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Micellar electrokinetic chromatography at low pH with polyelectrolyte-coated capillaries

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Abstract

The performance of capillaries coated with a poly(diallyldimethylammonium) (PDADMA) monolayer or poly(diallyldimethylammonium)–poly(styrenesulfonate) bilayer was investigated and compared under micellar electrokinetic chromatographic (MEKC) conditions. Both monolayer (positively charged) and bilayer (negatively charged) coatings with micellar (sodium dodecyl sulfate) electrolyte generated very stable and pH-independent cathodal electroosmotic flow (EOF). From the results obtained, it can be concluded that in a doubly coated capillary the second poly(styrenesulfonate) layer is replaced by sodium dodecyl sulfate micelles during flushing with micellar electrolyte. Consequently, in order to obtain a stable and pH-independent cathodal electroosmotic flow for the MEKC separations, the capillary coating with the second polyanion layer is not necessary. The importance of the PDADMA coating was illustrated by comparing MEKC separations of the common developing agents (hydroquinone, phenidone, pyrocatechol, pyrogallol and quinone) on a bare uncoated capillary with the coated capillary. The coating provides reproducible MEKC separations at low pH (pH 3.0) with relative standard deviation (R.S.D.) values for migration times and peak areas lower than 0.45 and 3.3%, respectively. Good linearities in the range from 5×10^{-5} to 2×10^{-3} mol l^{-1} were obtained for all five compounds, with correlation coefficients higher than 0.998. The detection limits were in the range from 5×10^{-6} mol l^{-1} for pyrocatechol to 2×10^{-5} mol l^{-1} for quinone. The proposed MEKC system was applied to the determination of hydroquinone and phenidone in X-ray photographic developer solutions.

Keywords: Coated capillaries; Electroosmotic flow; Hydroquinone; Phenidone

1. Introduction

Black and white photographic processes are carried out extensively by hospitals and many private medical companies to obtain X-ray plates. The main components present in a photographic developer are the developing agent (hydroquinone, phenidone, pyrocatechol, etc.) and an antioxidant (sulfite). The analysis of these baths is important in determining the quality and effectiveness of the developing process [1]. Moreover, in order to minimize unwanted environmental contamination, the effluents from used baths should be collected, and the harmful compounds should be converted to alternate forms (e.g. CO_2, N_2, H_2O, etc.). In the last decade various oxidation techniques have been investigated for this purpose [2–4]. The main problem of such investigations is the rapid and simple monitoring of the contaminants before and during their degradation.

In the last few years, capillary electrophoresis (CE) has been successfully introduced in the analysis of various photographic solutions [5–11]. However, since most of the developing agents are neutral compounds, micellar electrokinetic chromatography (MEKC) should be used for their separation. MEKC is a capillary electrophoretic technique for separation of uncharged compounds and was first reported by Terabe et al. [12]. In MEKC the separation of neutral analytes is based on analyte partition to micelles formed by addition of surfactants to the electrolyte. Sodium dodecyl sulfate (SDS) is the most commonly used surfactant in MEKC, and it forms negatively charged micelles that migrate towards the anode. Thus, the presence of an electroosmotic flow (EOF) is necessary in MEKC for transportation of the analytes to the detector at the cathode end. The rate of EOF depends strongly on the pH of the electrophoresis medium and decreases rapidly below pH 6, resulting in

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increased analysis time or even loss of the ability to analyze neutral compounds. For this reason, conventional MEKC separations are in practice often limited to higher pH values. Being strong reducing agents, all developers are very sensitive to oxygen, especially in alkaline solutions. Consequently an acidic pH range is better for the separation of these compounds. Elimination of the pH-dependency of the EOF requires a chemical modification of the capillary surface, and this has been accomplished by either covalent modifications or dynamical coatings [13]. Dynamic adsorption of ionic polymers offers some advantages with respect to the simplicity of the procedure. While many methods have been reported to produce neutral and positively charged capillary surfaces, relatively few methods have been reported to produce a pH independent, negatively charged capillary surface. The most commonly used approach is a bilayer capillary coating [14–19]. The two-stage process involves initially flushing the capillary with buffer containing the polycation. The multiply charged polycations coat the entire capillary wall, making it strongly positively charged. The capillary is then flushed with the buffer containing the polyanion. The polyanions adsorb to the positively charged layer and form a highly negatively charged layer, which is insensitive to pH changes, resulting in a strong and constant cathodal EOF.

Graul and Schlenoff [20] and Warner and co-workers [21] reported a similar coating procedure where alternating multilayers of polycation and polyanion were deposited to obtain a normal as well as reversed EOF, depending on the charge of the last deposited polyelectrolyte layer. Such coatings have been found to be robust and thus highly resistant to change and deterioration during use. Moreover, polyelectrolyte multilayers act as stationary phases and can be used for open-tubular capillary electrochromatographic separations.

However, little attention has been given to the applicability of dynamically coated capillaries in MEKC separations. To date, only few papers have reported MEKC separations in capillaries coated with polyelectrolytes [16,18,19]. Perhaps the most systematic study in this area was published by Bendahl et al. [18], who demonstrated that capillaries coated with polybrene/poly(vinylsulfonate) double layer can be successfully used in MEKC separations.

In the present work, the performance of capillaries coated with poly(diallyldimethylammonium) monolayer and poly(diallyldimethylammonium)/poly(styrenesulfonate) bilayer was investigated and compared under MEKC conditions. Another aspect of this work was to optimize the determination of common developing agents with the MEKC system at low pH.

2. Experimental

2.1. Instrumentation

Separations were performed on a P/ACE 2100 apparatus (Beckman Instruments Inc., Fullerton, CA, USA) equipped with a UV detector with wavelength filters (200, 214, 230 and 254 nm). Fused silica capillaries (Polymicro Technology, Phoenix, AZ, USA) of 57 cm (50 cm to the detector) 75 μm i.d. were used. Samples were injected in the hydrodynamic mode by overpressure (3.43×10^3 Pa). System Gold software (Beckman Instruments) was used for data acquisition. UV detection (214 nm) was employed at the cathode end. All experiments were conducted at 25 °C with a liquid thermostated capillary cartridge.

The HPLC instrumentation consisted of a Varian Model 5060 high-pressure pump, an injection valve equipped with a sample loop of 20 μl and a Waters 990 variable-wavelength UV detector set to absorb at 210 nm. The results and data were collected and plotted on a plotter/integrator SP 4290 (Spectrophysics, San Jose, CA, USA). HPLC separations were performed on a 5 μm Separon TM SGX C_{18} (150 mm × 3 mm i.d.) column (Tessek, Prague, Czeck Republic). The mobile phase flow rate was 0.25 ml min^{-1}.

2.2. Chemicals

All electrolyte and standard solutions were prepared with doubly distilled helium degassed water. Poly(diallyldimethylammonium chloride) (PDADMA, 20%, w/w, in water, average molecular mass ~100,000–200,000), poly(sodium-4-styrenesulfonate) (PSS, average molecular mass ~70,000) and SDS were purchased from Aldrich (Milwaukee, WI, USA). All other reagents were of analytical-reagent grade obtained from Merck (Darmstadt, Germany).

The stock analyte solutions (0.01 mol l^{-1}) were prepared daily by dissolution in water. All working solutions were prepared just before use by suitable dilution. Carrier electrolytes were prepared by neutralization of 25 mmol l^{-1} H_3PO_4 and 50 mmol l^{-1} SDS solution with NaOH to desired pH. The mobile phase system for HPLC analysis was 40% CH_3OH and 60% 0.01 mol l^{-1} K_2HPO_4, pH 6.5.

2.3. Procedures

All electrolyte and sample solutions were filtered through a 0.45 μm membrane filter. Each new fused-silica capillary was flushed with 1 mol l^{-1} NaOH for 30 min and then with deionized water for 15 min. The first layer of polymer was deposited by flushing the capillary with 0.5% (w/v) PDADMA, prepared in 0.5 mol l^{-1} NaCl, for 20 min followed by a 5 min water rinse. Next, the capillary was flushed with 0.5% (w/v) PSS, prepared in 0.5 mol l^{-1} NaCl, for 10 min and water for 5 min. Finally, the capillary was flushed with the carrier electrolyte for 10 min. Between all electrophoretic separations the capillary was rinsed with carrier electrolyte for 2 min.

3. Results and discussion

3.1. Coating performance

In order to investigate the surface behaviour, the effect of pH on the EOF mobility in both a PDADMA-coated

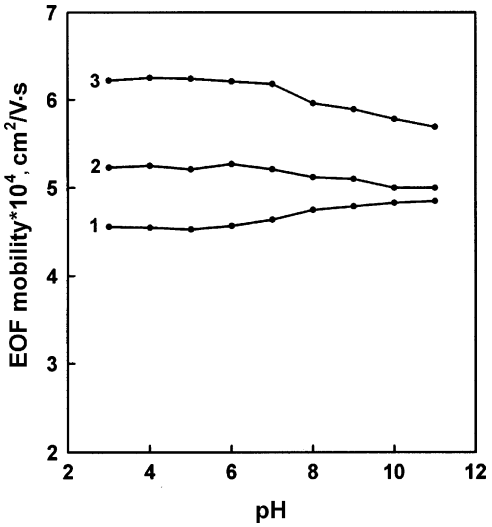

Fig. 1. Electroosmotic mobility as a function of pH in (1, 2) the PDADMA–PSS-coated capillary and (3) the PDADMA coated capillary. Electrolytes: (1) 25 mmol l^{-1} H_3PO_4 and (2, 3) 25 mmol l^{-1} H_3PO_4, 50 mmol l^{-1} SDS. Conditions: voltage, 25 kV; temperature, 25 °C; neutral marker, acetone.

capillary and a PDADMA–PSS-coated capillary was studied with a micellar electrolyte (for both capillaries) and an electrolyte without SDS (only for PDADMA–PSS-coated capillary). The EOF mobility was measured in the pH range 3.0–11.0 by five replicate injections of the EOF marker (acetone) at each pH level. At each pH point, the capillary was rinsed with a fresh electrolyte for 10 min. The results of this experiment are summarized in Fig. 1. As can be seen, in both capillaries the cathodic EOF is relatively constant over the entire pH range. It is clear that the PDADMA-coated capillary surface has a high density of positive charges that interact strongly with the negatively charged SDS micelles. The excess of negative surface charges gives cathodic EOF. Thus, in both capillaries the negative charge density on the capillary surface is provided by the sulfonic (PSS) or sulfate (SDS) groups, which are completely ionized in the pH range studied. However, the EOF in the doubly coated capillary was always slightly lower than that in the capillary coated with PDADMA. These results indicate that SDS micelles give higher negative charge density on the capillary surface than the PSS polyanion. Moreover, when the SDS-containing electrolyte is used with the PDADMA–PSS-coated capillary, the behavior of the EOF versus pH is somewhat different from that measured in the same capillary with electrolyte without SDS. At pH above 7, a slight decrease in EOF is observed with micellar electrolyte, whereas under non-micellar conditions an opposite trend takes place. However, the circumstances determining the pH dependence of the EOF for the coated capillaries are complex, and the reason for this behavior is difficult to explain. The relative standard deviations (R.S.D.s) of the EOF were less than 0.5% for both capillaries. Such stable and pH-independent EOF is a great asset that will provide

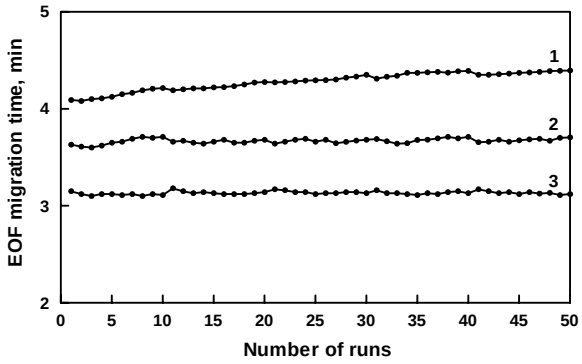

Fig. 2. Stability of the EOF in (1, 2) the PDADMA–PSS-coated capillary and (3) the PDADMA-coated capillary at pH 6.5. For conditions see Fig. 1.

reproducible migration behavior and also an opportunity to optimize the pH for best separation without altering the EOF.

Another important factor to consider when coated capillaries are used is the stability of the capillary coating. The stability of the capillaries was investigated by 50 consecutive measurements of the EOF at pH 6.5. The carrier electrolyte was changed every 10th run in order to minimize problems with buffer depletion. The EOF obtained for a large number of runs in both capillaries is compared in Fig. 2. The migration time of the EOF marker in the PDADMA–PSS-coated capillary increased gradually as the capillary was used with non-micellar electrolyte. Although the EOF decrease was faintly pronounced (about 12% relative to its initial value), this suggests slow detachment of the PSS layer. No systematic drifts of the EOF migration times were observed in either capillary with SDS-containing electrolyte. The differences in migration times between the first and the fiftieth run were less than 1.5%, indicating that negatively charged SDS micelles improve the stability of both coatings.

The effect of the SDS concentration on the EOF migration time in both capillaries is shown in Fig. 3. A rapid decrease in EOF migration time was observed for the PDADMA-coated capillary when the SDS concentration increased from

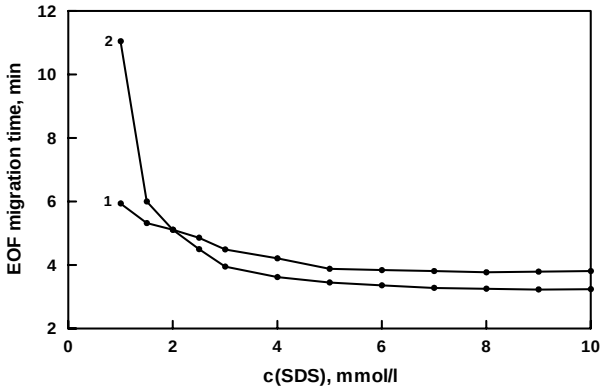

Fig. 3. Effect of SDS concentration on the EOF migration time in (1) the PDADMA–PSS-coated capillary and (2) the PDADMA coated capillary. Electrolyte: 25 mmol l^{-1} H_3PO_4, pH 3.0. Other conditions as in Fig. 1.

1 to about 4–6 mmol l^{-1}. A further increase of the SDS concentration up to 50 mmol l^{-1} did not show any statistically significant changes in the EOF velocity. Lucy and Underhill [22] reported that the critical micelle concentration (CMC) of cationic surfactants was consistent with the concentration at which the EOF became constant. Consequently, the use of polycation-coated capillaries should enable a simple determination of the CMC values of anionic surfactants. Specific and wider investigations of this aspect are in progress. As can be seen in Fig. 3, when the same electrolytes are used with the PDADMA–PSS-coated capillary the behavior of the EOF versus SDS concentration is similar but less pronounced. It is known that in bare-silica capillaries the EOF does not depend on the concentration of the anionic SDS. The results obtained in this study suggest that most likely SDS anions compete with PSS for the cationic PDADMA layer.

Tests were made to determine whether the SDS micelles replace the PSS coating. In this experiment, a negatively charged PDADMA–PSS coating was constructed, and three consecutive runs with 25 mmol l^{-1} phosphate electrolyte (pH 3.0) yielded an average EOF mobility of 4.59 $\times$ 10^{-4} cm^2 V^{-1} s^{-1}. The capillary was then flushed with micellar electrolyte (50 mmol l^{-1} SDS, 25 mmol l^{-1} phosphate, pH 3.0) for 20 min. Three consecutive runs resulted in an average EOF of 5.21 $\times$ 10^{-4} cm^2 V^{-1} s^{-1}. The same capillary was again flushed with phosphate electrolyte for 20 min, and the neutral marker was measured at the average mobility of -2.21×10^{-4} cm^2 V^{-1} s^{-1} for three runs, indicating an EOF reversal. The rinse with SDS electrolyte was repeated, and the EOF mobility was again directed towards the cathode (μ_{eo} = 5.18 $\times$ 10^{-4} cm^2 V^{-1} s^{-1}). These results clearly demonstrate that in doubly coated capillaries the second PSS layer is replaced by SDS micelles during flushing with micellar electrolyte. Consequently, in order to obtain stable and pH-independent cathodal EOF for the MEKC separations, the capillary coating with the second polyanion layer is not necessary. On the contrary, Katayama et al. [16] reported that polybrene/dextran sulfate bilayer is tolerant to SDS. Their conclusion, however, was based only on successful separation of cresol isomers by MEKC at low pH. Such experiment gives no evidence on the tolerance of the second dextran sulfate layer to SDS. If SDS does replace or does not the second layer during capillary preconditioning with SDS electrolyte, in both of the cases stable cathodal EOF which enables rapid MEKC separations in acidic electrolyte should form. Therefore, the discussion on the stability of other bilayer coatings under MEKC conditions needs additional experiments. Thus, a PDADMA-coated capillary was selected for the MEKC separations.

The long-term stability of the PDADMA-coated capillaries was investigated over a period of 5 days. When the capillary was stored in micellar electrolyte, no statistically significant changes ($\leq$2% R.S.D.) in electroosmotic mobility were observed over these 5 days. After these experiments, the capillary was flushed with water, dried and stored for three additional days. When it was refilled with electrolyte and tested, the resulting electroosmotic mobility was found to be the same as before drying. This possibility of capillary drying makes the handling of coated capillaries easier, since there is no need for capillaries to be stored in electrolytes when not in use.

It is well known that in uncoated fused-silica capillaries an increase in the ionic strength of the carrier electrolyte decreases the magnitude of the EOF. A possible effect of electrolyte ionic strength on the EOF mobility in a PDADMA-coated capillary was briefly investigated at pH 6.5. Electroosmotic mobility was measured at NaH_2PO_4 concentrations of 10, 20, 40, 60, and 80 mmol l^{-1}, the SDS concentration in the electrolyte being kept constant. The EOF mobility was roughly constant ($\leq$5% R.S.D.) with increasing electrolyte concentration, showing that the ionic strength did not seem to have any significant influence on the EOF. This is possible if significant flow penetrability is invoked [23]. The configuration of adsorbed polyelectrolytes can be described in terms of trains, loops and tails, i.e. the capillary surface coated with polyelectrolyte layer is not flat, so called a “hairy” layer. Electroosmotic flow inside the hairy layer is less subject to double layer compression with increasing ionic strength. More detailed information on this is available in [23].

3.2. MEKC separation of developers

Four developing agents, hydroquinone, phenidone, pyrogallol and pyrocatechol, were used to test the performance of MEKC in the PDADMA-coated capillaries. In addition, the main component that forms during the developing process, quinone, was also studied. The importance of the capillary coating in optimizing MEKC separation was demonstrated by comparison of separations on a coated capillary and an uncoated capillary. Direct comparison of the separation performance of the two capillaries at low pH is complicated by the fact that the pH range of the uncoated capillary is limited to neutral and alkaline medium. For this reason, comparison was performed at different pHs. Fig. 4 shows the electropherograms of a test mixture of four developing agents separated under conventional MEKC conditions at pH 9.0 (Fig. 4a), and with a PDADMA-coated capillary at pH 3.0 (Fig. 4b). Quinone was not added to the test mixture because phenidone reduces it to hydroquinone. As can be seen, complete separation of all four developers was observed with both MEKC systems, but the migration order of phenidone and pyrocatechol has changed, probably because of partial ionization of the latter analyte in the alkaline medium. Relative to the uncoated capillary, the efficiencies were slightly higher (about 10–20%) for hydroquinone, phenidone and pyrocatechol and almost the same for pyrogallol. The improvement in the separation selectivity under acidic conditions was much more evident when the separation of the hydroquinone/quinone redox pair was compared

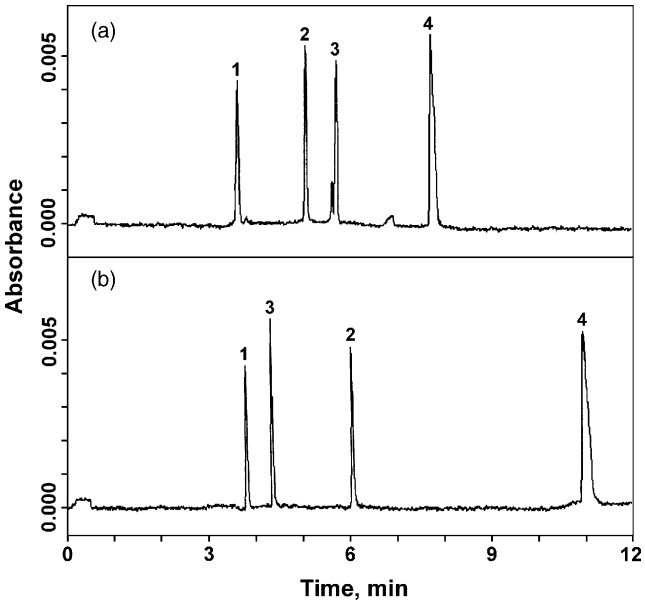

Fig. 4. Separation of four developers in (a) the uncoated capillary and (b) the PDADMA coated capillary. Electrolytes: (a) 25 mmol l^{-1} $Na_2B_4O_7$, 50 mmol l^{-1} SDS, pH 9.0 and (b) 25 mmol l^{-1} H_3PO_4, 50 mmol l^{-1} SDS, pH 3.0. Conditions: voltage, 25 kV; UV detection at 214 nm. Peaks: 1 = hydroquinone; 2 = phenidone; 3 = pyrocatechol; 4 = pyrogallol.

(Fig. 5). These results clearly demonstrate the importance of the coating in the resolution of some analytes.

The effect of temperature on the separation of developers was also briefly studied. The temperature for this study was varied from 25 to 50 °C. As the temperature was increased, the migration times decreased as a result of the decrease in electrolyte viscosity. The separation at 50 °C was complete in about 60% of the time required for the same separation at 25 °C. Although increasing the temperature resulted in higher efficiencies, a loss in the resolution of quinone and pyrocatechol took place. It should be also noted that no effect of the temperature on the coating stability was observed.

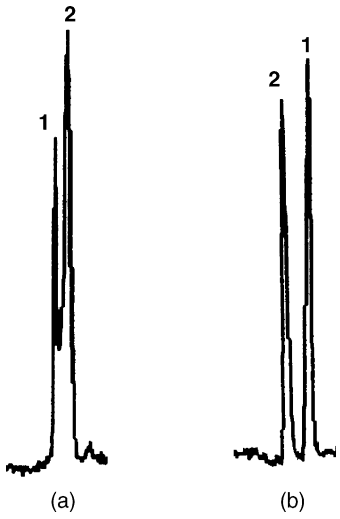

Fig. 5. Separation of quinone and hydroquinone in (a) the uncoated capillary and (b) the PDADMA-coated capillary. For conditions see Fig. 4. Peaks: 1= quinone; 2 = hydroquinone.

Table 1
Repeatability of migration times and peak areas of neutral analytes in PDADMA coated capillary ($n = 5$)[a]

Analyte	Migration time R.S.D. (%)	Peak area R.S.D. (%)
Hydroquinone	0.26	3.24
Quinone	0.21	2.51
Pyrocatechol	0.30	1.85
Phenidone	0.28	2.40
Pyrogallol	0.42	2.75

[a] Experimental conditions as in Fig. 4b.

3.3. Analytical performance

With the optimal experimental parameters described above (a carrier electrolyte of 50 mmol l^{-1} SDS in 25 mmol l^{-1} NaH_2PO_4, pH 3.0, and applied voltage of 25 kV) several analytical performance characteristics important for quantitative analysis were measured. To determine the migration time and peak area repeatability, solutions containing 0.25 mmol l^{-1} of each analyte were analyzed sequentially six times. The RSD obtained are summarized in Table 1. As can be seen, the proposed method gives repeatability comparable to or even better than that obtained by conventional MEKC techniques.

The linearity of the calibration curve was tested in the range from 5×10^{-5} to 2×10^{-3} mol l^{-1} by triplicate injections (10 s) of six standard solutions. Good linearities were obtained for all five compounds, with correlation coefficients higher than 0.998. The detection limits determined for 15 s hydrodynamic injection (three times the baseline noise) were in the range from 5×10^{-6} mol l^{-1} for pyrocatechol to 2×10^{-5} mol l^{-1} for quinone. Although the detection limits achieved were relatively high, such sensitivity was sufficient for the monitoring of commercial processing solutions.

To evaluate the proposed CE system for real samples, it was applied to the analysis of a commercial X-ray developer solution containing hydroquinone and phenidone. The only sample pre-treatment stage involves filtration of the sample through a 0.45 μm pore filter and appropriate dilution. Fig. 6 shows the electropherograms obtained for 1:200 diluted X-ray developer samples collected at different developing process run times. Quinone was not found in the samples. Most likely, it reacts with sulfite ions present in the developing solutions, forming hydroquinone sulfonate.

Two samples were analyzed by the proposed MEKC method and by HPLC. The results are compared in Table 2. As can be seen, the MEKC method showed good agreement with data obtained with the HPLC technique. The comparison of means with a *t*-test has shown that there is no statistically significant difference between them at a confidence level of 0.05.

In conclusion, this report demonstrates the significance of the use of polyelectrolytes as a capillary coating in MEKC

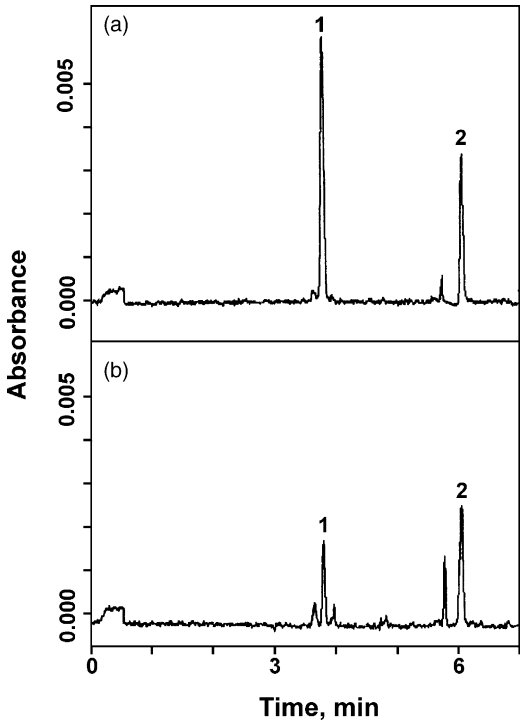

Fig. 6. Electropherograms of 1:200 diluted (a) fresh and (b) spent X-ray developer samples. For conditions see Fig. 4b. Peaks: 1 = hydroquinone; 2 = phenidone.

Table 2
Results of the determination of developing agents ($g l^{-1}$) in X-ray developer solutions ($n = 3$)

Sample	Analyte	MEKC	HPLC
Fresh	Hydroquinone	10.8	10.3
	Phenidone	2.82	2.85
Spent	Hydroquinone	2.06	2.00
	Phenidone	1.98	1.93

separation systems. These coatings are especially effective in analyzing neutral compounds at low pH values.

Acknowledgements

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