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Separation of six phenylureas and chlorsulfuron standards by micellar, mixed micellar and microemulsion electrokinetic chromatography

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Abstract

The usefulness of electrokinetic chromatography (EKC) for the determination of phenylureas and chlorosulfuron herbicides was investigated. Micellar, mixed micellar and microemulsion EKC were examined for this purpose and compared systematically. Micelles formed from sodium dodecyl sulfate (SDS), mixed micelles formed from SDS and polyethylene glycol 400 monolaurate and oil-in-water microemulsions formed from SDS, *n*-butanol and *n*-octane were mainly employed. Capacity factors showed that the separation of the herbicides by ECK employing SDS follows an interaction mechanism similar to that in reversed-phase HPLC. Although there was not much difference in the elution order of the herbicides among the three modes of EKC, there were differences in separation selectivity. The separation efficiencies in mixed micellar and microemulsion EKC were higher than that in micellar EKC. Under the same separation conditions, the migration window in mixed micellar EKC was narrower than that in micellar EKC, but the migration window in microemulsion EKC was wider than that in micellar EKC and was also much easier to extend by changing the SDS concentration and applied voltage. The effects of the separation conditions on the separation of the herbicides by micellar EKC and microemulsion EKC were investigated.

1. Introduction

Substituted phenylureas are selective herbicides used extensively in agriculture and are fairly persistent in the aquatic environment. This has led to the development of many analytical procedures for determining phenylurea residues in aqueous samples. Mostly gas chromatography (GC) and high-performance liquid chromatography (HPLC) have been used.

Owing to the thermoinstability of phenylurea herbicides, their direct determination by means of GC poses a problem. Degradation results in the often uncontrollable formation of isocyanates and/or anilines and prevents quantitative analysis. Direct derivatization of the NH moiety via silylation [1] or alkylation [2–5] improves the thermal stability and chromatographic properties of the phenylureas, but the sensitivity towards sensitive electron-capture detection (ECD) is not increased. The required sensitivity for trace analysis is only reached via derivatization with electrophilic reagents such as halogen-containing acid anhydrides and subsequent GC with elec-

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tron-capture detection (ECD) [6–9,26], but acylation often requires an anhydrous medium, consistent reaction times and controlled conditions for the removal of the excess of the acylation reagent. An alternative GC technique is based on the hydrolysis of the phenylureas to their corresponding anilines [10–13], which are then derivatized and analysed by GC–ECD. However, this technique has a distinct lack of selectivity because different phenylurea can form the same aniline as hydrolysis products.

The use of HPLC to separate them may circumvent the derivatization step used in GC, and most applications make use of normal-phase chromatography [9,12,17] or reversed-phase chromatography [15–26]. However, lack of adequate sensitivity often makes HPLC unsuitable for trace analytical work, and consequently a preconcentration procedure is required [22–26].

Chlorsulfuron is one of the recently developed sulfonylurea herbicides which are efficient at levels 100 times less than earlier herbicides. Because of their extreme thermolability, the determination of sulfonylurea herbicides by GC is impossible. The method of choice is HPLC [27,28]. Recently, Carcia and Henion [29] reported their capillary zone electrophoretic separation (CZE).

Electrokinetic chromatography (EKC), which was developed in 1984 by Terabe et al. [30], is a separation method in which electrophoresis and chromatography are combined. EKC uses the CZE technique but its separation principle is the same as that of chromatography. In EKC, a pseudo-stationary phase is distributed homogeneously in carrier electrolyte solution of CZE and moves electrophoretically in the opposite direction to the electroosmotic flow. Then electrically neutral or non-ionic solutes can partition between the pseudo-stationary phase and carrier electrolyte solution, resulting in separation. The type of pseudo-stationary phase is important in manipulating the selecting in EKC. Micelles, cyclodextrin derivatives having ionic groups, polymer ions and microemulsions, etc., have been successfully employed as pseudo-stationary phase in EKC. Among several modes of EKC, micellar EKC, which uses a running solution of micelles of an ionic surfactant as a pseudo-stationary phase, has been intensively studied [31,32]. Recently, the use of an oil-in-water (O/W) microemulsion as a pseudo-stationary phase in EKC was reported [33–35], but more systematic and intensive studies are still required. A major advantage of EKC over HPLC is its separation efficiency: whereas HPLC separations typically exhibit theoretical plate numbers of 5000–25 000, EKC can generate as many as 50 000–500 000 theoretical plates. Such efficiencies are competitive with those of capillary GC and are necessary for the resolution of complex mixtures. Similar efficiency is obtainable with capillary liquid chromatography but at the expense of a longer analysis time.

As EKC has many advantages, such as high separation efficiency, easy operation and low running costs, we focused on evaluating its potential for the separation of phenylureas and chlorsulfuron. Micellar, mixed micellar and microemulsion EKC were examined and compared systematically. A comparison between the present EKC results and previous results obtained by HPLC was also carried out.

2. Experimental

2.1. Apparatus

The capillary electrophoresis apparatus used in this study was constructed at the Beijing Institute of New Technology Application (Beijing, China). It consists of a high-voltage d.c. power supply delivering up to 30 kV, a UV detector which has several optional wavelengths with fixed and removable devices for on-column detection, a Plexiglas box with a safety interlock and a syringe installation used to flush the capillary. The electrophorograms were recorded with an HP 3390A integrator (Hewlett-Packard, Avondale, PA, USA).

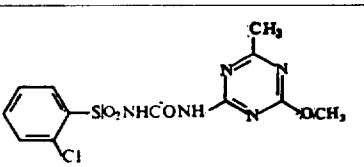
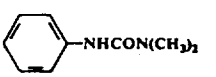
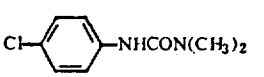
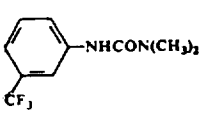
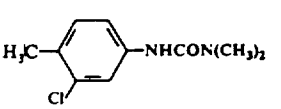
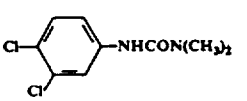
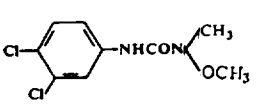
2.2. Reagents and materials

Six phenylurea herbicides, fenuron, monuron, fluometuron, chloroturon, dinuron and linuron, were obtained from various commercial sources.

Chlorsulfuron was synthesized at the Department of Applied Chemistry, Shandong Institute of Building Materials (Jinan, Shandong, China). The names and structures of the herbicides are given in Table 1. Potassium dihydrogenphosphate, sodium borate, *n*-butanol, *n*-octane and N,N-dimethylformamide were of analytical-grade and purchased in China. Polyethylene glycol 400 monolaurate was purchased in China as a chromatographic stationary phase. Sodium dodecyl sulfate (SDS) was of chemical grade and recrystallized twice from ethanol. In all experiments, deionized water was used.

Total dissolution of the herbicides tested required the use of N,N-dimethylformamide. Stock standard herbicide solutions with individual concentration of about 0.3–0.5 mg/ml were prepared. Stock standard solutions of 400 m*M* potassium dihydrogenphosphate, 100 m*M* sodium borate and 250 m*M* SDS were prepared. Running solutions of micelles were mixed with 1.56 ml of stock standard potassium dihydrogenphosphate solution, 1.89 ml of stock standard borate solution and a certain volume of stock standard SDS solution, then diluted to 50 ml. Running solutions of mixed micelles were prepared by adding appropriate volumes of polyethylene glycol 400 monolaurate to the required

Table 1
Systematic names and structures of the herbicides studied

Herbicide	Chemical name	Structure
Chlorsulfuron (CF)	{2-Chloro-N-[(4-methoxy--6-methyl-1,3,5-triazin--2-yl)amino]carbonyl} benzenesulfonamide	
Fenuron (FE)	N′-Phenyl-N,N--dimethylurea	
Monuron (MO)	N′-4-Chlorophenyl--N,N-dimethylurea	
Fluometuron (FL)	N′-3-Trifluoro--methylphenyl-N,N--dimethylurea	
Chloroturon (CT)	N′-3-Chloro-4--methylphenyl-N,N--dimethylurea	
Dinuron (DI)	N′-3,4-Dichlorophenyl--N,N-dimethylurea	
Linuron (LI)	N′-3,4-Dichlorophenyl--N-methoxy-N-methylurea	

running solution of SDS micelles. Running solutions of microemulsions were mixed with the same volumes of stock standard solutions as used above to prepare the running solution of micelles and additional appropriate volumes of *n*-butanol and *n*-octane. The pH of the running solutions was maintained at 7.0 by the adjusting the concentration ratio of potassium dihydrogenphosphate and sodium borate (12.4 and 3.8 m*M*, respectively). Sample injection was accomplished by siphoning for 10 or 20 s at a 10-cm height.

2.3. Electrophoresis

A polyimide-coated fused-silica capillary column (Yongnian Optic Fibre Factory, Hebei, China) of 50 μm I.D. and 375 μm O.D. was used with a total length 75 cm and with the detector located 55 cm from the capillary inlet. Detection at 254 nm was used for all herbicides. A new capillary was flushed with 1 *M* KOH for 1 h and then equilibrated with running solution overnight by using a syringe to force the solution through it before use. In micellar EKC, the capillary was flushed with one capillary volume of running solution of micelles between two runs. In microemulsion EKC, however, the capillary was flushed successively with one capillary volume each of deionized water, 1 *M* KOH, deionized water and the running solution of microemulsions between two runs. Otherwise, there might be no signal response on detector after an injection. All separations were run at ambient temperature.

3. Results and discussion

3.1. Separation of herbicides by micellar, mixed micellar and microemulsion EKC

Both micelles and microemulsions are thermodynamically stable aggregates of surfactant molecules in aqueous solutions. However, micelles are formed only from surfactant and microemulsions from surfactant, cosurfactant and oil. The choice of microemulsion components (surfactant, cosurfactant and oil) can follow the rule of equivalent alkyl carbon number: the carbon number of the surfactant is equal to the carbon number of the cosurfactant plus the carbon number of the oil. When this rule is obeyed, the boundary membrane between water and oil can organize in good order and be very firm. According to this rule, *n*-octane, SDS and *n*-butanol were chosen to be the oil, surfactant and cosurfactant respectively. The structure of an O/W microemulsion is similar to that of a micelle, except that the microemulsion has a core of a minute droplet of an oil. The surfactant and the cosurfactant are located on the surface of the oil droplet to stabilize the droplet, as shown in Fig. 1.

Micelles of microemulsions can differentially solubilize and bind a variety of solute molecules via hydrophobic and hydrogen-bonding interactions. In this experiment, micelles, mixed micelles and microemulsions were prepared by using the same surfactant, SDS, and separations of the herbicides were as shown in Fig. 2. It can be seen that all the peaks showed different extents of fronting. This was possibly caused by sample overloading, which was similar to that reported by Carcia and Henion [29]. In this experiment, the concentrations of injected herbicides were 1.2–3.0 m*M*.

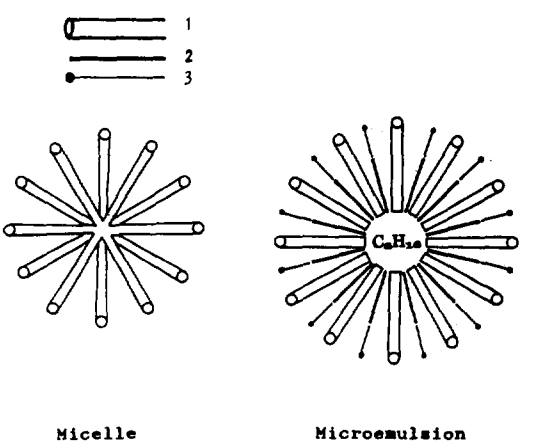


Fig. 1. Schematic diagrams of a micelle and a O/W microemulsion. 1 = Molecule of SDS; 2 = molecule of *n*-octane; 3 = molecule of *n*-butanol.

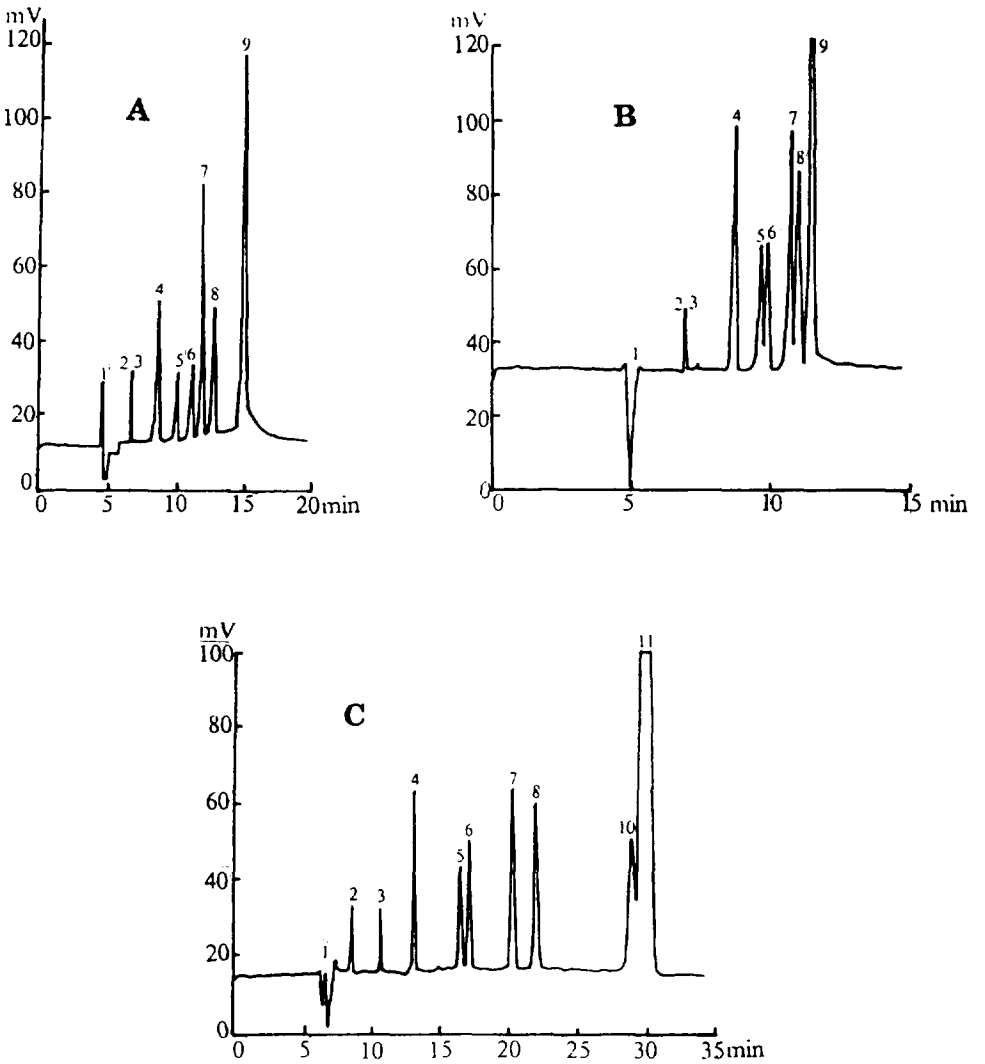


Fig. 2. Separation of the herbicides by micellar, mixed micellar and microemulsion EKC. Applied voltage, 24 kV. (A) Micellar EKC; running solution of micelles, 50 m*M* SDS–12.4 m*M* KH_2PO_4–3.8 m*M* $Na_2B_4O_7$ (pH 7.0). (B) Mixed micellar EKC; running solution of mixed micelles, 50 m*M* SDS–2% (v/v) polyethylene glycol 400 monolaurate–12.4 m*M* KH_2PO_4–3.8 m*M* $Na_2B_4O_7$ (pH 7.0). (C) Microemulsion EKC; running solution of microemulsions 50 m*M* SDS–800 m*M* *n*-butanol–70 m*M* *n*-octane–12.4 m*M* KH_2PO_4–3.8 m*M* $Na_2B_4O_7$ (pH 7.0). Peaks: 1 = N,N-dimethylformamide; 2 = chlorsulfuron; 3 = fenuron; 4 = monuron; 5 = fluometuron; 6 = chloroturon; 7 = dinuron; 8 = linuron; 9 = Sudan III; 10 = impurity in *o*-diphenylbenzene; 11 = *o*-diphenylbenzene.

3.2. Chromatographic behaviour of the herbicides

For electrically neutral solutes separated by EKC, Terabe et al. [30] derived the following equation to describe its retention:

$$k' = \frac{t_R - t_0}{t_0(1 - t_R/t_m)} \quad (1)$$

where k' is the capacity factor, t_R is the migration time of the solute, t_0 is the migration time of water and t_m is the migration time of micelles or

microemulsions. Generally, t_R is greater than t_0 and smaller than t_m. Hence EKC has a limited migration window for neutral solutes between t_0 and t_m. In this experiment, t_0 was experimentally determined by the migration of N,N-dimethylformamide, which was assumed not to interact with the micelles or microemulsions. The parameter t_m was determined by the migration of Sudan III, which was assumed to be fully solubilized by the micelles, and the migration of *o*-diphenylbenzene, which was assumed to be fully solubilized by the microemulsions.

For the six phenylurea herbicides shown in Fig. 2, the logarithms of the capacity factors were calculated and are presented in Table 2 together with the logarithms of the capacity factors previously determined by reversed-phase and normal-phase HPLC and the logarithms of the octanol–water partition coefficients. It was observed from Table 2 that the partitioning of the phenylurea herbicides in EKC employing SDS follows an interaction mechanism similar to that of reversed-phase HPLC. The relative retention order observed in Fig. 2 followed the lipophilicity of the phenylurea herbicides given by the logarithms of the octanol–water partition coefficients in Table 2. Retention of the phenylurea herbicides generally increased with increasing number of chlorine atoms. A trifluoromethyl substituent contributed more than a chlorine atom to retention. A methoxy substituent on the urea nitrogen increased the retention more than a methyl group. This was probably caused by interactions between the neighbouring oxygen and nitrogen atoms, which decreased the solute polarity. The resulting migration order was fenuron, monuron, fluometuron, chloroturon, dinuron and linuron.

It was also observed from Table 2 that microemulsion EKC gave slightly lower capacity factors than micellar EKC under the same conditions, except for fenuron. For a strict comparison, the distribution coefficient, K, should be employed, which can be calculated by

$$K = \frac{k'}{V_m/V_{aq}} \tag{2}$$

where V_m and V_{aq} are the volumes of microemulsions or micelles and of the aqueous phase, respectively. As the same SDS concentration was used, the volume of the microemulsion was evidently larger than that of the micelle, as illustrated in Fig. 1. Hence the distribution coefficients of the six herbicides in microemulsion EKC were smaller than those in micellar EKC. Maybe even though the looser surface of the microemulsions made it easier to solubilize the herbicides, the aqueous solution containing *n*-butanol resulted in even easier solubilization,

Table 2
Logarithms of capacity factors in different chromatographic modes and logarithm of octanol–water partition coefficients (log *p*) of the phenylurea herbicides

Herbicides	Logarithm of capacity factor[a]					Log *p*
	A	B	C	D	E	
Fenuron	−0.102	0.063	−0.040	0.184	1.118	1.18
Monouron	0.312	0.534	0.223	0.279	0.896	1.91
Fluometuron	0.541	0.798	0.498	–[b]	0.562	–[b]
Chlotoluron	0.733	0.893	0.549	0.543	0.755	2.55
Diuron	0.866	1.259	0.776	0.686	0.667	2.68
Linuron	1.064	1.476	0.910	0.840	0.033	2.76

[a] A = Micellar EKC, determined in this work; B = mixed micellar EKC, determined in this work; C = microemulsion EKC, determined in this work; D = reversed-phase HPLC, 1-μm LiChrosorb RP-18 column, ethanol–water (55:45) mobile phase, taken from Ref. [19]; E = normal-phase HPLC, Silasorb S column, *n*-propanol–*n*-hexane (15:85) mobile phase, taken from Ref. [14].

[b] Not determined.

and hence decreased distribution coefficients. In contrast, mixed micellar EKC gave higher capacity factors than micellar EKC, as shown in Table 2. Considering that the same concentration of SDS and additional polyethylene glycol 400 monolaurate was used in the running solution of mixed micelles, the result obtained was reasonable according to Eq. 2.

Although the elution orders of the herbicides by micellar, mixed micellar and microemulsion EKC were very similar, as shown in Fig. 2, there were differences in the resolutions of adjacent herbicides. The resolution was calculated using

$$R_s = \frac{2\Delta t_R}{W_{b1} + W_{b2}} \quad (3)$$

where Δt_R is the difference between migration times of two peaks and W_{b1} and W_{b2} are the baseline widths of the two peaks, respectively. Although Eq. 3 is typically the standard for measuring experimental resolution, the high efficiencies often observed in EKC and the fronting peaks in Fig. 2 would limit the accuracy of resolution calculated with it. However, the calculation is adequate for the purpose of comparison. The resolutions of adjacent herbicides in micellar, mixed micellar and microemulsion EKC shown in Fig. 2 were calculated and are given in Table 3. It was observed that the use of a nonionic surfactant as one component of a running solution of mixed micelles had an effect on the separation selectivity as described by Rasmussen et al. [36]. When using mixed micellar EKC, the resolutions of two pairs, FE–MO and CT–DI, were increased, whereas those of two other pairs, FL–CT and DI–LI, were decreased and the resolution of MO–FL remained almost constant. It was also observed from Table 3 that although the same surfactant, SDS, was used, microemulsion EKC had a different separation selectivity to micellar EKC. When using microemulsion EKC, the resolutions of four pairs, CF–FE, FE–MO, MO–FL and CT–DT, were greatly increases whereas the resolution of DI–LI was slightly increased and that of FL–CT was greatly decreased. These changes in selectivity suggested that there were differences in interaction mechanism among micellar, mixed micellar and microemulsion EKC. This may be due to the differences in the structures of micelles, mixed micelles and microemulsions.

The limited migration window in EKC also contributes to resolution, as shown in the resolution equation [38]

$$R_S = \frac{\sqrt{N}}{4}\left(\frac{\alpha - 1}{\alpha}\right)\left(\frac{k_2'}{1 + k_2'}\right)\left[\frac{1 - t_0/t_m}{1 + (t_0/t_m)k_1'}\right] \quad (4)$$

where R_S is the resolution, N is the plate number and α is the separation factor, which is equal to k_2'/k_1'. The smaller the ratio of t_0/t_m, the higher is the resolution. In Fig. 2, micellar EKC gave $t_0/t_m = 0.320$, mixed micellar EKC gave $t_0/t_m = 0.410$ and microemulsion EKC gave $t_0/t_m = 0.229$. It was understandable that mixed micellar EKC gave a higher value of t_0/t_m than micellar EKC because the mixed micelles had a higher mass-to-charge ratio (m/z) than the micelles. The t_0/t_m value in microemulsion EKC was lower than that in micellar EKC, and the mass-to-charge ratio of the microemulsions was then lower than that of the micelles. The reason for this involves the degree of counter-ion binding of an aggregate. In the formation of an aggregate, although hydrophobic interactions between sur-

Table 3
Resolution of adjacent herbicide peaks in Fig. 2

EKC mode	Resolution adjacent herbicides					
	CF–FE	FE–MO	MO–FL	FL–CT	CT–DI	DI–LI
Micellar	0	6.93	3.27	2.61	1.69	2.21
Mixed micellar	0	8.72	3.26	0.91	2.79	0.93
Microemulsion	9.45	8.79	8.19	1.41	5.64	2.77

factant molecules can stabilize it, the repelling effect resulting from the charged head of the surfactant molecules as shown in Fig. 1 can make it unstable. Therefore, an aggregate will attract counter ions to stabilize itself. The degree of counter-ion binding is defined as the ratio of counter ions and surfactant ions in an aggregate and has a general value of 50–80%. Then, the higher the degree of counter-ion binding of an aggregate, the higher its mass-to-charge ratio will be. In comparison with micelles, microemulsions have *n*-butanol molecules between SDS molecules which can decrease the repelling effect of SDS molecules, and they then have a lower degree of counter-ion binding and consequently a lower mass-to-charge ratio.

The separation efficiencies of the herbicides in Fig. 2 were calculated and are presented in Table 4. Because fenuron and chlorsulfuron co-eluted in micellar and mixed micellar EKC, the separation efficiency of fenuron was determined when there was no chlorsulfuron present in the separated herbicides mixture. It was observed that fenuron had a higher separation efficiency than other herbicides, which may be attributed to the lack of a halogen atom in its molecule. It was also observed from Table 4 that mixed micellar and microemulsion EKC gave higher separation efficiencies than micellar EKC. A similar result was reported by Little and Foley [37] for mixed micellar EKC, but a different result was obtained by Terabe et al. [35] for microemulsion EKC. It was also noted that the plate numbers in Table 4 are low in comparison with the reported values for EKC, so there was the possibility of sample overloading, as indicated above. If the possibility of sample overloading was precluded, the increased separation efficiency was mainly due to differences in the structures of micelles and microemulsions, perhaps in the kinetics of solute sorption–desorption. Because SDS micelles had a higher charge density on their surface than mixed micelles and microemulsions, it was speculated that the activation barriers to sorption–desorption might be also greater.

3.3. Effect of conditions on separation of the herbicides

The effects of the applied voltage on migration times and capacity factors of the herbicides in microemulsion EKC are shown in Fig. 3. An increase in applied voltage caused a significant decrease in the migration times of the herbicides (Fig. 3A), did not cause a decrease in the capacity factors of the herbicides in the low-voltage region, but caused a decrease in the capacity factors of some herbicides in the high-voltage region (Fig. 3B). In micellar EKC, smaller decreases in migration times and larger decreases in capacity factors were observed. That was because microemulsions are much larger than micelles, hence microemulsions are less affected by Joule heating caused by an increase in applied voltage.

The effects of SDS concentration on migration times and capacity factors of the herbicides in microemulsion EKC are shown in Fig. 4. Almost the same trends were observed as in micellar EKC.

The effect of the concentration of polyethylene glycol 400 monolaurate on the migration times and capacity factors of the herbicides in micellar EKC is illustrated in Fig. 5. A higher

Table 4
Separation efficiency of the herbicides at 24 kV

EKC mode	Theoretical plate number ($\times 10^4$)						
	CF	FE	MO	FL	CT	DT	LI
Micellar	–	6.21	1.04	1.80	1.53	2.79	2.18
Mixed micellar	–	6.39	2.57	2.65	3.13	2.85	3.17
Microemulsion	2.52	9.03	3.30	3.62	3.17	2.94	3.00

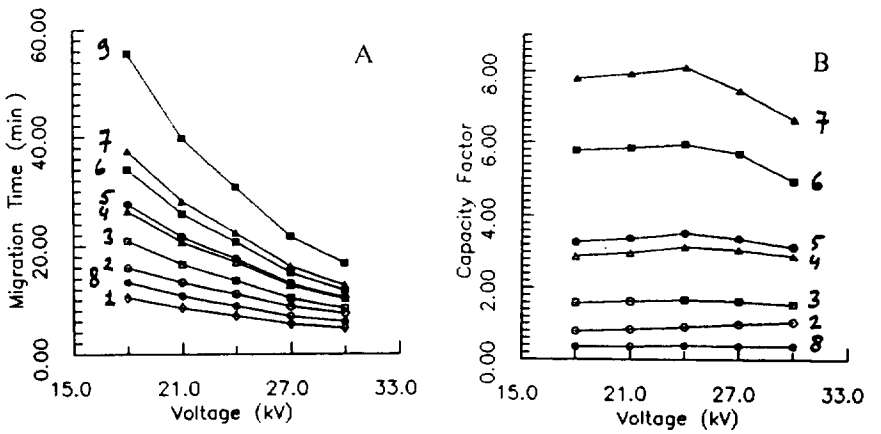


Fig. 3. Effects of applied voltage on (A) migration times and (B) capacity factors of the herbicides in microemulsion EKC. Running solution of microemulsions 50 m*M* SDS–800 m*M* *n*-butanol–70 m*M* *n*-octane–12.4 m*M* KH_2PO_4–3.8 m*M* $Na_2B_4O_7$ (pH 7.0). 1 = N,N-Dimethylformamide; 2 = fenuron; 3 = monuron; 4 = fluometuron; 5 = chloroturon; 6 = diuron; 7 = linuron; 8 = chlorsulfuron; 9 = *o*-diphenylbenzene.

concentration led to higher t_0/t_m and capacity factors.

The effect of the concentration of *n*-butanol on the capacity factors of the herbicides and the effect of the concentration of *n*-octane on the migration times of the herbicides in microemulsion EKC are shown in Figs. 6 and 7. An increase in the concentration of *n*-butanol caused an increase in the capacity factors of the herbicides, which could be attributed to the increased volume of the microemulsions. However, an increase in the concentration of *n*-octane did not cause much change in the migration times of the herbicides and consequently did not have much effect on the capacity factors.

In comparison with micellar EKC, the most significant improvement in microemulsion EKC was in the easily extended migration window. As mentioned by Watarai [33,34], the migration window was easily extended by increasing the SDS concentration, which is illustrated in Fig. 8A. Increasing the SDS concentration could

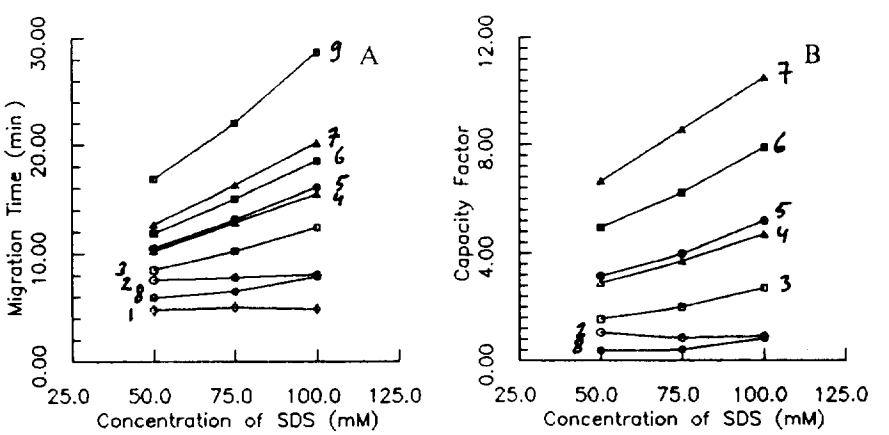


Fig. 4. Effects of SDS concentration on (A) migration times and (B) capacity factors of the herbicides in microemulsion EKC. Running solution, 800 m*M* *n*-butanol–70 m*M* *n*-octane–12.4 m*M* KH_2PO_4–3.8 m*M* $Na_2B_4O_7$ (pH 7.0). Applied voltage, 30 kV. Curves as in Fig. 3.

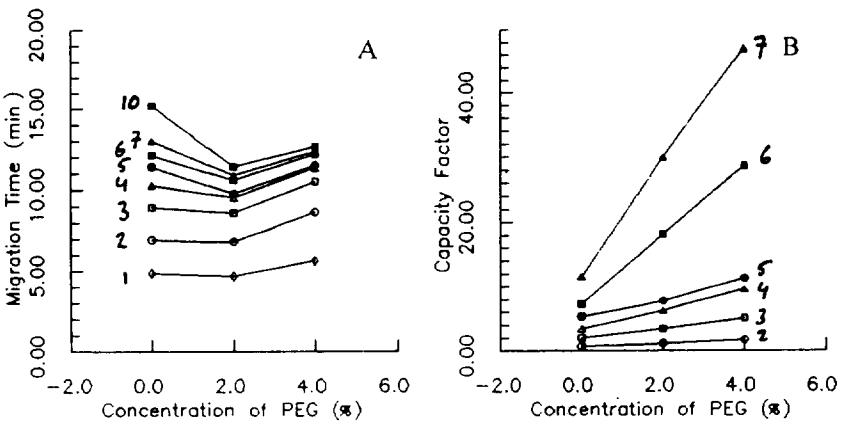


Fig. 5. Effects of the concentration of polyethylene glycol 400 monolaurate on (A) migration times and (B) capacity factors of the herbicides in mixed micellar EKC. Running solution of micelles, 50 m*M* SDS–12.4 m*M* KH_2PO_4–3.8 m*M* $Na_2B_4O_7$ (pH 7.0). Applied voltage, 24 kV. Curves in Fig. 3; 10 = Sudan III.

increase the number of SDS molecules in an aggregate. The distance between the charged head of the surfactant molecules in a larger aggregate was longer, hence the repelling effect resulting from the charged head of the surfactant molecules was lower. Therefore, increasing the SDS concentration would cause the degree of counter-ion binding of an aggregate to decrease, thus causing its mass-to-charge ratio to decrease. The larger the aggregate was, the greater the decrease in its extent of counter-ion binding would be, and hence the greater the decrease in its mass-to-charge ratio would be. Microemulsions were much larger than micelles, and therefore their mass-to-charge ratio was easily decreased by increasing the SDS concentration and the migration window was also easily extended.

The migration window was also easily manipulated by changing the applied voltage in microemulsion EKC, which is illustrated in Fig. 8B. An increase in applied voltage caused a greater

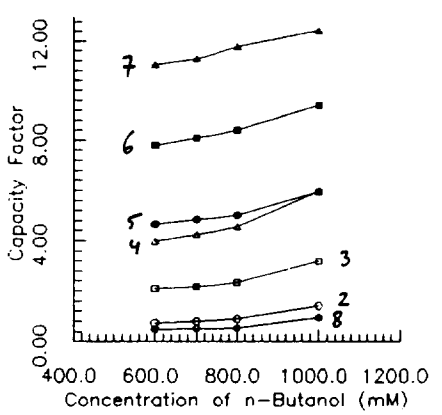


Fig. 6. Effect of *n*-butanol concentration on capacity factors of the herbicides. Running solution, 75 m*M* SDS–70 m*M* *n*-octane–12.4 m*M* KH_2PO_4–3.8 m*M* $Na_2B_4O_7$ (pH 7.0). Applied voltage, 27 kV. Curves as in Fig. 3.

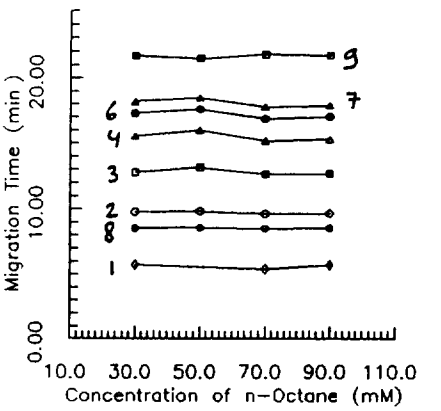


Fig. 7. Effect of *n*-octane concentration on the migration times of the herbicides in microemulsion EKC. Running solution: 75 m*M* SDS–1000 m*M* *n*-butanol–12.4 m*M* KH_2PO_4–3.8 m*M* $Na_2B_4O_7$ (pH 7.0). Applied voltage, 27 kV. Curves as in Fig. 3.

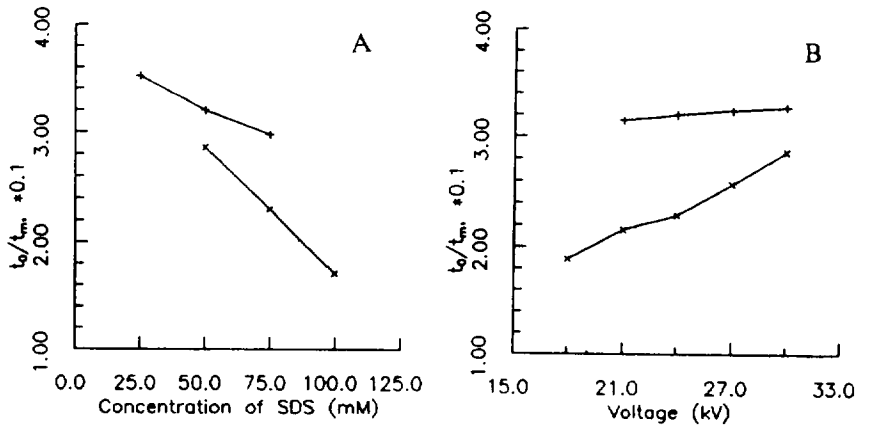


Fig. 8. Migration window as functions of (A) SDS concentration and (B) applied voltage. (A) Conditions for micellar EKC: running solution of micelles, 12.4 m*M* KH_2PO_4–3.8 m*M* $Na_2B_4O_7$ (pH 7.0); applied voltage, 24 kV; conditions for microemulsion EKC as in Fig. 4. (B) Conditions for micellar EKC: running solution of micelles, 50 m*M* SDS–12.4 m*M* KH_2PO_4–3.8 m*M* $Na_2B_4O_7$ (pH 7.0); conditions for microemulsion EKC as in Fig. 3. + = Micellar EKC; × = microemulsion EKC.

increase in t_0/t_m in microemulsion EKC than in micellar EKC, which suggested that the increased applied voltage cause a larger increase in the extent of counter-ion binding of the microemulsions than that of the micelles. Probably the higher applied voltage made the microemulsions or micelles unstable, and because the microemulsions had a much larger volume and smaller extent of counter-ion binding than the micelles, they had more steric freedom to associate more counter-ions to stabilize themselves at higher applied voltages.

4. Conclusions

Although phenylureas and chlorsulfuron herbicides have traditionally been mainly determined by GC and HPLC, this work showed that EKC is also a worthwhile method for their determination. Micelles formed from SDS, mixed micelles formed from SDS and polyethylene glycol 400 monolaurate and O/W microemulsions formed from SDS, *n*-butanol and *n*-octane were successfully used as pseudo-stationary phases in EKC to separate these herbicides. The capacity factors showed that the separation of the herbicides by EKC employing SDS followed an interaction mechanism similar to that of reversed-phase HPLC. When using different pseudo-stationary phases there were differences in the separation selectivity, which was observed from the resolutions of adjacent peaks. The separation efficiencies in mixed micellars and microemulsion EKC were higher than that in micellar EKC. Under the same separation conditions, the migration window in mixed micellar EKC was narrower than that in micellar EKC, but the migration window in microemulsion EKC was wider than that in micellar EKC and was also much easier to extend by changing the SDS concentration and applied voltage. Although EKC is not suitable for trace analysis at present, the data showed that it is a method worth considering further for trace analysis studies by improving the sensitivity of the detection technique or using a sample preconcentration method.

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