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Separation of eleven central nervous system drugs by capillary zone electrophoresis

V. Pucci[a,1], M. Raggi[b], E. Kenndler[a,*]

[a]*Institute for Analytical Chemistry, University of Vienna, Währingerstr. 38, A 1090 Vienna, Austria*
[b]*Dipartimento di Scienze Farmaceutiche, University of Bologna, Bologna, Italy*

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Abstract

Several strategies to improve the separation of 11 central nervous system drugs (antipsychotics and antidepressants) with capillary zone electrophoresis were applied: the variation of the pH of the buffering background electrolyte, its ionic strength, addition of inclusion-complex forming β-cyclodextrin or polyvinylpyrrolidone (PVP), respectively, as a replaceable, soluble, polymeric pseudo-stationary phase. Best separation was achieved at pH 2.5 and 35 mmol/l ionic strength (phosphate buffer), with 0.5% (w/v) PVP. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Antidepressants; Antipsychotics; Cyclodextrins

1. Introduction

An important task in clinical and/or forensic toxicology is the development of reliable analytical methods for the screening and quantitation of antipsychotic and antidepressant agents. In fact a disproportionate use of these drugs can lead to serious risks for patients, especially at an inappropriate dosage. However, even in the right dosage some drugs can induce disorders, e.g. clozapine that may cause agranulocytosis [1,2] when applied in order to treat the negative symptoms of schizophrenia such as social withdrawal, blunting effect or poverty of speech. Olanzapine, [3] despite displaying some structural and pharmacological similarities with clozapine [4] does not cause this blood disorder. Moreover, all the classical antipsychotics used to treat the positive symptoms of schizophrenia (hallucinations, delusions and conceptual disorganization) as haloperidol, can cause extrapyramidal motor side effects such as tremor and rigidity [5]. Paroxetine, e.g. a new antidepressant that influences the re-uptake of serotonine, also used for a variety of psychiatric conditions [6], may induce anticholinergic side effects that are dose related [7], and has a high affinity for muscarinic receptors.

The importance of monitoring relevant physiological and psychiatric parameters during all the period of treatment is obvious. Furthermore, it is necessary to check the amount of the drug or of the various drugs that the patients everyday have to ingest. It

*Corresponding author. Tel.: +43-1-313-67-2405; fax: +43-1-319-6312.
E-mail address: ernst.kenndler@univie.ac.at (E. Kenndler)
[1]Permanent address: University of Bologna, Bologna, Italy.

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follows that accurate and fast techniques are needed for the analytical control of all pharmaceutical formulations that could contain one or more of these substances. As some of these drugs have similar chemical structures, e.g. olanzapine, clozapine and loxapine, or mipramine and protriptyline, appropriate analytical methods which can separate and quantify these compounds would be required.

Several papers on the simultaneous chromatographic determination of some of the present drugs have been published [8–10], mostly based on HPLC separation associated with dual [11] or multiple [12] wavelength detection. Tricyclic antidepressants and antipsychotics have been simultaneously resolved by micellar electrokinetic capillary chromatography [13–15].

All pharmaceuticals under consideration are ionogenic in structure, and are therefore potentially separable by capillary electrophoresis. However, only few papers report on this topic and in some of them the substances have been separated using three different conditions for the different families of drugs [16–20].

As all of the substances are amines, separation by adjustment of the pH seems obvious. However, due to the similar structure of some of the agents a fine-tuning of the separation conditions might be necessary. In the present paper beside the pH, the variation of the ionic strength, the application of a complex forming additive (β-cyclodextrin), the interaction with a pseudo-stationary phase is used to resolve the compounds of interest by capillary zone electrophoresis (CZE). Assays are in progress, in order to apply this method to the determination of these drugs in human plasma, and to extend the separation capabilities of the method to drugs metabolites. Based on the development of the appropriate separation conditions, the topic of the present paper, these assays can focus to sample pretreatment and preconcentration methods.

2. Experimental

2.1. Reagents

The following pharmaceuticals were used: olanzapine (Eli Lilly, Indianapolis, IN, USA); flurazepam (from Dalmadorm® capsules, Roche, Milan, Italy); loxapine (from Loxapac® capsules, Lederle Laboratories, Gosport, UK); clozapine (Novartis, Italy); chlorpromazine, protriptyline, fluphenazine, imipramine and haloperidol (Sigma, St. Louis, MO, USA); paroxetine (Smith Kline Beecham Pharmaceuticals, Melbourne, Australia); risperidone (Jansen-Cilag, Sydney, Australia).

Sodium hydroxide (analytical grade) and orthophosphoric acid (85%) as constituents of the buffers were purchased from E. Merck (Darmstadt, Germany). β-Cyclodextrin (purum>99%) was from Fluka (Buchs, Switzerland), polyvinylpyrrolidon (PVP 25) from Serva (Heidelberg, Germany). Water was doubly distilled from a quartz apparatus. CZE buffers were filtered (0.45 μm, Minisart RC25, Sartorius, Göttingen, Germany) prior to use.

The standard solutions were prepared by diluting suitable amounts of stock solution. All stock solutions were stored at −18°C for 1 month at most, the standard solution at 4°C at most for a week.

Stock solutions of antipsychotic and antidepressant agents obtained from pharmaceutical formulations were prepared by finely grinding of capsules. An amount of powder was weighed, transferred into the appropriate volume of phosphate buffer (50 mmol/l, pH 2.5), and filtered after agitation with vortex for 15 min. This solution consisted of declared concentration of 1 mg/ml drug. Stock solutions from pure standard compounds were prepared at the same concentration in BGE (phosphate 50 mmol/l, pH 2.5). The working solutions were prepared by dilution with double distilled water.

2.2. Apparatus

Capillary zone electrophoresis was carried out with a home made instrument using a 50-μm I.D. uncoated fused-silica capillary (Composite Metal Services, Hallow, UK) with a total length of 43.2 cm and an effective length of 23.0 cm. The sample solutions were loaded into the capillary by electrokinetic (2 kV for 5 or 10 s) or siphoning injection (height difference of the reservoirs of 10 cm for 10 s). The compounds were detected at 206 or 245 nm, respectively, using a UV-Vis detector (Spectra System UV 2000, Thermo Separation Products, Riviera Beach, FL, USA). The instrument was operated at 15

kV with currents typically less than 40 μA, generated by a high-voltage power supply (2127 Tachophor, LKB, Bromma, Sweden). The electropherograms were recorded and processed with a dual-channel interface (35900, Hewlett-Packard, Waldbronn, Germany).

2.3. Procedure for capillary preparation and handling

Before use the capillary was rinsed for 10 min with deionized water, 10 min with 0.1 mol/l sodium hydroxide, 5 min with 1 mol/l sodium hydroxide, 10 min with 0.1 mol/l sodium hydroxide and 30 min with water before filling in the CZE buffer.

After each run the capillary was rinsed with water (2 min) and with buffer (5 min). After a maximum of 30 injections the capillary was rinsed again with water, 0.1 mol/l sodium hydroxide, water and refilled with CZE buffer. For storage overnight, the capillary was additionally washed with water.

3. Results and discussion

Two parameters are responsible for separability in CZE: the different migration of the separands, and their peak dispersion (cf. e.g. [21]). The migration is governed by the total mobility, μ_i, of the analyte, which is composed by its effective mobility, $\mu_{\mathrm{eff},i}$, and by the mobility of the electroosmotic flow, if occurring. The effective mobility can be influenced by a number of variables, with the pH of the BGE as one of the most important system properties. However, there is also a number of other possibilities, namely the ionic strength of the BGE, at least for those cases where the sample components have different charge numbers, organic solvents that affect the p*K* values and the actual mobilities [22,23], other forms of equilibria than acido–basic reactions, e.g. complexation as one of the most important principles for the separation of chiral compounds, and pseudophases such as micelles or soluble polymers which introduce a chromatographic aspect in addition to the electrophoretic separation principles.

With the exception of organic solvents, all of these possibilities were applied in the present investigation to find those experimental conditions that allow separating all analytes in one run.

3.1. Effect of pH on selectivity

The selectivity of two separands, *i* and *j*, is described by the selectivity coefficient, r_{ij}, the ratio of the total mobilities, μ_i, given by

$$r_{ij} = \mu_i/\mu_j \quad (1)$$

In CZE the pH of the BGE determines the effective mobility of the separands by the adjustment of the degree of ionization. It is clear that the most pronounced influence on the effective mobility takes place at pH values close to the p*K* of the analytes. All of the separands under investigation have at least one aliphatic amino group in their molecules (see Fig. 1), which has typically pK_a values around 9 or 10. It is apparently favorable to work at a pH in this range to establish the highest separation selectivity possible.

However, there are some practical limitations concerning this pH range. The one is the increased possible adsorptivity of the cationic analytes onto the negatively charged wall of the fused-silica capillary. This effect might lead to severe adsorption due to the electrostatic attraction occurring, causing peak distortion or even the disappearance of separands by irreversible adsorption. Adsorption is pronounced especially for higher charged cations [24]. Some analytes under investigation can form such cations even at moderate pH. Note that the capillary wall is negatively charged also in the lower pH range (the inflection point of the curve relating the mobility of the electroosmotic flow to the pH is in the pH range between 5 and 6; this means that in this range half of the dissociable silanol groups are in fact negatively charged). Adsorption effects were observed indeed during our investigation of structurally similar metabolites of olanzapine. These effects caused a severe peak tailing of some compounds, a retention of these solutes due to the chromatographic effect occurring, and even to a drastic reduction of the peak areas by irreversible adsorption.

Another reason for us to avoid high pH ranges was the disadvantage of the occurrence of the electroosmotic flow (EOF), which is directed towards the

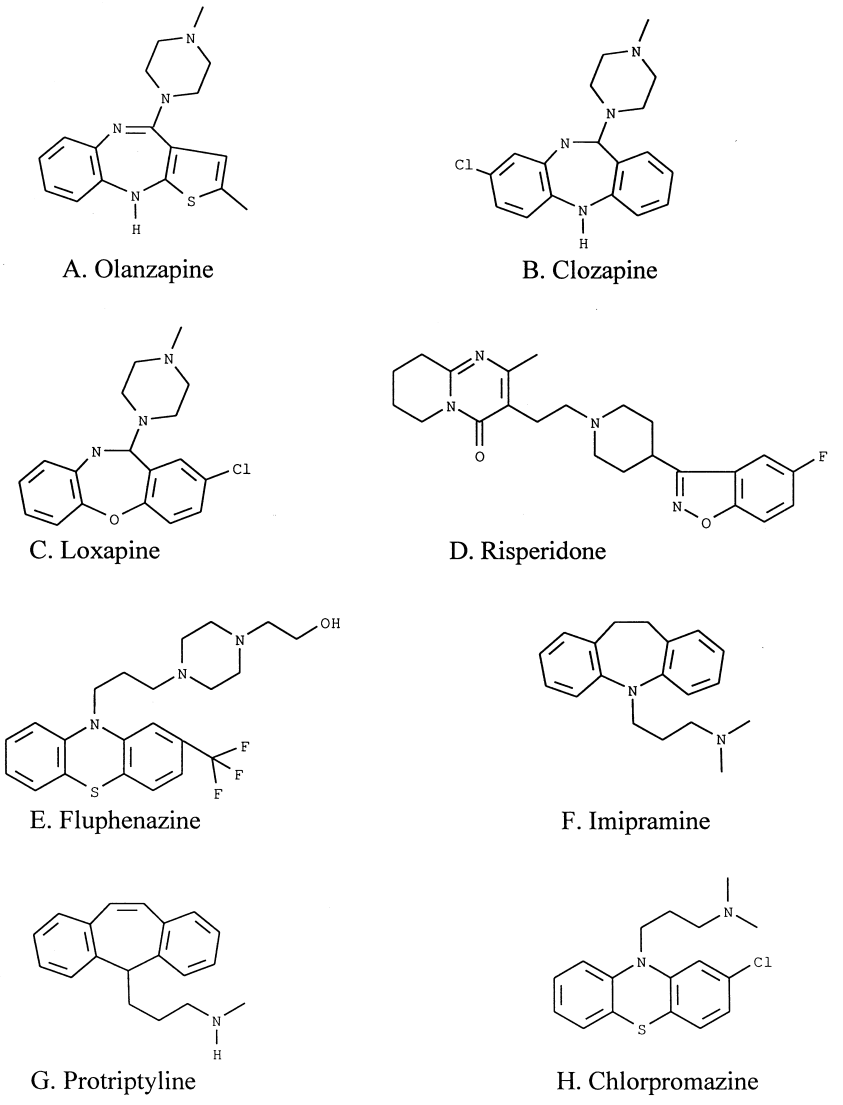
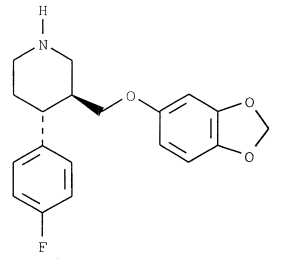


Fig. 1. Structural formulae and symbols of the separands.

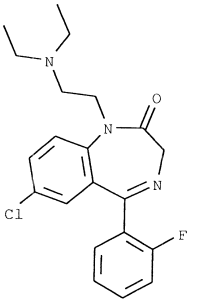
cathode. This EOF, despite increasing the separation efficiency, will always lead to a reduction of the resolution for the cationic separands under investigation, compared to the situation without EOF [25,26]. For crucial separations it is thus more favorable to work under experimental conditions where the EOF is reduced as much as possible.

A third reason to avoid a too high pH was the low solubility of the non-ionic species of some pharmaceuticals. It is clear that the cationic form, which is in equilibrium with the more lipophilic non-charged species, is better water-soluble. Indeed it was found that the (corrected) peak area of some agents decrease with increasing pH of the BGE, seemingly due to the loss of the substance caused by its precipitation inside the capillary.

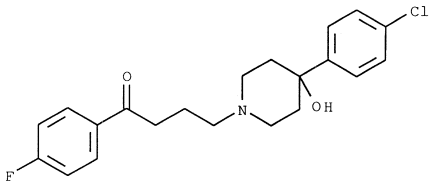
For these reasons low values of the pH were chosen in the present investigation. We realize that under these conditions the potential for the adjustment of the separation selectivity is limited to the sub-optimal region of mobilities with more or less fully protonized solutes.



I. Paroxetine



J. Flurazepam



K. Haloperidol

Fig. 1. (*continued*)

In Fig. 2 the selectivity coefficients, r_{Aj}, of the separands are given depending on the pH of the BGE. The selectivity coefficients were related to the compound with the highest mobility at all pH, olanzapine (compound A in Fig. 1). The pH was varied in the region between 2.4 and 3.5. The total ionic strength was 34.5 mmol/l. At such a low pH around 2 the analytes exhibit nearly their actual mobilities, which are given by the size and shape of the ions and their charge number. Indeed it was found (and is reflected by the order of the values of the selectivity coefficients) that the sequence of the mobilities follows roughly the number of nitrogen atoms in the molecule. Haloperidol with one N-atom has the smallest mobility on the one hand, olanzepine and clozapine with four N-atoms (whereas not all are ionizable in this pH range) have the highest mobility, on the other hand. Note that in Fig. 2 the selectivity coefficient is based on the total mobility, composed by the individual mobility, and the non-specific mobility of the EOF. The latter is very low in the acidic pH region under consideration. Therefore it was not determined, because extremely long time would be necessary for measurement. However, for the separation the total mobility is of more relevance than the actual mobility; therefore the

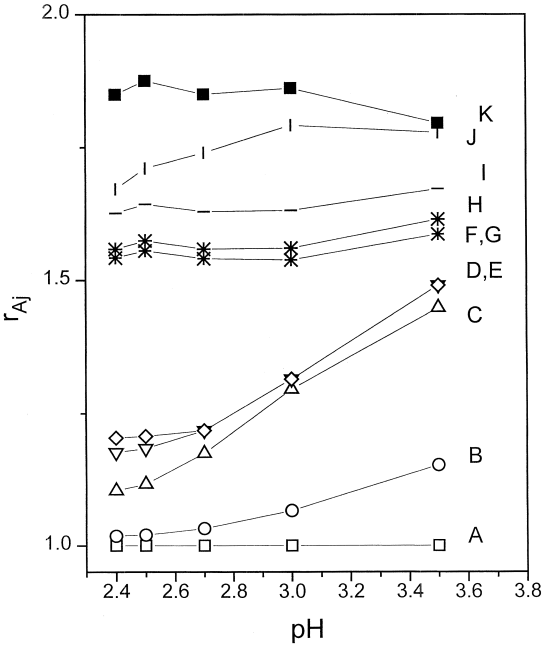


Fig. 2. Plot of the selectivity coefficient, r_{ij}, vs. pH. Buffer: phosphate with 50 mmol/l total phosphate concentration. The pH of a solution of phosphoric acid was adjusted by sodium hydroxide. For the definition of r_{ij} see Eq. (1). Clozapine (A in Fig. 1) is taken as reference component, *i*, in all cases. The symbols for the substances (index *j* in the selectivity coefficient) are according to Fig. 1.

consideration of the former is adequate for this discussion.

Without going into detail concerning the effect of the pH on the particular selectivity coefficients (the p*K* values of the individual amino groups are hard to predict) one can derive from Fig. 2 that the largest number of compounds are separated at pH 2.5. This is the pH that the further investigation will concentrate on.

3.2. Effect of ionic strength on mobility

The ionic strength is one variable that might enable a finer tuning of the mobilities of the separands, at least for sample mixtures where the particular analytes have different charge numbers. In this case the ionic strength of the BGE may influence the mobilities to a different extent, because the actual mobility depends on the charge number, *z*, and the ionic strength, *I,* according to Ref. [27]

$$\mu_{\mathrm{act},i} \propto -0.77 \exp\sqrt{z_i I} \qquad (2)$$

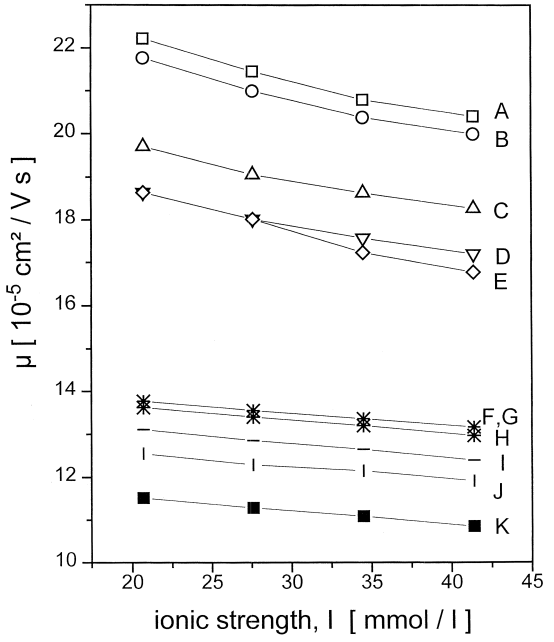


Fig. 3. Total mobility, μ, in dependence on the ionic strength, *I*, of the BGE. Phosphate buffer with varying concentration of phosphoric acid, adjusted to pH 2.5 with sodium hydroxide. Symbols of the solutes as in Fig. 1.

The total ionic concentration was varied at pH 2.5 between 30 and 60 mmol/l phosphate. It can be seen from Fig. 3 that, according to theory, the mobility decreases with increasing ionic strength. Indeed the separands with a small number of ionizable nitrogen atoms (compounds F and K) and therefore with presumably smaller charge numbers, exhibit a flatter μ vs. *I* curve. Those with the larger charge numbers (and with the larger mobilities) have steeper μ vs. *I* curves (compounds A–E), indicating at least roughly the validity of Eq. (2). Interestingly risperidon and fluphenazine (D, E), having the same mobilities at low ionic strength reach different mobilities at ionic strengths, *I*, larger than 30 mmol/l. This means that at *I* of, e.g. 35 mmol/l or higher these two solutes can be separated. As at 42 mmol/l the migration time is the highest, *I* of 35 mmol/l and pH 2.5 can be considered as favorable conditions concerning these two experimental variables. The resulting

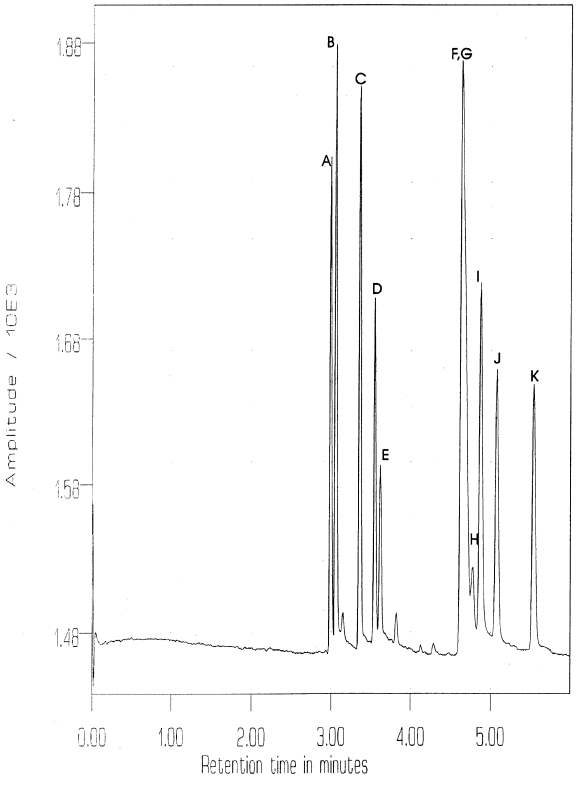


Fig. 4. Electropherogram of the sample components at pH 2.5 and ionic strength of 35 mmol/l (phosphate buffer). Capillary: uncoated fused silica, 43.2 cm total length, 23 cm to detector, I.D. 50 μm. Voltage, 15 kV. Detection at 206 nm. Analyte concentration, 1 μg/ml. Symbols of the solutes as in Fig. 1.

electropherogram is shown in Fig. 4. It can be seen that it is possible to identify 10 analytes in less than 6 min. However, even under these conditions the peaks of imipramine and protriptyline (F and G) comigrate, and also that of chlorpromazine (H) is not fully resolved. Further improvement of the separation is therefore needed.

3.3. Effect of complexation on mobility

The use of complex-forming agents to affect the selectivity is a well-established strategy to improve the separation of compounds with pharmaceutical interest (for recent reviews, cf. Refs. [28,29]). In the present investigation β-cyclodextrin is applied for these purposes. In Fig. 5 the effect of the concentration of this additive on the mobility of the separands is shown. A strong reduction of the mobility is found for compounds E–I, leading to a pronounced change of the separation selectivity, and even of the migration order. From these results it can be concluded that the β-cyclodextrin concentration most favorable for separation is 3.5 mmol/l. The electropherogram obtained under these conditions is shown in Fig. 6. Full separation for all compounds except F, G and H is established. This triplet is not fully baseline resolved. Although the accuracy of the determination of the position of the peak maxima (for identification) and of the peak areas (for quantitation) will suffice in many cases, a somewhat unusual alternative to influence the separation selectivity is applied: a soluble, replaceable pseudo-stationary phase.

3.4. Effect of pseudo-stationary phase on mobility

Polyvinylpyrrolidone was found to introduce a specific interaction with sample components in previous work when used as pseudo-stationary phase, and to improve the separation selectivity [30–34]. The effect of the concentration of PVP on the mobility is shown in Fig. 7. Indeed, even a change in the mobility sequence is observed. Especially the mo-

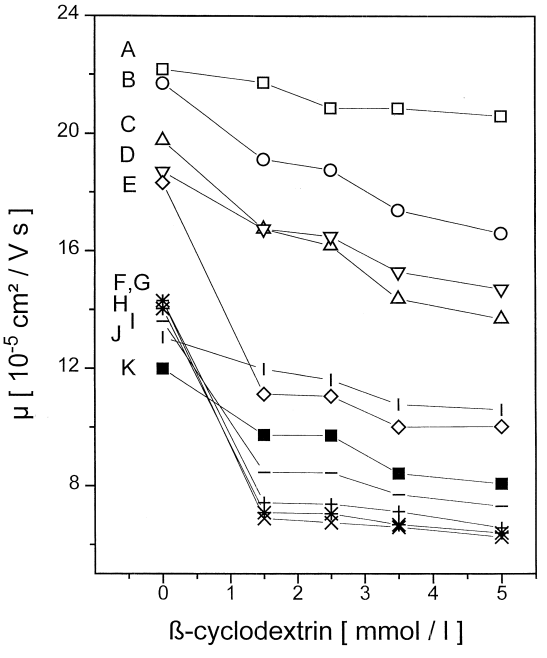


Fig. 5. Total mobility, μ, as function of the concentration of β-cyclodextrin (phosphate buffer, pH 2.5, ionic strength 35 mmol/l). Symbols of the solutes as in Fig. 1.

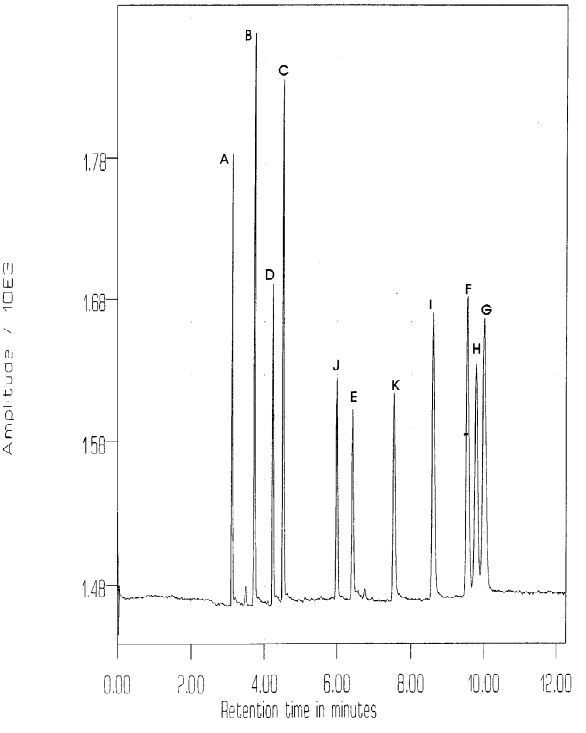


Fig. 6. Electropherogram of the sample components at β-cyclodextrin concentration of 3.5 mmol/l, pH 2.5, ionic strength 35 mmol/l (phosphate buffer). Experimental conditions as in Fig. 4. Analyte concentration 10 μg/ml. Symbols of the solutes as in Fig. 1.

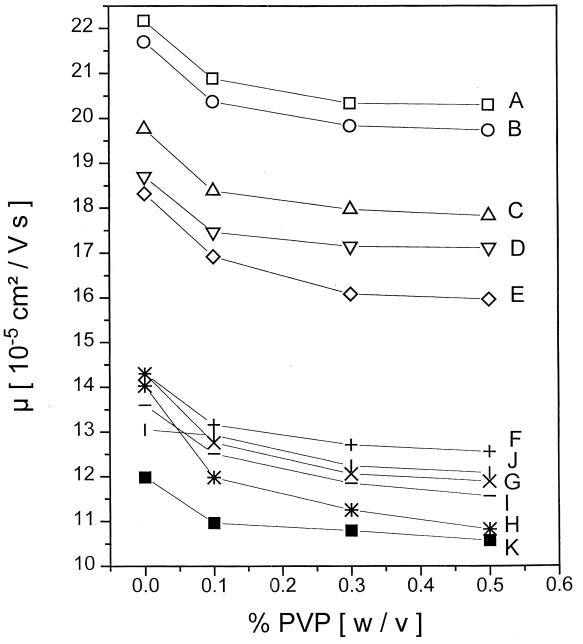


Fig. 7. Total mobility, μ, as function of the concentration of PVP (phosphate buffer, pH 2.5, ionic strength 35 mmol/l). Symbols of the solutes as in Fig. 1.

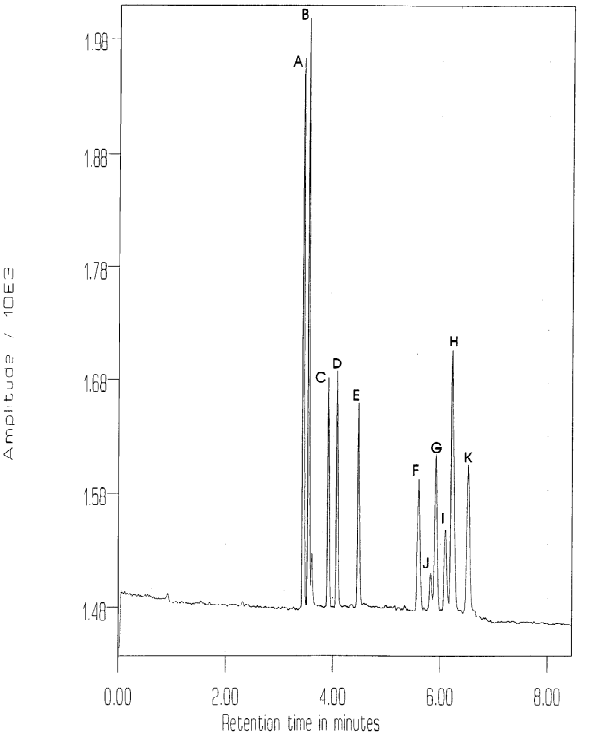


Fig. 8. Electropherogram of the sample components at 0.5% PVP, pH 2.5, and ionic strength 35 mmol/l (phosphate buffer). Experimental conditions as in Fig. 4. Detection at 245 nm. Analyte concentration, 20 μg/ml. Symbols of the solutes as in Fig. 1.

bilities for separands F, G and H (the compounds that nearly comigrate in the electropherogram obtained with β-cyclodextrin as additive, see Fig. 6) are strongly affected. This increase in selectivity results in a full separation of these three compounds. From Fig. 8 the improvement of the separation becomes visible at a PVP concentration of 0.5% (w/v). In contrast to the other systems, the electropherograms were run here at 245 nm, because PVP has a considerable UV-absorbance at 206 nm. This stronger absorbance leads to a larger noise of the baseline compared to the other systems, a disadvantage that should be mentioned. However, we think that this disadvantage is overcompensated by the unusual selectivity aspect of PVP as pseudophase.

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