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On-line coupling of micellar electrokinetic chromatography to electrospray mass spectrometry

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Abstract

The possibility of selectivity enhancement in capillary electrophoresis–mass spectrometry (CE–MS) by hyphenating micellar electrokinetic chromatography (MEKC) and electrospray mass spectrometry (MS) is described for two quaternary ammonium compounds. Direct coupling of MEKC to MS is hazardous because of the contamination of the ion source due to presence of an excess of micelle forming agent in the MEKC buffer. Therefore, a coupled-capillary setup with the possibilities of voltage switching and buffer renewal has been designed. Such a system allows on-line heartcutting of the zones of interest in the MEKC capillary with subsequent transfer via a second capillary to the mass spectrometer.

1. Introduction

Since the introduction of micellar electrokinetic chromatography (MEKC) by Terabe et al. [1,2], the scope of capillary electrophoretic separation techniques has broadened significantly. The additional separation mechanism of MEKC, based on the differential partition between the micellar phase and the surrounding aqueous phase, introduces new possibilities concerning the analysis of neutral compounds that cannot be separated by capillary zone electrophoresis (CZE), and concerning the analysis of ionic compounds that prove to be difficult to separate on the basis of their electrophoretic properties only. In the recent past, MEKC has found many applications, ranging from the analysis of ionic and non-ionic compounds in a single run [3], bases, nucleosides and oligonucleotides [4], peptides [5] and proteins [6] to the determination of drug metabolites in urine [7], hereby demonstrating its value in the field of pharmaceutical and bioanalysis. For an overview of the application of MEKC in pharmaceutical analysis, see Nishi and Terabe [8].

For capillary zone electrophoresis–mass spectrometry (CZE–MS), the choice of buffers to achieve a particular separation is limited to the more volatile ones, such as ammonium acetate and triethylamine. As a consequence of this restriction, selectivity tuning for CZE–MS is not as straightforward as it is for CZE in combination with other detection techniques, where buffer additives mostly do not interfere with detection.

Direct coupling of MEKC to MS with the aim of selectivity enhancement in the separation step is hazardous because of the influence of the micelles in the MEKC buffer on MS performance, resulting in loss of sensitivity and ion

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source contamination [9]. However, the combination of these two techniques is very interesting from the viewpoint of selectivity enhancement for capillary electrophoresis combined with MS for the analysis of compounds difficult to separate with CZE.

In this paper attention is focused on the coupling of MEKC to electrospray MS, with the aim of selectivity enhancement for capillary electrophoretic separation techniques coupled to mass spectrometry. In analogy to the phase system switching approach in liquid chromatography [10], a strategy which enables the separation of the micelles or any other buffer additive from the analytes prior to mass spectrometric detection has been followed. In order to achieve this, a coupled-capillary setup with the possibilities of voltage switching and buffer r enewal has been developed, which allows heart-cutting of the zones of interest in the MEKC capillary with subsequent transfer via a second capillary to the MS. The system is described for two model compounds of the quaternary ammonium type that are difficult to separate by CZE, and will be evaluated regarding the use for other separation techniques that are difficult to couple to mass spectrometry.

2. Experimental

2.1. Experimental setup

A programmable injector for capillary electrophoresis (PrInCE, Lauerlabs, Emmen, Netherlands) was used for pressurized injection and power supply at the inlet of the capillary in which MEKC was performed. The capillary setup consisted of two fused-silica capillaries (SGE, Ringwood, Australia), that were coupled via an open liquid junction. Capillary dimensions were 575 mm × 75 μm I.D. for the first capillary, in which MEKC was carried out, and 325 mm × 75 μm I.D. for the second capillary, where the capillary zone electrophoretic transfer of the analytes to the mass spectrometer was effected. The custom-made liquid junction coupling device was constructed from a 7.5 × 7.5 × 2.5 mm piece of polyethylene with two perpendicular channels with different diameters drilled in it. The narrow bore channel functioned to keep the capillaries perfectly aligned opposite each other, with an opening between them as small as possible to enable liquid flow from the buffer reservoir. To provide free buffer contact at the junction a wide bore channel perpendicular to the first was drilled.

The junction was placed in a custom-made perspex connection buffer vial, which had a built-in electrode with a connection to an external power supply (Spellman CZE 1000R, Plainview, NY, USA). With an outlet buffer exchange unit (Butler, Lauerlabs, Emmen, Netherlands) the buffer in the connection vial could be changed from MEKC buffer to CZE buffer, depending on the stage of analysis. The experimental setup is schematically represented in Fig. 1.

An SSQ 710 single-quadrupole mass spectrometer (Finnigan MAT, San José, CA, USA) equipped with an electrospray interface (Analytica of Branford, USA) was used in the positive ion mode. For electrical contact at the tip of the electrospray needle a sheath liquid was used, delivered at a flow-rate of 1 μl min^{-1} by a Model 2400 syringe pump (Harvard Apparatus, Edinbridge, UK). Nitrogen was used as drying gas. The electrospray tip was held at ground potential, while the electrospray counterelectrode was set at −4.0 kV. The analytes were monitored in the multiple ion detection (MID) mode, using 0.2 s per mass.

2.2. Micellar electrokinetic chromatography

The buffer used to obtain a micellar electrokinetic separation was a mixture of ammonium acetate buffer (10 m*M*, pH 4.5) and methanol (3:2, v/v). As the micelle forming agent, sodium dodecylsulphate (SDS) at a concentration of 25 m*M* was added to the ammonium acetate buffer. For MEKC–UV experiments, a Spectra 100 UV–Vis absorbance detector (Spectra-Physics, Mount View, CA, USA) was used at a wavelength of 210 nm.

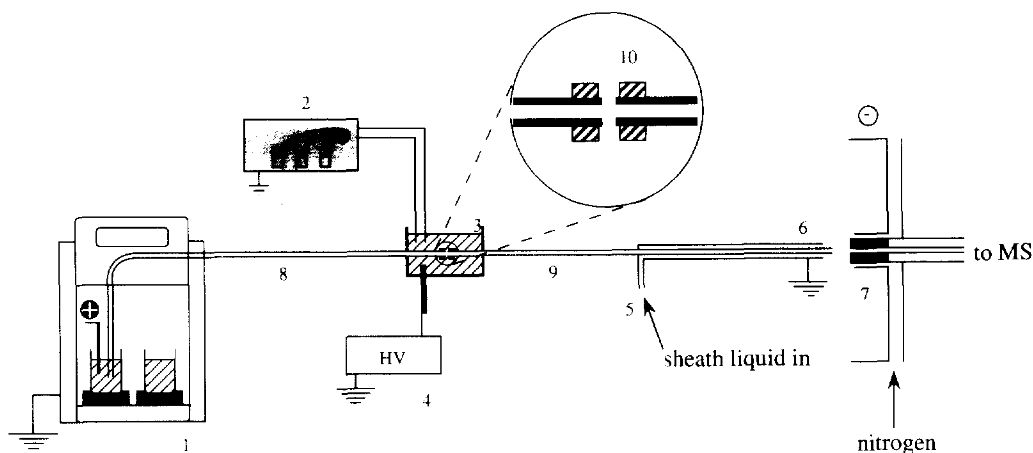

Fig. 1. Schematic representation of the MEKC–MS setup: (1) programmable injector for capillary electrophoresis with internal high-voltage power supply; (2) outlet buffer exchange unit; (3) custom-made perspex connection vial with electrode connection; (4) external high-voltage power supply; (5) inlet for the sheath liquid; (6) grounded electrospray needle; (7) electrospray sampling capillary; (8) MEKC capillary; (9) CZE capillary; (10) capillary coupling device.

2.3. Capillary zone electrophoretic transfer of the analytes to the mass spectrometer

Transfer of the analytes via the second capillary to the mass spectrometer was carried out with a solution of ammonium acetate buffer (50 m*M*, pH 4.5) and methanol (3:2, v/v). The sheath liquid, necessary to maintain electrical contact at the electrospray tip, also consisted of ammonium acetate (100 m*M*, pH 4.5) and methanol (1:4, v/v).

2.4. Chemicals

Mepenzolate and pipenzolate (for chemical structures, see Fig. 2) were obtained from Sigma (St. Louis, MO, USA). SDS was purchased from Serva Feinbiochemica (Heidelberg, Germany), and ammonium acetate was from Merck (Darmstadt, Germany). Methanol and acetic acid were analytical grade, obtained from Biosolve (Barneveld, Netherlands) and Baker (Deventer, Netherlands), respectively.

In all experiments deionized water was used, obtained with a Milli-Q system (Millipore, Bedford, MA, USA). All samples were dissolved in the MEKC buffer.

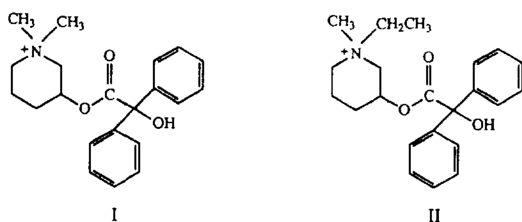

Fig. 2. Chemical structures of the compounds studied. I = mepenzolate, M_r = 340.2; II = pipenzolate, M_r = 354.2.

3. Results and discussion

The direct coupling of MEKC to mass spectrometric detection is hazardous because of the contamination of the ion source due to the presence of an excess of micelle forming agent in the MEKC buffer. In order to overcome the problems associated with introduction of, in this case, SDS into the mass spectrometer, a coupled-capillary setup with the possibilities of voltage switching and buffer renewal has been developed. With this system, a MEKC separation with mass spectrometric detection can be achieved without causing contamination of the ion source, thus ensuring long-term stable operation of the mass spectrometer.

With the coupled-capillary setup described here, MEKC–MS can be divided in three stages.

The first is the actual MEKC separation in the first capillary, followed by a transfer of the zones of interest to the second capillary. Subsequently, the micelles are separated from the analytes by means of CZE.

In Fig. 3, the principle of this approach is represented schematically. Throughout the whole procedure the electrospray tip is held at ground potential and a stable electrospray is established. Before the analysis is started, the first capillary and the connection vial are filled with the MEKC buffer, while the second capillary is filled with the CZE buffer. After pressurized injection of the sample, +15 kV is applied at the inlet of the MEKC capillary, while the connection vial is placed at −10 kV (step I in Fig. 3). The negative voltage applied at the connection vial is necessary to preserve an electroosmotic flow in the second capillary from the electrospray tip towards the connection vial, thus preventing any MEKC buffer constituent from entering the second capillary. At the moment that the first compound of interest reaches the end of the MEKC capillary, which is depicted in step II, the voltages on the MEKC capillary inlet and the connection vial are switched off. With the outlet buffer exchange unit (see Fig. 1) the connection vial is emptied and the MEKC buffer is replaced by CZE buffer. In this way, a continuous buffer system in the connection vial and the second capillary is established. Ideally, the CZE buffer and the electrospray sheath liquid should be of the same composition in order to obtain optimum electrophoretic performance with regard to plate numbers and resolution, but the relatively high water content in the CZE buffer prohibited the establishment of a stable electrospray for a prolonged period of time. For that reason, a choice was made for a sheath liquid with higher organic modifier content and an ammonium acetate buffer of high concentration to make up for differences in conductivity between the CZE buffer and the sheath liquid. After replacement of the buffer in

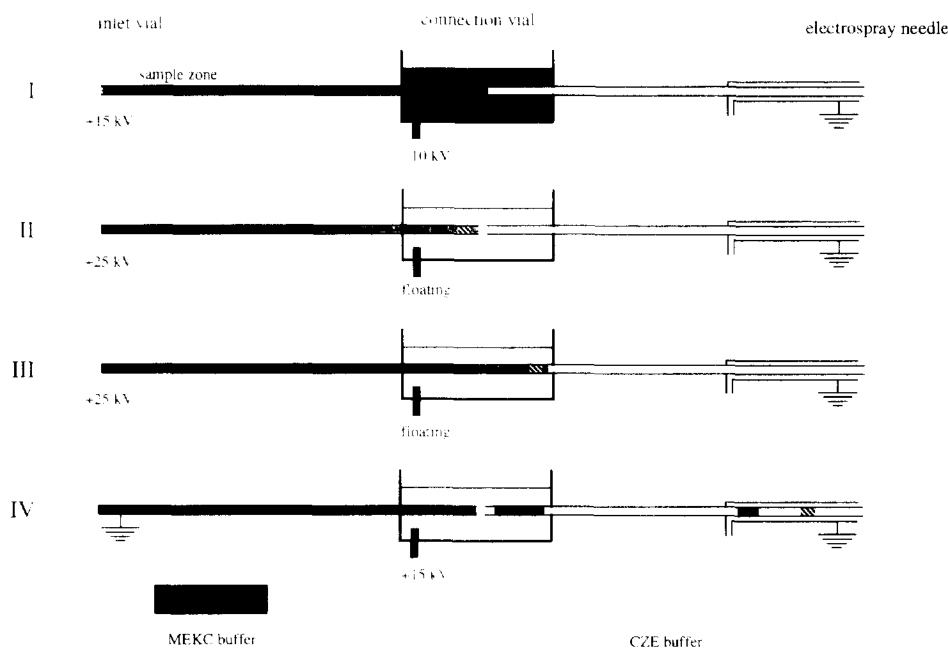

Fig. 3. Schematic representation of the principle of buffer exchange and voltage switching for the coupling of MEKC to MS. For reasons of clarity the capillary coupling device has not been drawn in the picture (see Fig. 1). See text for a detailed explanation of the procedure.

the connection vial, a voltage of +25 kV is applied at the MEKC capillary inlet, while the voltage in the connection vial is not controlled externally. Regarding the passage of the analytes through the liquid junction interface it can be stated that no losses occur. This is based on the effect that the electric field lines are in the direction of the transfer capillary, which has been realized by using the transfer capillary end as the cathodic end. For this reason the center electrode in the connection vial is floating during the passage of analytes through the liquid junction. When the connection vial would not be disconnected physically from the external high-voltage power supply, it would serve as an additional ground, resulting in an electrical split between the connection vial and the second capillary. When the analytes are transferred over the liquid junction into the second capillary, a situation represented in step III in Fig. 3, the voltage at the inlet of the MEKC capillary is switched off. In step IV, a voltage of +15 kV is applied at the connection vial via the external high-voltage power supply, and capillary zone electrophoretic transport of the analytes to the mass spectrometer is effected.

The electrophoretic transfer of the analytes to the mass spectrometer (represented by step IV) can be regarded as conventional CZE of a zone that has been heartcut from the MEKC capillary. The positively charged analytes that have already been separated in the MEKC step migrate further towards the electrospray tip as a result of their own electrophoretic mobility and the electroosmotic flow. The negatively charged SDS micelles have an electrophoretic mobility in the direction of the connection vial, which may be overcome by the larger electroosmotic flow. From our experiments so far it has not become completely clear what happens with the SDS plug, apart from that it does not reach the electrospray tip within reasonable time (<1 h) and does not disturb the electrospray process. We assume that at the boundaries of the heartcut zone in the second capillary the micelles break up due to dilution of the zone in the CZE buffer where no SDS is present. Below the critical micellar concentration of 8 m*M* (in aqueous solution) the micelles are disrupted and the SDS exists as free anions in solution.

In the experiments described here, charged analytes have been used to characterize the system, but obviously MEKC is extremely suitable for the separation of neutral compounds that cannot be separated by CZE. In that case, transfer of the analytes to the MS is based solely on electroosmotic flow, ionization for mass spectrometric detection has to take place in the electrospray process.

The choice of the CZE buffer has proven to be of the utmost importance with regard to the maintenance of the electrophoretic peaks in the CZE capillary. The transfer of a zone from the MEKC capillary to the CZE capillary can be considered as the injection of a sample plug containing several types of ions (sodium, dodecylsulphate, ammonium, acetate, the analytes, and their counterions). When the ionic strength of the CZE buffer is chosen too low, severe fronting of the analyte occurs [11] and difficulties may arise for the assignment of electrophoretic peaks. To overcome this problem, the concentration of ammonium acetate in the CZE buffer was taken significantly higher than that in the MEKC buffer.

An important parameter to influence the separation is the use of an organic modifier to control the extent of interaction between the analytes and the micelles. This has been reported extensively in the literature [12–15], and is especially useful in the coupling with electrospray mass spectrometry, because of the greater spray stability when spraying organic solvents such as methanol.

Another aspect that needs attention is the timing of the events. When MEKC is carried out too long, the analytes will migrate into the connection vial and are lost for mass spectrometric detection. However, when the MEKC time is chosen too short, a large plug of MEKC buffer enters the CZE capillary before the compounds of interest are transferred, which might seriously hamper the electrophoretic transport of the analytes, and even result in introduction of SDS into the MS. From this viewpoint it is also clear that only a small separation window from the

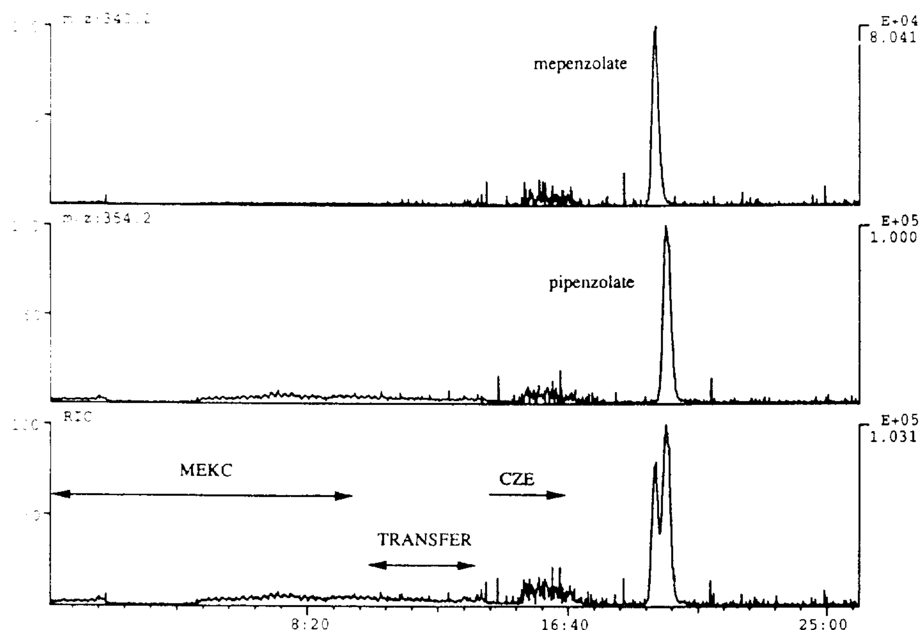

Fig. 4. Mass electropherogram obtained after 9 min MEKC, 3 min transfer and subsequent CZE transport of a mixture of mepenzolate (m/z 340.2) and pipenzolate (m/z 354.2) in a concentration of 8 μg/ml of each compound. The analytes were dissolved in the MEKC buffer, and the volume injected was approximately 25 nl, corresponding to an injected amount of 200 pg.

MEKC capillary can be transferred to the CZE capillary. In practice, this means that only transfer of analytes that are just resolved is feasible.

In Fig. 4 the mass electropherogram is shown that was obtained after 9 min MEKC and 3 min transfer of the zone of interest to the CZE capillary, carried out according to the procedure described above. The injected sample consisted of mepenzolate and pipenzolate in a concentration of 8 μg/ml each, and was prepared in MEKC buffer. The injected volume was approximately 25 nl, corresponding to an injected amount of 200 pg of each compound. The noise at $t = 16$ min is probably due to sodium that is logically present in the sample plug as a counter-ion for the dodecylsulphate ions. From the reconstructed ion current it can be seen that the two compounds are not completely resolved. That this is a problem that arises from the procedure of transferring the analytes to the second capillary can be concluded from the electropherogram obtained with MEKC–UV experiments (Fig. 5) for which an identical buffer composition was used. This electropherogram shows baseline separation of mepenzolate and pipenzolate.

It should be noted that the efficiencies obtained with the coupled-capillary system are

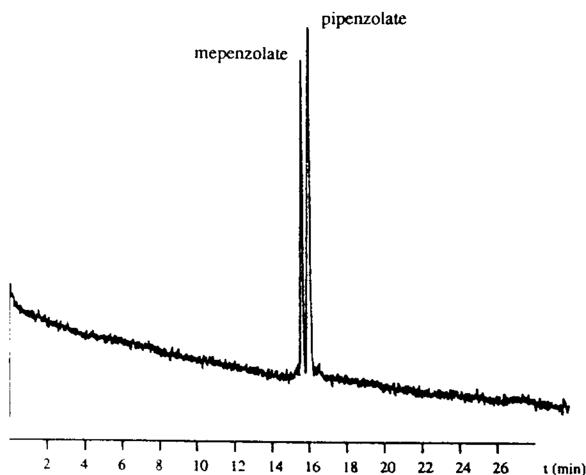

Fig. 5. MEKC–UV electropherogram showing baseline resolution of mepenzolate and pipenzolate. The injected amount was 0.5 ng per compound. Experimental conditions: capillary, 750 mm × 75 μm I.D. fused-silica (600 mm to detector); voltage, +30 kV; UV detection at 210 nm.

lower than those obtained with the single-capillary MEKC–UV setup. One reason is that the applied voltage in the former case was 25 kV, while in the latter setup 30 kV was used. Since the efficiencies are directly proportional to the applied voltage, it may be expected that the resolution will differ. Taking into account the different applied voltages, a loss in efficiency of about 20% should be observed. In fact, the efficiencies differ about 50%. This can be explained by additional band broadening in the coupled-capillary setup caused by the application of the liquid junction, and partly by the diffusion of the zones in the rather long time needed to empty the connection vial from MEKC buffer and refill it with CZE buffer. Optimization of both these aspects is currently under development.

4. Conclusions

The coupled-capillary approach described here enables the micellar electrokinetic separation coupled to mass spectrometric detection of charged analytes that are difficult to separate by capillary zone electrophoresis. During the experiments, no detrimental effects of the presence of SDS on MS performance has been observed, ensuring long-term stable operation conditions. The application of this system to the separation of neutral compounds is currently under investigation.

Analogous to the phase system switching developed in our laboratory [10], the setup used throughout these experiments opens up possibilities for other capillary electrophoretic separation systems which cannot be directly coupled to the mass spectrometer due to the presence of certain buffer additives that are necessary to obtain the required separation. In future research, special attention will be paid to separation systems utilizing chiral separators such as cyclodextrins. Obviously, the coupling of chiral separation systems to mass spectrometry is of great interest in pharmaceutical analysis, because of the necessity of unequivocal mass determination of analytes paired to the inability of the mass spectrometer of discrimination between two enantiomers which are not electrophoretically resolved.

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