine and 5-ethyl-2-methylpyridine can be achieved using Na^+ and quaternary ammonium cations except of TMA^+. The separation of the pair 2,4,5-trimethylpyridine and 2-propylpyridine allow the use of Na^+, TPA^+ and TBA^+. Finally, 4-*tert*.-butylpyridine and 2,6-dimethyl-4-ethylpyridine were fully resolved using Li^+ and TMA^+. It can be concluded that for the model mixture is the most suitable buffer containing TBA^+ as co-ion (Fig. 7), where the complete separation was achieved except of four compounds (2-ethylpyridine, 2,3-, 2,4- and 2,5-dimethylpyridine). However, the highest concentration of PEG and increasing ionic diameter of co-ion unfavourably decrease driving current (7 μA).

The method was applied to control of the industrial sample which was formed by the pyridine fraction obtained from coal tar. The pyridine fraction was isolated by fraction distillation as a mixture in the DEZA Valašské Meziříčí. The results were compared with GLC (Table 1). 12.5% PEG solution allows the separation and identification of 2-methylpyridine, 3,5-dimethylpyridine, 2,3,6 and 2,4,6-trimethylpyridine. The determination of minor compounds-pyridine, 4-ethylpyridine, 3-ethyl-

Table 1
Determination of pyridine bases in industrial mixture

Base	CZE–PEG (%)		GLC (%)	
Pyridine	0.20		0.10	
2-Methylpyridine	10.57		10.45	
3-Methylpyridine	11.57		11.70	
4-Methylpyridine	9.52		18.17	
2,6-Dimethylpyridine	55.26[a]		10.55	51.27[a]
2,3-Dimethylpyridine			4.50	
2,4-Dimethylpyridine			27.75	
2,5-Dimethylpyridine			8.47	
3,5-Dimethylpyridine	0.18			
3-Ethylpyridine	0.13			
4-Ethylpyridine	0.23	4.21[b]	3.35[b]	
2,3,6-Trimethylpyridine	0.75			
2,4,6-Trimethylpyridine	2.92			

[a] Sum of 2,6-dimethylpyridine, 2,3-dimethylpyridine, 2,4-dimethylpyridine, 2,5-dimethylpyridine.
[b] Sum of 3,5-dimethylpyridine, 3-ethylpyridine, 4-ethylpyridine, 2,3,6-trimethylpyridine and 2,4,6-trimethylpyridine.

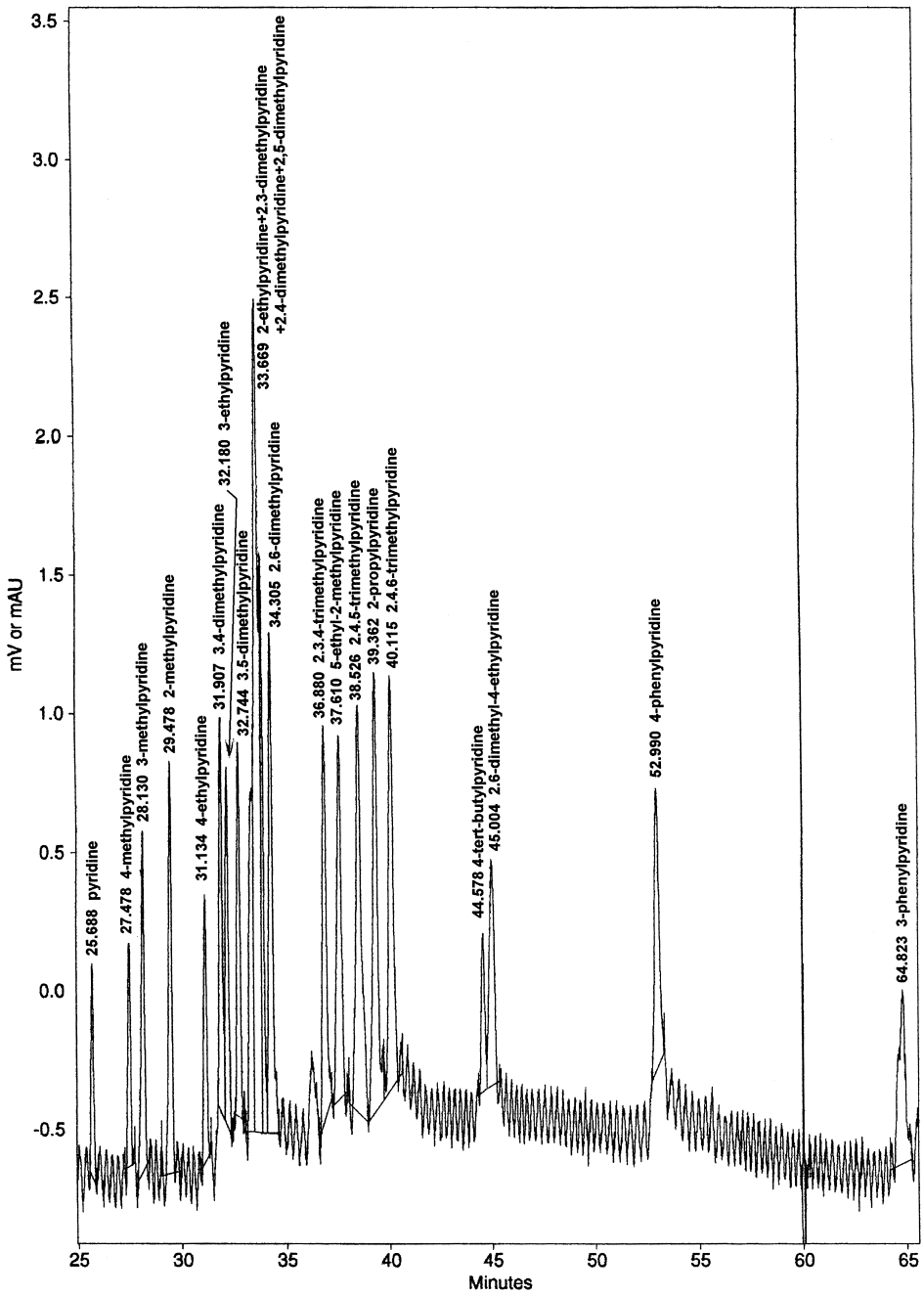

Fig. 7. Model mixture separation of alkyl and aryl pyridine derivatives. BGE: 50 m*M* phosphate buffer (TBA^+) pH=2.5+37.5% PEG 2000, vacuum injection 0.5 s, U=25 kV.

pyridine and 3,5-dimethylpyridine-was done with 25% solution of PEG in 1:100 diluted sample (Fig. 8). As in the case of the model mixture, the highest concentration of additive is necessary for the separation of methylpyridines (Fig. 9). Dimethylpyridines can be CZE determined only in

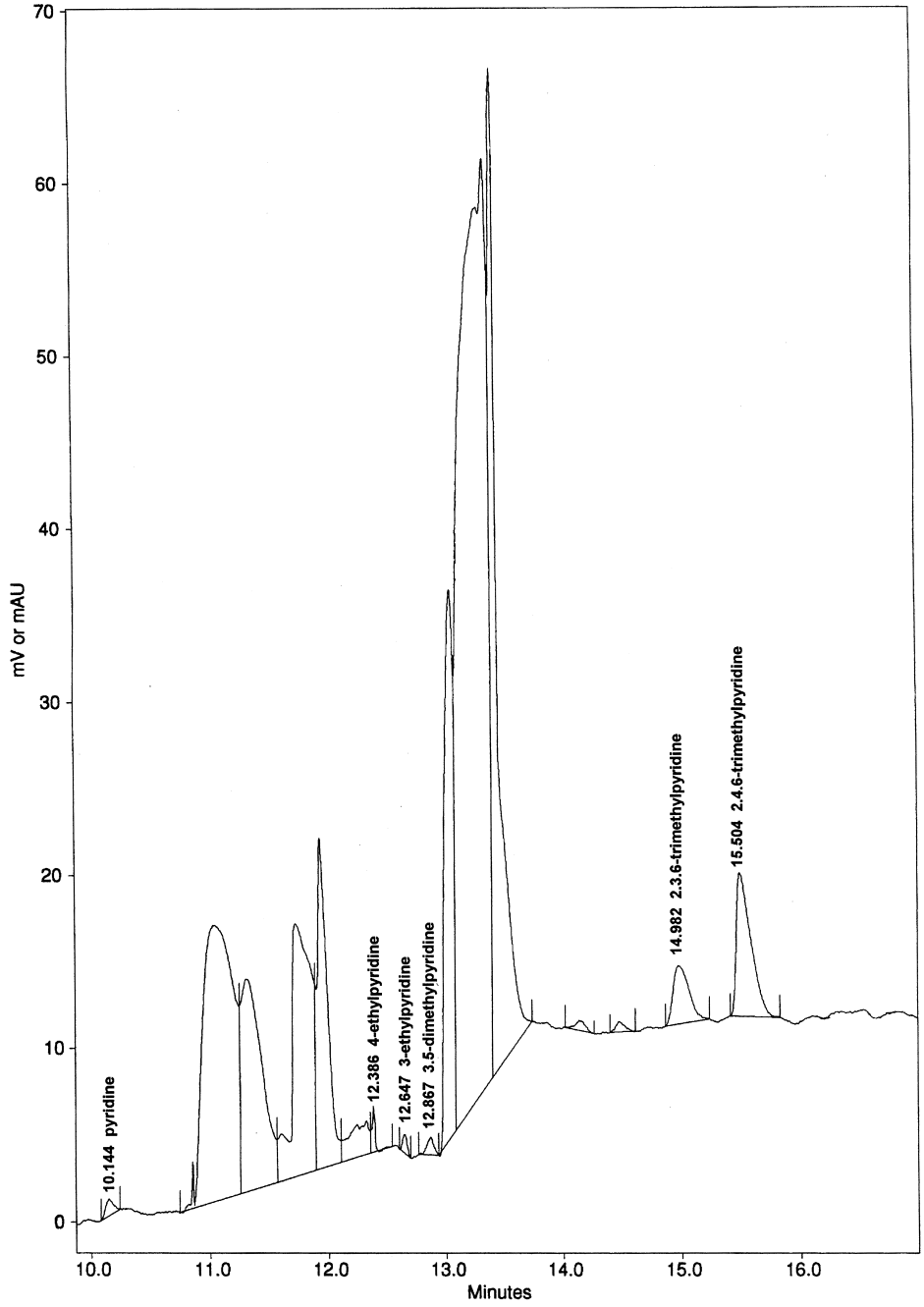

Fig. 8. Separation of DEZA product. BGE: 50 m*M* phosphate buffer (Na^+) pH=2.5+25% PEG 2000, vacuum injection 1 s, U=25 kV, analyte was diluted 1:100 by phosphate buffer.

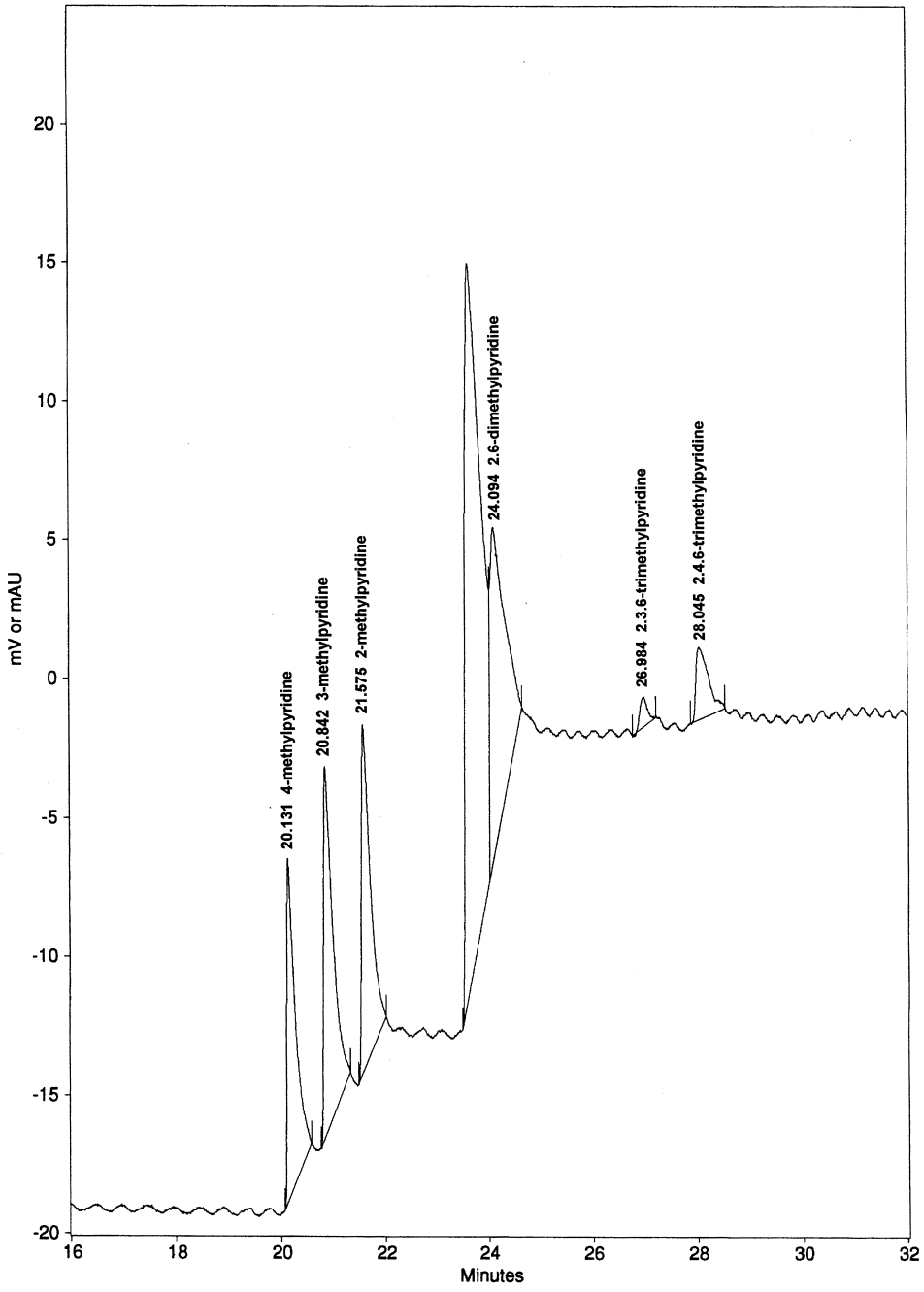

Fig. 9. Separation of DEZA product. BGE: 50 m*M* phosphate buffer (Na^+) pH=2.5+37.5% PEG 2000, vacuum injection 1 s, U=30 kV, analyte was diluted 1:100 by phosphate buffer.

sum, except of 3,5-dimethyl derivative. The agreement of quantitative values is good, except of found content of 4-methylpyridine.

4. Conclusions

It was shown that the polyethylene glycol, as the additive to the background electrolyte, is useful for CZE separations of protonated organic bases in relatively high concentration. The effect of the addition of PEG 2000 into the background electrolyte was studied, the conditions for the separation of 19 components in model mixture containing 23 alkyl and aryl derivatives of pyridine were found. An industrial sample of pyridine bases was analysed. Contents of pyridine, all isomers of methylpyridine, 3- and 4-ethylpyridine, 3,5-dimethylpyridine, 2,3,6- and 2,4,6-trimethylpyridine were determined. Other dimethylpyridines could be determined only in total because of their poor separation.

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