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Use of a partial filling technique and reverse migrating micelles in the study of *N*-methylcarbamate pesticides by micellar electrokinetic chromatography–electrospray ionization mass spectrometry

Manuel Molina[a,b], Susanne K. Wiedmer[a,*], Matti Jussila[a], Manuel Silva[b], Marja-Liisa Riekkola[a]

[a]*Laboratory of Analytical Chemistry, Department of Chemistry, POB 55, FIN-00014 University of Helsinki, Helsinki, Finland*
[b]*Analytical Chemistry Division, Department of Analytical Chemistry and Ecology, University of Córdoba, E-14004 Córdoba, Spain*

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Abstract

This study describes three ways to couple micellar electrokinetic chromatography (MEKC) on-line with electrospray ionization mass spectrometry (ESI-MS) for the analysis of *N*-methylcarbamate pesticides. The methods involved the use of a partial filling (PF) technique under basic conditions and the use of reverse migrating micelles (RMMs) under acidic and basic conditions. The use of RMMs in basic electrolyte solutions required coated capillaries with low electroosmotic flows, and capillaries coated with anionic poly(sodium 2-acrylamide-2-methylpropanesulfonate) were selected for the purpose. Before the on-line MEKC–ESI-MS coupling, the MEKC and MS conditions were separately optimized under off-line conditions. The methods were compared in terms of detection limits and the stability of the electrospray process. The PF method offered good separation but poorer stability of the electrospray relative to the other methods. A more stable electrospray performance was obtained with use of RMMs in acidic electrolyte solutions, but some of the analytes were protonated and could not be detected due to the increase in their retention factors. However, with the use of anionic polymer-coated capillaries and RMMs at pH 8.5, all analytes were successfully separated. The high-salt stacking method was applied to improve the sensitivity of MEKC–ESI-MS and the detection limits were in the range of 0.04–2.0 μg/ml.

Keywords: Partial filling micellar electrokinetic chromatography; Coated capillaries; Micellar electrokinetic chromatography; Stacking; Reverse migrating micelles; Methylcarbamates; Pesticides

1. Introduction

N-Methylcarbamates (NMCs), which are well-known pesticides, are extensively used as insecticides, acaricides, nematocides, and molluscicides for the protection of a wide variety of crops. Although they are readily degraded by atmospheric and biological agents, there is a growing interest in the study of their environmental implications because some of them show marked toxicity. International

*Corresponding author. Tel.: +358-9-1915-0264; fax: +358-9-1915-0253.
E-mail address: susanne.wiedmer@helsinki.fi (S.K. Wiedmer).

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organizations regulate the levels of NMC residues observed in drinking water and have set limits of 0.1 μg/l for individual pesticides and 0.5 μg/l as a total. The corresponding values for crops are in the range 0.02–5 μg/g. To allow the analysis of NMCs in water, foodstuff, and environmental matrixes, a number of research groups have focused on the development of powerful analytical tools.

Chromatographic techniques have been widely applied to the determination of NMCs in a variety of matrixes. Gas chromatography (GC) has provided good results [1,2], but problems sometimes arise because of the thermolability of the analytes. Lately, high-performance liquid chromatography (HPLC), which is both simple and compatible, has become an accepted method for the separation and determination of NMC pesticides [3–7]. However, some pairs of NMCs (e.g. carbofuran and propoxur) are not easily separated by this technique [6]. Among the various detection systems available, mass spectrometry (MS) is currently the most interesting option because of its identification power and the fact that problems initially imposed by HPLC–MS interfaces have virtually been overcome [3,4,8–10].

For the determination of NMCs, micellar electrokinetic chromatography (MEKC) [11] is a promising alternative to HPLC in terms of flexibility in selectivity adjustment and efficiency [12–16]. One of the major advantages of MEKC compared to HPLC is the inherently higher column efficiency. Although many efforts have been made to increase the sensitivity of MEKC determinations of NMCs [13–16], the use of MS detection has not yet been reported. Extensive work has been done on coupling CE to electrospray ionization (ESI) MS, but only a few papers have been published on MEKC–ESI-MS. The lesser interest is mainly due to the presence of surfactants, which may cause problems in the performance of the ESI-MS, such as instability of the electrospray, contamination of the MS detector, and loss in sensitivity. Several approaches have been developed to overcome the negative effects of nonvolatile surfactants on the electrospray process [17–24]. Most of them involve preventing the micelles from entering the mass spectrometer. Examples are the use of reverse migrating micelles (RMMs) [18], the partial filling (PF) technique [19–22], and the heart-cut technique [17]. With RMMs (or anodically migrating micelles), passage of the surfactant to the MS is prevented by adjusting the electroosmotic flow lower than the mobility of the micelles (in order to have reverse migrating micelles). With sodium dodecylsulfate (SDS), for example, this means keeping the pH of the electrolyte solution lower than ~5. The PF technique, in which only a part of the capillary is filled with a solution containing micelles or other additives, has been fairly widely applied. The technique is simple to use and has proven to be highly useful for MEKC–ESI-MS analyses, mainly because it offers long-term operational stability as the nonvolatile additives cannot interfere with the electrospray process. The major drawback of the heart-cut technique is that it requires a somewhat modified coupled-capillary set-up to enable voltage switching and buffer renewal. High-molecular-mass surfactants [23,24] have also been employed as pseudostationary phases for MEKC–ESI-MS. However, this alternative has not been widely applied, mainly because a decrease in the MS sensitivity and of the electrospray stability may occur when the polymeric surfactants enter the MS. Considering the advantages and drawbacks of the approaches mentioned above, the PF technique and RMMs are the most attractive options because they can be applied to MEKC–ESI-MS analyses without instrumental modification.

Coating of capillaries has been useful in CE both to prevent molecules from adsorbing onto the inner capillary wall during analysis and to control the electroosmotic flow (EOF). In on-line CE–ESI-MS studies, the presence of nonvolatile additives in electrolyte solutions, which usually is the case with dynamic coatings, is unacceptable and statically coated capillaries are therefore preferred. The use of coated capillaries and RMMs together allows the use of any charged carrier over a wide pH range [25].

We investigated three ways of coupling MEKC on-line with ESI-MS for the determination of NMC pesticides. These were the PF technique and the use of RMMs under acidic and basic conditions. The PF and RMM approaches were first investigated and compared with use of uncoated fused-silica capillaries. To achieve performance with RMMs under basic conditions, capillaries were coated with anionic poly(sodium 2-acrylamide-2-methylpropanesulfonate) [26,27], and the EOF of the capillary was determined

over a wide pH range (2.0–9.0). The use of RMMs in controlled electroosmotic flow capillaries is shown to be an effective MEKC–ESI-MS method for the analysis of NMC pesticides. In addition, the usefulness of the high-salt stacking method [28,29] to increase the sensitivity of the methods is verified.

2. Materials and methods

2.1. Chemicals

SDS of electrophoresis grade was purchased from Bio-Rad (Hercules, CA, USA). NMCs were obtained from Riedel-de Haën (Seelze, Germany) and methanol and acetone (HPLC grade) from Mallinckrodt Baker (Deventer, The Netherlands). Timepidium bromide (JP grade), used as a micelle marker, was a gift from Dr. Hiroyuki Nishi (Analytical Research Laboratory, Tanabe Seiyaku, Osaka, Japan). Acrylamide (99%, Merck, Darmstadt, Germany), acrylamido-2-methylpropanesulfonic acid (AMPS, 99%, Merck), 3-(trimethoxysilyl)propyl methacrylate (Bind-Silane, 98%, Polysciences, Washington, PA, USA), *N,N,N′,N′*-tetramethylethylenediamine (TEMED, 99.5%, Sigma, Dorset, UK) and ammonium peroxodisulfate (APS, 98%, Merck) were used to coat the capillaries. All chemicals were used as received.

Stock solutions of the pesticides were prepared in methanol at a concentration of 1 mg/ml and stored at 4°C in the dark. Standard solutions containing 0.01–10 μg/ml were prepared by diluting an appropriate volume of the standard solution with 50 m*M* ammonium acetate or water. The required volume of 1 g/l solution of timepidium bromide in water was added to the sample up to a final concentration of 20 mg/l to determine the mobility of the micelles. Methanol was used as electroosmotic flow marker.

2.2. Apparatus

MEKC separations were carried out using uncoated fused-silica capillaries from Composite Metal Services (Worcestershire, UK) with dimensions of 50 μm I.D.×360 μm O.D. The length of the capillary to the detector was 50 cm (58.5 cm total length) in the preliminary MEKC–UV experiments, and 88-cm long capillaries (20 cm to UV detector) were used in the MEKC–ESI-MS analyses. HP ^{3D}CE equipment (Agilent, Waldbronn, Germany) was used for all the analyses. The UV absorbance wavelength on the diode-array detection system provided with the CE instrument was set at 202 nm.

The mass spectrometer was a Bruker Esquire (Bruker-Daltonics, Bremen, Germany) instrument equipped with an electrospray ionization source (Analytica of Branford, Branford, CT, USA) operating in the positive ion mode. The spectrometer comprised a hexapole and an ion trap mass analyzer. Nitrogen was used as drying gas at a temperature of 200°C and a flow-rate of 150 l/h. Nitrogen was also supplied as sheath (nebulizing) gas at a pressure of 1 bar. The sheath liquid (water–methanol, 1:1, v/v) was infused at 150 μl/h. The voltages set for the capillary, the end plate, and the cylinder were −3.9, −3.3 and −2.5 kV, respectively.

Conductivity measurements were made with a CDM3 conductivity meter and cell (Radiometer, Copenhagen, Denmark). A Jenway pH meter and electrode (Jenway, Felsted, UK) were used to adjust the pH of the electrolyte solutions. All micellar and electrolyte solutions were filtered through 0.45-μm PTFE filters (Gelman Sciences, Ann Arbor, MI, USA) and degassed by ultrasonication before use. Distilled water was further purified with a Water-I system from Gelman Sciences.

2.3. Capillary coating procedure

The two-step procedure for coating the capillaries with a stable covalent polymer layer was slightly different from that proposed by Hjertén [30] and modified by Landman and coworkers [26,27]. To coat the capillaries, new bare fused-silica capillaries were successively rinsed with acetone, 5 *M* hydrochloric acid, and 5 *M* sodium hydroxide (2 min, 95 kPa), and then with water (5 min). Residual water was removed by flushing the capillaries for 20 min with a gentle stream of nitrogen. In the first step, the capillary was filled with Bind-Silane solution, capped, and allowed to stand overnight at room temperature. Small pieces were cut off from the ends of the capillaries and unbound silane was removed by flushing with acetone. The capillary was dried with nitrogen for 20 min before the polymerization

reaction. In the second step, the capillary was filled with the monomer solution, capped, and allowed to react overnight. The monomer solution was prepared as follows: 25 mg of AMPS were dissolved in 500 μl of 0.35 *M* NaH_2PO_4–Na_2HPO_4 buffer (pH 8.5) and degassed under vacuum; a 150-μl volume of this solution was mixed with 6 μl of APS solution (5%, w/v, in water) and then 6 μl of TEMED (5%, v/v, in water) was added to the mixture. To achieve good reproducibility of the polymerization reaction, solutions were always freshly prepared. Before each run the excess polymer was removed with a stream of nitrogen, and the coated capillaries were conditioned by flushing for 5 min with water and 2 min with running buffer. The capillaries were stored in water.

2.4. Safety considerations

The reagents employed to coat the capillary are hazardous and all contact with skin, eyes, and mucous tissue must be avoided. Wearing of protective gloves and eye protection are recommended. Some of the NMCs are highly toxic and care must be taken in handling them. If necessary, they can be decomposed under strongly alkaline conditions.

3. Results and discussion

The usefulness of three different approaches for the on-line MEKC–ESI-MS analysis of NMC pesticides was evaluated. Initially, off-line MEKC studies were carried out to optimize the PF conditions, and off-line MS studies were done to optimize the composition of the sheath liquid and other MS parameters. The high-salt sample stacking procedure was tested in the on-line PF-MEKC–ESI-MS studies. RMMs were investigated under both acidic and basic conditions. Coated capillaries that provided low electroosmotic flows were used in the RMM studies under basic conditions. As in PF-MEKC–ESI-MS, the high-salt stacking method was tested in the on-line RMM-MEKC–ESI-MS studies.

3.1. MEKC–UV studies

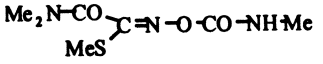
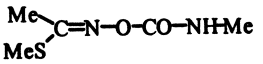
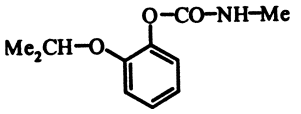
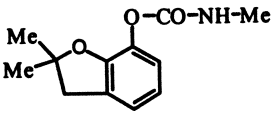
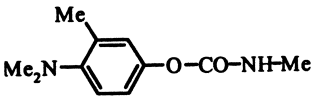
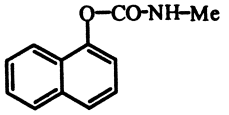
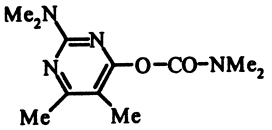
The structures and common names of the analytes are shown in Table 1. All the pesticides except for aminocarb and pirimicarb (pK_a 5.6 and 4.4, respectively) are neutral at pH values from 3.0 to 10.0. First, the off-line MEKC–UV conditions were selected, keeping in mind the MEKC–ESI-MS studies to follow. SDS was chosen as the micelle-forming compound, because of its availability and popularity. Although some papers have demonstrated the separation of NMCs by MEKC using borate and phosphate buffers [14–16], these are nonvolatile buffers and cannot be employed in the coupling of CE to ESI-MS. Hence, more volatile ammonium acetate was selected as the buffer. The concentrations of ammonium acetate (5–50 m*M*) and SDS (10–60 m*M*) in the running buffer were tuned to achieve maximum efficiency in the shortest possible time. With a running buffer containing 10 m*M* ammonium acetate and 40 m*M* SDS, adjusted to pH 9.0 with 0.1 *M* ammonia, successful separation of the pesticides was achieved within 10 min using a voltage of 30 kV at 25°C (Fig. 1A). The sample solution was injected for 10 s at 5 kPa. The retention factors of the NMCs, calculated from their migration times and those of the micelles and the electroosmotic flow marker, ranged from 0.3 to 9.1. The wide range of hydrophobicities made the NMCs an interesting challenge for MEKC–ESI-MS studies.

3.2. Off-line MS studies

Preliminary MS experiments were required to obtain a good performance of the ESI interface used for coupling of MEKC to ESI-MS. Thus, remembering that the compounds would be ionized by positive electrospray ionization, methanol, isopropanol, and their mixtures with water were tested as sheath liquid. Addition to the sheath liquid of ammonium acetate up to a concentration of 5 m*M* and acetic acid up to 1% (v/v) did not offer any improvement in the signal intensities. Methanol–water (50:50, v/v) was eventually selected as sheath liquid because it provided the best sensitivity.

The position of the capillary tip with respect to the grounded coaxial steel needle was of crucial importance for the sensitivity of the ESI-MS. Optimum signals were obtained when the capillary tip was extended about 0.5 mm beyond the grounded needle. Typical fragments of the NMC pesticides (determined by off-line MS studies at concentrations of 5

Table 1
NMC pesticides with corresponding structures, molecular masses and quantification masses[a]

NMC	Structure	M_r	Quantification masses
Oxamyl		219	72 ($[(CH_3)_2NCO]^+$), 242* ($[M+Na]^+$)
Methomyl		162	88 ($[M-(CH_3NHCOOH)+H]^+$), 106 ($[M-(CH_3NCO)+H]^+$), 128* ($[M-(CH_3NCO)+Na]^+$), 185 ($[M+Na]^+$)
Aldicarb		190	89 ($[M-(CH_3NHCOONCH_2)+H]^+$), 116 ($[M-(CH_3NHCOOH)+H]^+$), 213* ($[M+Na]^+$)
Propoxur		209	93 ($[M-((CH_3)_2CHOH)-(CH_3NCO)+H]^+$), 111* ($[o\text{-}(HO)_2(C_6H_4)+H]^+$), 168 ($[M-((CH_3CH_2CH)+H]^+$), 232 ($[M+Na]^+$)
Carbofuran		221	123 ($[M-(CH_3CHCH_2)-(CH_3NCO)+H]^+$), 165 ($[M-(CH_3NCO)+H]^+$), 222 ($[M+H]^+$), 244* ($[M+Na]^+$)
Aminocarb		208	152* ($[M-(CH_3NCO)+H]^+$), 209 ($[M+H]^+$), 231 ($[M+Na]^+$)
Carbaryl		201	145* ($[M-(CH_3NCO)+H]^+$), 224 ($[M+Na]^+$)
Pirimicarb		238	182 ($[M-(CH_3NCO)+H]^+$), 239 ($[M+H]^+$), 261* ($[M+Na]^+$)

[a] Base peak is marked with an asterisk.

μg/ml) under positive ESI-MS conditions are listed in Table 1. Fig. 2 shows the individual mass spectra of all pesticides investigated. The analytical signal for each pesticide was the sum of the counts of its characteristic fragments.

3.3. Partial filling technique

In the PF technique, a (short) plug of a micelle (or other additive) solution is introduced to a capillary which has been filled with a background electrolyte solution free of surfactant (or additive). Introduction of the sample solution follows. Once the neutral analytes have migrated through the micellar pseudo-stationary phase and separated from each other according to their partition coefficients, they reach the surfactant-free background electrolyte solution, where they migrate with the EOF to the ESI-MS system. Preliminary experiments aimed at optimizing the PF-MEKC separation conditions were conducted using UV detection. As a starting point, a micelle solution of the same composition as the running buffer described above, i.e. 10 m*M* ammonium acetate and 40 m*M* SDS at pH 9.0 (1.62 mS cm^{-1}) and a background electrolyte solution containing ammonium acetate (pH 9.0) were used. A 20 m*M* ammonium acetate background electrolyte solution, which provided a similar conductivity (1.54 mS

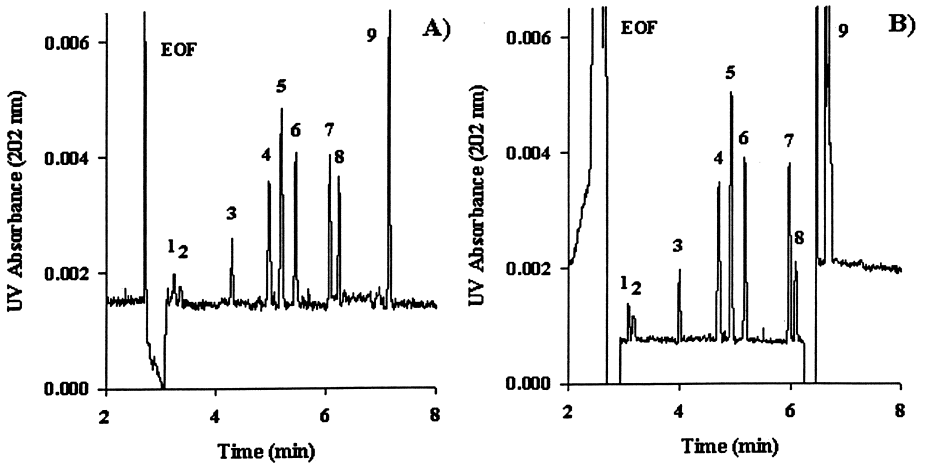


Fig. 1. Separation of NMCs by conventional MEKC (A) and PF-MEKC (B). Length of capillary (uncoated fused-silica, 50 μm I.D.×360 μm O.D.) to the UV detector 50 cm (58.5 cm total length); applied voltage 30 kV; temperature 25°C; detection wavelength 202 nm. Peaks: 1=oxamyl, 2=methomyl, 3=aldicarb, 4=propoxur, 5=carbofuran, 6=aminocarb, 7=carbaryl and 8=pirimicarb. The concentration of each analyte was 10 μg/ml and hydrodynamic sample injection was carried out for 10 s at 5 kPa. Methanol and timepidium bromide were used as EOF and micelle markers (9), respectively. Running buffer (A): 10 m*M* ammonium acetate, 40 m*M* SDS, pH 9.0. Running buffer (B): background electrolyte 20 m*M* ammonium acetate, pH 9.0; micellar solution (injection for 150 s at 30 kV) running buffer (A). Further explanations can be found in the text.

cm^{-1}) as the micelle solution, was employed to avoid loss of resolution due to conductivity differences at the front of the micelle zone. The length of the micelle plug and the procedure for its injection into the capillary were investigated to obtain maximum efficiency. It was essential that the injected plug should be short enough that it would not enter the MS.

First, hydrodynamic injection at 5 kPa was studied using injection times of 100–500 s. At this pressure the average velocity of the micelle plug was 0.088 cm s^{-1}. The efficiency of the separation increased up to an injection time of 300 s (when about 50% of the capillary was filled with micellar solution); above this the performance was not significantly improved. Electrokinetic injection was then evaluated, as a means of decreasing the injection time, and hence total analysis time, and improving the separation. Initially, an injection voltage of 15 kV was used. The results resembled those obtained with hydrodynamic injection at 5 kPa (300 s) and again the optimum injection time was about 300 s. Under these conditions, the micelle front, assuming a net micelle mobility of $\sim 3 \cdot 10^{-8}$ m^2 V^{-1} s^{-1}, was 24 cm from the inlet tip of the capillary, which was close to the micelle plug position under optimum hydrodynamic injection conditions. The injection voltage was increased to 30 kV to decrease the injection time as much as possible (150 s), keeping the amount of micelles injected constant. These conditions were selected as optimal as they provided the best performance with minimal injection time.

A typical separation obtained under selected PF conditions (micelle plug injected electrokinetically for 150 s at 30 kV) is shown in Fig. 1B. The separation pattern resembles that of conventional MEKC, showing that the micelle plug was long enough for successful separation of all the pesticides. The drops in the baseline indicate the point at which the micelle plug passed the UV detector. Namely, in this case, using a 58.5-cm (effective length 50 cm) capillary, ≈60% of the capillary was initially filled with the micelle solution, so that the micelle plug passed the detector window during the separation.

3.4. On-line PF-MEKC–ESI-MS studies

Next, a longer capillary (88 cm) was taken into use, and PF-MEKC–ESI-MS studies were carried out under the earlier used PF-MEKC conditions

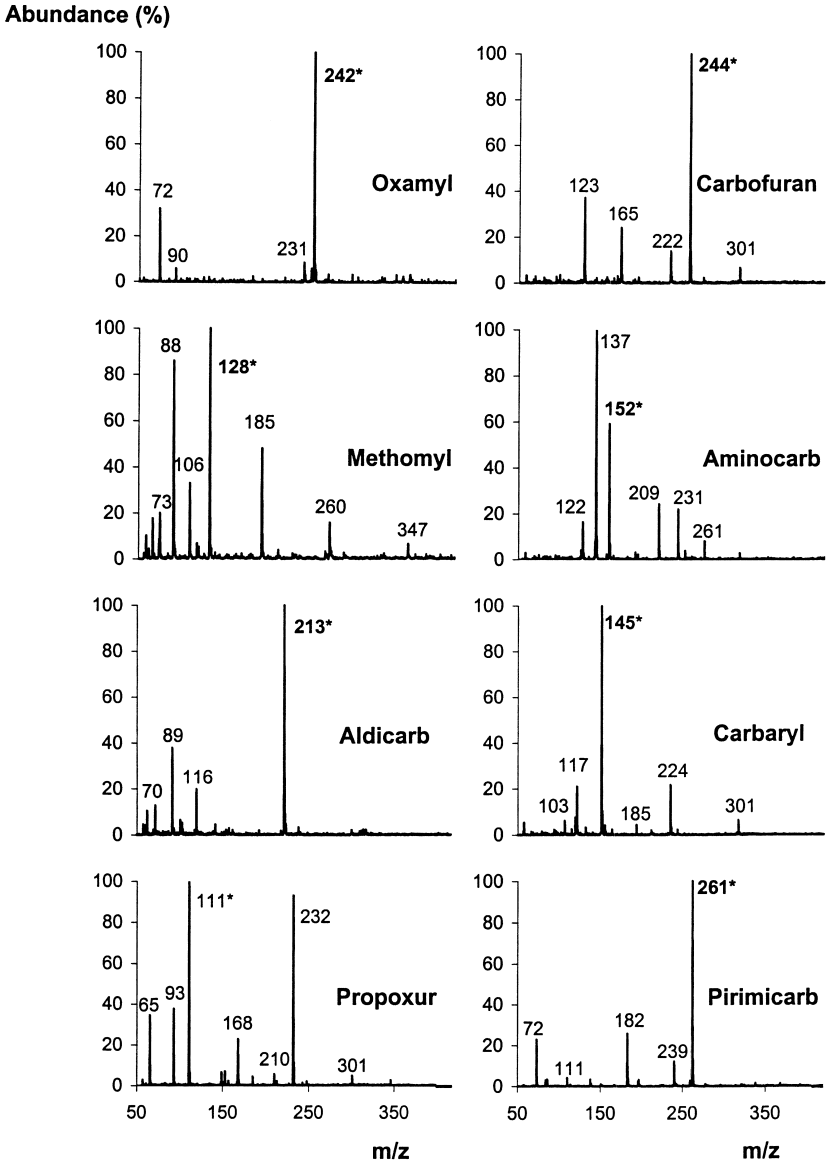


Fig. 2. Individual mass spectra of investigated pesticides.

(with a 58-cm capillary). The separation under PF-MEKC–ESI-MS conditions is presented in Fig. 3A. The resolution was somewhat reduced, relative to the off-line PF-MEKC separation using only UV detection (compare peaks 1 and 2 in Figs. 1B and 3A). This is caused by the dispersion that may occur at the electrospray interface and the lower separation efficiency of the analytes in a shorter micelle zone. The lower separation efficiency was due to the lower electric field strength (and, hence, lower electroosmotic flow) employed during the injection of the micelle plug (the applied micelle injection voltage was the same as with the shorter PF-MEKC–UV capillary). As expected, the separation window was widened, not only because of the longer distance to the detector but also because of the lower electric field strength. When the length of the capillary was increased up to 88 cm, the micelle plug filled only

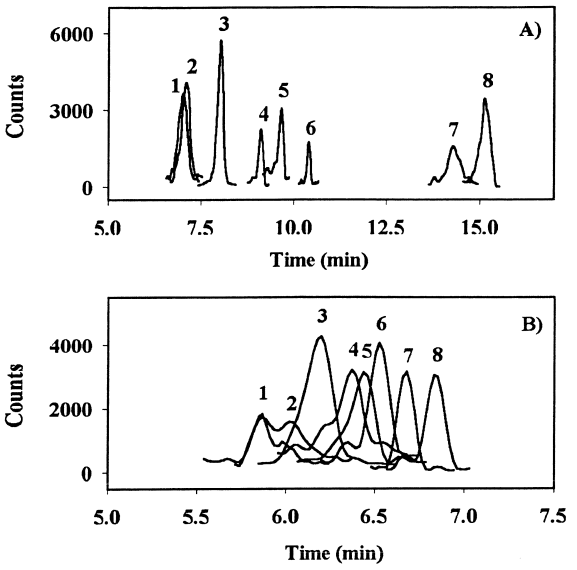


Fig. 3. Reconstructed ion electropherogram of NMCs by PF-MEKC–ESI-MS with 40 m*M* (A) and 20 m*M* SDS (B). The total length of the capillary was 88 cm and the separation voltage was 25 kV. The background electrolyte solution consisted of 20 m*M* ammonium acetate, pH 9.0. The micelle plug was injected for 150 s at 30 kV. (A) Micelle solution 10 m*M* ammonium acetate, 40 m*M* SDS, pH 9.0; the sample (30 μg/ml of each analyte in water) was hydrodynamically injected for 10 s at 5 kPa (non-stacking conditions). (B) Micelle solution 10 m*M* ammonium acetate, 20 m*M* SDS, pH 9.0; the sample (the concentration of compounds 1–3 was 5 μg/ml, and that of compounds 4–8 was 1 μg/ml) was prepared in 50 m*M* ammonium acetate and injected under stacking conditions for 60 s at 5 kPa. The counts correspond to the sum of the quantification masses depicted in Table 1. Peak numbering and other conditions as in Fig. 1.

about 17% of the capillary, allowing for the determination of all the analytes before the micelles reached the cathode and, accordingly, the MS.

Some problems associated with high micelle concentrations in PF-MEKC–ESI-MS have been described in the literature [18,22,23] and a lower SDS concentration may be advantageous. In fact, after several runs we observed a decrease in the performance of the electrospray. One possible explanation for this is that although the micelles, theoretically, never reach the ESI interface during the run, the monomer of the surfactant may reach the MS system [21]. This may affect subsequent experiments due to the contamination of the MS system with non-volatile ions. Moreover, the presence of a high concentration of sodium ions (originating from SDS) could also affect the electrospray, partially suppress the ionization, and reduce the sensitivity. When the concentration of SDS in the micelle plug was decreased to 20 m*M*, good repeatability of MS was achieved, but the resolution dramatically decreased (Fig. 3B). In the end since stable operation of ESI-MS could not be obtained in combination with good efficiency, the separation afforded by the PF-MEKC approach did not meet our expectations. Our results show that in using the PF technique one has to compromise between good resolution and reliable operation of the ESI-MS, whenever there is a danger of contamination of the ESI-MS system with non-volatile species.

In view of the relatively low sensitivity provided by the PF-MEKC technique, we next investigated the use of a recently introduced high-salt stacking approach [28,29] as an alternative way to enhance the sensitivity. This method allows the on-line preconcentration of neutral analytes in MEKC using a high conductivity sample matrix. Owing to the higher conductivity of the sample zone (locally decreased electric field strength), the negatively charged micelles are stacked in front of the sample plug. This, in turn, causes the stacking of analytes while they migrate with the EOF into the micelle zone. Because there is an optimum in the ratio of sample conductivity to running buffer conductivity, changes in the composition of the running buffer and pH may affect the efficiency of the stacking process for some analytes (due to changes in the sample conductivity/buffer conductivity ratio). If the conductivity of the running buffer is modified without simultaneous optimization of the sample conductivity, slight differences in the stacking may occur, depending on the retention factors of the analytes. In previous studies on pesticides [31] the optimum conductivity ratio was found to be 2.5.

Because of its volatile nature, we chose ammonium acetate to increase the sample conductivity. The optimum concentration was 50 m*M* when hydrodynamic injection was applied for 60 s (5 kPa). With the use of a micellar solution consisting of 10 m*M* ammonium acetate and 20 m*M* SDS, pH 9, the conductivity ratio between the micellar solution and the sample solution was 2.7, which is fairly close to the earlier found optimum value. Since the stacking

effect is due to the conductivity ratio between the micellar and sample solutions, the possible effect of the background electrolyte solution (20 m*M* ammonium acetate) under the PF conditions was not taken into account. This approach afforded limits of detection for the pesticides below 2 μg/ml (see Table 2) without significant loss of resolution relative to normal injection (i.e. 10 s at 5 kPa). The limits of detection were calculated from the peak heights using a S/N ratio of three. This stacking injection procedure was used in further studies unless otherwise stated.

3.5. Reverse migrating micelles with acidic running buffer

The use of RMMs to avoid contamination of the ESI-MS system with surfactants is based on the decrease of the electroosmotic flow when either acidic running buffers or coated capillaries are used, so that the EOF is slightly lower than the mobility of the micelles. Thus, the mobility of the additives (e.g. micelles) is reversed with respect to conventional MEKC separations and the additives migrate away from the cathode and the MS system [18].

Table 2
Limits of detection for the NMCs with MEKC–ESI-MS using the high-salt stacking technique[a]

Compound	Limits of detection (μg/ml)		
	PF[b]	RMM[c]	RMM and coated capillaries[d]
Oxamyl	0.9	2.0	1.6
Methomyl	1.8	1.1	1.4
Aldicarb	0.4	0.4	0.5
Propoxur	0.2	0.1	0.2
Carbofuran	0.1	0.07	0.1
Aminocarb	0.1	ND	0.06
Carbaryl	0.08	0.04	0.1
Pirimicarb	0.1	ND	0.1

ND, not detected.

[a] Stacking conditions: sample prepared in 50 m*M* ammonium acetate, hydrodynamic injection for 60 s (5 kPa).

[b] PF conditions: micellar solution 10 m*M* ammonium acetate, 20 m*M* SDS, pH 9.0; running electrolyte solution 20 m*M* ammonium acetate, pH 9.0.

[c] Running buffer 10 m*M* ammonium acetate, 10 m*M* SDS, pH 5.0.

[d] Running buffer 10 m*M* ammonium acetate, 10 m*M* SDS, pH 8.5.

When the PF technique described above did not offer fully satisfactory results for the analysis of NMCs by MEKC–MS, we investigated the possibility of using RMMs. Initial experiments were carried out to determine the mobility of the SDS micelles at running conditions (10 m*M* ammonium acetate and 40 m*M* SDS at pH 5–10), using timepidium bromide as micelle marker. A mobility of $-4.56\cdot10^{-8}$ $m^2\ V^{-1}\ s^{-1}$ was obtained, which remained constant between pH 5 and 10. Earlier studies have shown the SDS mobility ($-4.6\cdot10^{-8}$ $cm^2\ V^{-1}\ s^{-1}$) to be constant in the pH range 5.5–9 [18,32]. The micelle mobility could not be directly measured at pH 5.0 because the migration time of the micelle marker was too long. Instead the micelle mobility was calculated from the migration times of the first analyte and the EOF marker and the retention factor of the first analyte, which was assumed to remain constant between pH 5.0 and 6.0. In other words, the pH of the running buffer was optimized to obtain an EOF slightly lower than the mobility of the micelle and a running buffer of pH 5.0, providing an electroosmotic flow mobility of $4.4\cdot10^{-8}$ $m^2\ V^{-1}\ s^{-1}$, was eventually selected.

Under these conditions, the migration times of the analytes strongly depend on their retention factors, and so on the surfactant concentration. On the other hand, the surfactant concentration may also alter the efficiency as well as the migration time window. Very high retention factors will lead to too long migration times and distorted peak profiles. To avoid this, the surfactant concentration was lowered to 10 m*M*, which led to lower retention factors for all the analytes except aminocarb and pirimicarb (peak numbers 6 and 8, respectively, in the figures). Owing to protonation at acidic pH values (pK_a values 5.6 and 4.4, respectively) the retention factors of these two analytes sharply increased. These compounds migrated with the micelles away from the detector and, as shown in Fig. 4A, could not be detected by RMM-MEKC.

3.6. RMM-MEKC–ESI-MS studies

The potential of using acidic electrolyte solutions and reverse migrating micelles on-line with ESI-MS was then investigated. A typical reconstructed ion electropherogram of RMM-MEKC–ESI-MS, using

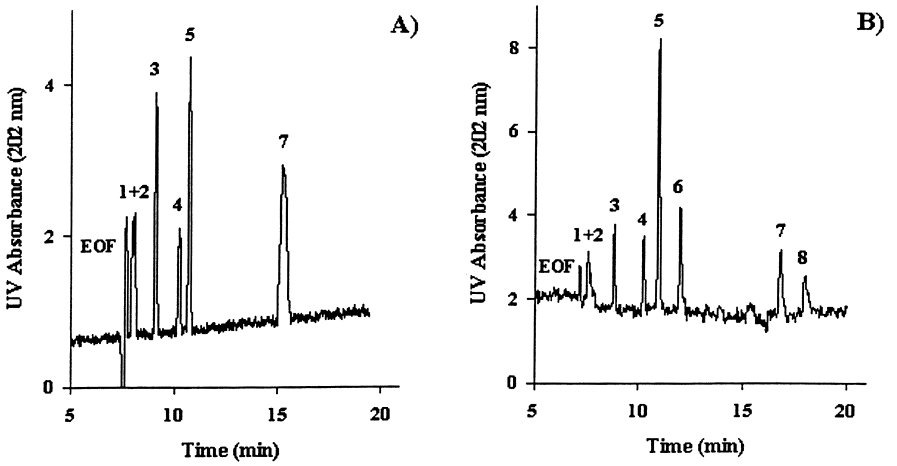


Fig. 4. Separation of NMCs by RMM-MEKC in (A) uncoated fused-silica capillaries and (B) AMPS-coated capillaries. The separation voltage was 20 kV. Running buffer (A) 10 m*M* ammonium acetate, 10 m*M* SDS, pH 5.0; running buffer (B) 10 m*M* ammonium acetate, 10 m*M* SDS, pH 8.5. Other conditions as in Fig. 1.

the optimized running buffer (10 m*M* ammonium acetate, 10 m*M* SDS, pH 5.0) is shown in Fig. 5A. For the sake of clarity the baseline is only shown for one of the compounds. The baseline noise was similar for the rest of the compounds. The separation time was about that in MEKC–UV even though the capillary was longer. This is because of (1) the higher voltage needed to obtain electric field strengths like those in the RMM-MEKC–UV studies and (2) the higher migration velocities of the analytes, after moving out of the micelle zone. The velocities are higher because, after the analytes have passed the micelle zone, they will reach the MS with the velocity of the EOF. The efficiency of the separation was higher than that obtained by PF-MEKC–ESI-MS, and the stability of the electrospray

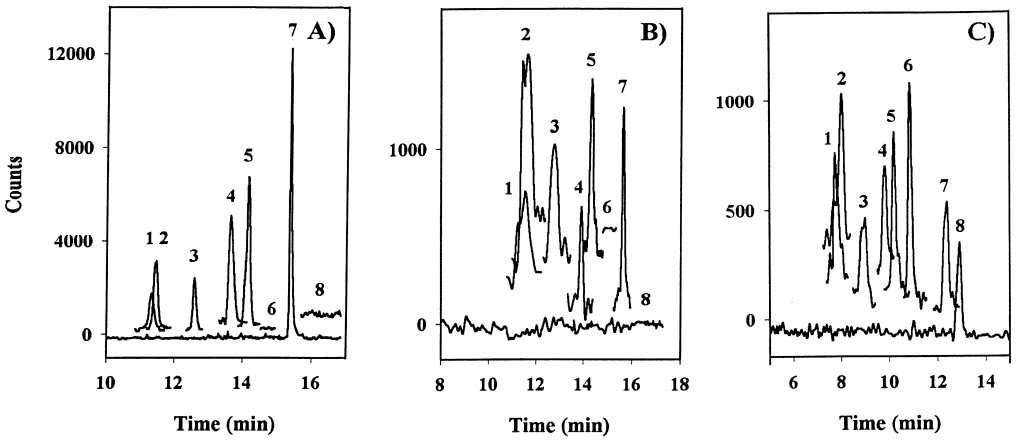


Fig. 5. Reconstructed ion electropherogram of NMCs by RMM-MEKC–ESI-MS. Running buffer 10 m*M* ammonium acetate, 10 m*M* SDS. The buffer pH was 5.0 in (A) and (B), and 8.5 in (C). (A) Uncoated capillary; the sample (30 μg/ml of compounds 1–3 and 10 μg/ml of 4–8 in water) was injected for 10 s at 5 kPa. (B) Uncoated capillary; the sample (5 μg/ml of compounds 1–2, 1 μg/ml of compound 3, and 0.2 μg/ml of compounds 4–8 in 50 m*M* ammonium acetate) was injected for 60 s at 5 kPa. (C) AMPS-coated capillary; the sample (5 μg/ml of compounds 1–3 and 1 μg/ml of compounds 4–8 in 50 m*M* ammonium acetate) was injected for 60 s at 5 kPa. Other conditions as in Fig. 3.

did not decrease as a function of time. The higher resolution was due to the longer micelle plug.

The high-salt stacking procedure was also investigated in the on-line RMM-MEKC–ESI-MS studies. Fig. 5B shows the excellent performance of the stacking technique (see Fig. 5A and B), especially for later migrating analytes possessing higher retention factors. The fact that the stacking process works better for more hydrophobic analytes has been noted in several reports. The limits of detection with use of the high-salt stacking effect are listed in Table 2.

3.7. Reverse migrating micelles in coated capillaries

From the results described above, it is clear that basic electrolyte solutions must be used to separate all eight pesticides. To enable the separation of all the analytes we investigated a modified RMM technique relying on polyacrylamide-based coated capillaries, while aiming at achieving very low electroosmotic flow values over a wide pH range. An electroosmotic flow lower than the SDS mobility was needed to provide the separation of all the compounds by RMMs. Thus, in order to have negatively charged capillaries with a low EOF, capillaries were coated (in situ polymerized) with a mixture of linear polymers of acrylamide–AMPS (30:70, w/w) or with pure AMPS (100%). Pure AMPS solution provided an electroosmotic flow of about $4.45 \cdot 10^{-4}$ $cm^2\ V^{-1}\ s^{-1}$, i.e. an EOF of about 60–70% of that in uncoated fused-silica capillaries, which was in good accordance with previous results [26,27]. The EOF in the AMPS-coated capillary was independent of the buffer pH in the range 2.0–9.0.

For preliminary RMM-MEKC–UV experiments with basic running buffers (10 m*M* ammonium acetate, 10 m*M* SDS, pH 8.5), a 58-cm capillary was polymerized in situ with AMPS. Even though it was shown that pH values as high as 9.0 can be used, such high pH values may reduce the stability of the polymer layer. Hence, the pH of the running buffer was adjusted to 8.5, where the buffering capacity of the ammonium acetate was good and the separation of all the analytes was still successful.

A typical RMM-MEKC–UV electropherogram of the separation of the NMCs, using an AMPS-coated capillary filled with a basic running solution, is shown in Fig. 4B. A comparison of this separation (Fig. 4B) with that where an uncoated fused-silica capillary and RMMs under acidic conditions were used (Fig. 4A), shows that the efficiency of the separation is similar and, now, as expected, the aminocarb and pirimicarb are also detected. However, the sensitivity was slightly lower because the baseline noise was higher, probably due to the presence of a polymer layer in the UV detection window (the polymer may interfere with the detection of the analytes by scattering of light). No loss in sensitivity was observed in the RMM-MEKC–ESI-MS experiments because the polymer is covalently linked to the capillary wall and does not affect the MS sensitivity.

All NMCs were successfully separated with use of a coated capillary, a basic running buffer at pH 8.5, and RMM-MEKC–ESI-MS (Fig. 5C). A slight decrease was observed in the sensitivity of some of the analytes (Table 2), most probably due to the lower pH of the running buffer (i.e. 8.5 and not 9.0). The small change in pH did not significantly change the ratio of sample conductivity to running buffer conductivity. A preconcentration method (continuous solid-phase extraction) was earlier developed for some NMCs in environmental waters [15]. After the preconcentration step, which provided an enrichment factor of ~1250, the extracts were separated by MEKC–UV. If such a preconcentration step were to be applied in this study, and assuming a similar enrichment for all the NMCs, the limits of detection (LODs) of at least four of the analytes would be below 0.1 μg/l (the regulation value for individual pesticides in drinking water). Even though the analysis of environmental water samples was outside the scope of this study, the results show that the developed technique, in combination with a preconcentration step, would be suitable for the determination of NMCs in environmental water samples.

4. Conclusion

Three different ways of coupling MEKC on-line with MS were investigated for the determination of *N*-methylcarbamates. These were the PF technique under basic conditions and reverse migrating mi-

celles under acidic and basic conditions. The latter, used with coated capillaries, was the most successful of the techniques. Reverse migrating SDS micelles and capillaries coated with AMPS, provided an optimum EOF of $4.45 \cdot 10^{-8}$ m^2 V^{-1} s^{-1}, which is approximately 65% of that in uncoated capillaries. For successful ESI-MS on-line coupling, low-concentration ammonium acetate buffers were applied throughout the work. The use of coated capillaries in RMM-MEKC–MS under basic conditions allowed the determination of a wide range of analytes without the troubles commonly associated with the presence of surfactants in the running buffer. With this study we have demonstrated that use of the RMM-MEKC–ESI-MS technique with basic electrolyte solutions and coated capillaries allows simultaneous MEKC separation and MS identification of analytes to be carried out over a wide pH range, broadening the potential field of application of the technique. Use of the high-salt stacking mode as well much improves the sensitivity of the determinations. The technique is an excellent alternative for the sensitive determination of NMC pesticides and since it is neither analyte- nor surfactant-specific, it promises to dramatically broaden the scope of on-line MEKC–MS analysis.

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