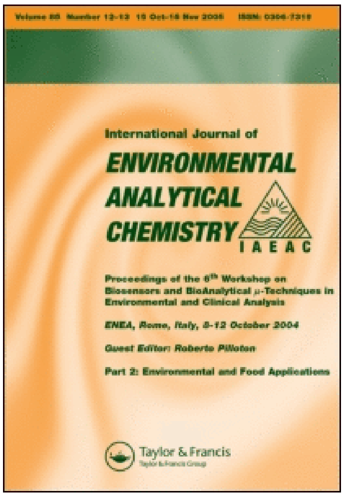


International Journal of Environmental Analytical Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713640455

Simultaneous Separation Of Neutral and Acidic Herbicides by Micellar Electrokinetic Capillary Chromatography

A. Farran[a]; S. Ruiz[a]
[a] Department of Chemical Engineering (ETSEIB-UPC), Barcelona, Spain

To cite this Article Farran, A. and Ruiz, S.(1995) 'Simultaneous Separation Of Neutral and Acidic Herbicides by Micellar Electrokinetic Capillary Chromatography', International Journal of Environmental Analytical Chemistry, 58: 1, 121 — 129

To link to this Article: DOI: 10.1080/03067319508033118

URL: http://dx.doi.org/10.1080/03067319508033118

Intern. J. Environ. Anal. Chem., Vol. 58, pp. 121-129
Reprints available directly from the publisher
Photocopying permitted by license only

SIMULTANEOUS SEPARATION OF NEUTRAL AND ACIDIC HERBICIDES BY MICELLAR ELECTROKINETIC CAPILLARY CHROMATOGRAPHY

A. FARRAN and S. RUIZ

Department of Chemical Engineering (ETSEIB-UPC),
Diagonal, 647. 08028 Barcelona, Spain.

(Received, 10 September 1993; in final form, 14 October 1993)

Micellar electrokinetic capillary chromatography (MECC) is used for the simultaneous separation of mixtures of phenylurea and phenoxyalkyl acid herbicides. The influence of buffer and micellar concentration on the separation of the herbicides has been studied and the obtained results are compared with those obtained by using capillary zone electrophoresis (CZE). By using the MECC system, the total separation of a mixture of nine different herbicides in 16 minutes has been achieved.

In addition, from the experimental results, the distribution coefficient (K) of each herbicide between micellar and aqueous phases, and the critical micelle concentration (CMC) under the experimental conditions, have been calculated.

KEY WORDS: Micellar electrokinetic capillary chromatography, phenylurea herbicides, phenoxyalkyl acid herbicides, capillary electrophoresis.

INTRODUCTION

The phenylurea and the phenoxyalkyl acid derivatives are two of the most used herbicides. Commercial formulations include, in most cases, mixtures of these compounds[1].

The determination of these compounds in environmental samples has been routinely carried out by means of gas chromatography. However, the problem of the thermal instability of phenylurea herbicides and the low vapour pressure of phenoxyalkyl acids makes it necessary to employ derivatization[2].

So, the analysis of these compounds is in general simpler by means of liquid chromatography. The best results have been obtained by using reversed phases, with acidified eluents in the case of acid compounds[3].

However, an analytical technique capable of to determine phenylurea and phenoxyalkyl acid herbicides in a simple and single way has not been developed yet.

In last years capillary zone electrophoresis (CZE) has become a powerful tool of separation. Some recent works have demonstrate that CZE is a feasible method for the determination of acidic herbicides[4–6]. Neutral molecules present a special problem for CZE. They do not separate from each other but migrate as a group with the electroosmotic flow. Enhancement of selectivity can be obtained by the addition of modifiers to the electrophoretic medium. Various types of modifiers have been proposed for CZE separation[7–10]. When micelles are used, the technique is called micellar electrokinetic capillary chromatography (MECC). MECC involves the addition of surfactant ions to the mobile phase at concentration above their critical micelle concentration. The separation is based on the differential partitioning of the solute between an electroosmotically pumped aqueous mobile phase and the hydrophobic interior of the micelles, which are moving at a velocity different from that of the mobile phase due to electrophoretic effects. In this work, the use of anionic micelles as modifiers in the capillary electrophoretic separation of mixtures of phenylurea and phenoxyalkyl acid herbicides has been investigated. The influence of micellar and buffer concentrations on the separation efficiency of the nine herbicides has been studied.

EXPERIMENTAL

Instrumentation

An integrated capillary electrophoresis system (ISCO Model 3850) (ISCO, Lincoln, NE) equipped with a fused silica capillary (55 cm length, 50 μm i.d.) was used for the separation.

Detection was carried out by on-column measurement of UV absorption at 205 nm at a placed position of 40 cm from the anode. Electropherograms were registered by a Spectra-Physics SP-4270 integrator (San Jose, CA).

The sample was introduced into the system by a split-flow manual injector using an HPLC-style syringe. In all experiments the amount of sample loaded into the column was 1.5 nL and a constant voltage of +15 kV was applied.

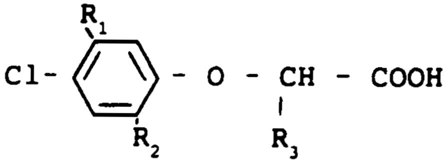
Chemicals

The nine herbicides used were purchased from Riedel-de Haën (Heelze-Hannover, Germany). All the herbicides were used as they were received and dissolved in methanol.

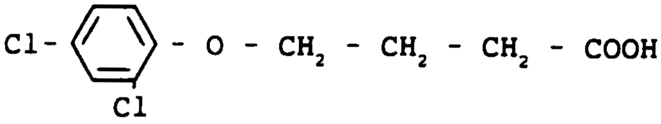
All other chemicals were obtained from Merck (Darmstadt, Germany). The buffer solutions were prepared from sodium dihydrogenphosphate and sodium tetraborate, by fixing the pH at 7.

The electrolyte and sample solutions were filtered through a 0.3 μm membrane from Lida (Kenosha, WI) and were degassed by ultrasonication.

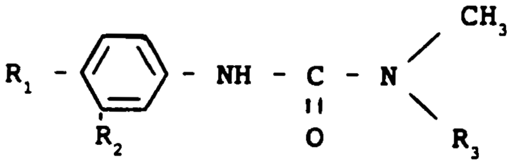
Table 1 Chemical structures of the investigated herbicides.



1. 2,4-D : R_1 = H, R_2 = Cl, R_3 = H
2. 2,4-DP : R_1 = H, R_2 = Cl, R_3 = CH_3
3. 2,4,5-T : R_1 = Cl, R_2 = Cl, R_3 = H



4. 2,4-DB



5. Fenuron : R_1 = H, R_2 = H, R_3 = CH_3
6. Monuron : R_1 = Cl, R_2 = H, R_3 = CH_3
7. Diuron : R_1 = Cl, R_2 = Cl, R_3 = CH_3
8. Monolinuron : R_1 = Cl, R_2 = H, R_3 = OCH_3
9. Linuron : R_1 = Cl, R_2 = Cl, R_3 = OCH_3

RESULTS AND DISCUSSION

A test mixture of four phenoxyalkyl acid and five phenylurea herbicides was used to investigate the retention behaviour of herbicides in MECC. The chemical structures of the investigated compounds are shown in Table 1. Previous results obtained in our laboratory demonstrated the feasibility of CZE to separate phenoxyalkyl acid herbicides. As it can be seen in Figure 1, when no modifiers were added to the buffer, the four phenoxyalkyl acids 2, 4-DB, 2, 4-DP, 2, 4, 5-T and 2, 4-D migrate in this order, according to their molecular size, from the anode to the cathode. Despite the high efficiency obtained for the anionic solutes, this technique, as it can be observed in the same Figure, is not very effective at separating neutral compounds as phenylurea herbicides, since they are transported through the capillary strictly by electroosmotic flow and do not separate from each other. Previous works[9, 11-13] developed the use of SDS micelles to obtain the separation of neutral molecules. The separation is based on the differential distribution of the solute molecule between the aqueous and the micellar phases. In our work, when SDS was added to the buffer, the electroosmotic flow opposes the electrophoretic flow of the micelles and is of greater magnitude. Consequently, two distinct phases, aqueous and micellar, migrate at different velocities toward the cathode. Solutes injected into the anodic end of the column are eluted with migration times which range from the migration time of a solute which is not solubilized by the micelles (t_{eo}) to the migration time of a totally solubilized solute (t_{mc})[14]. Sudan III was added to the test mixture as a tracer of the micelle and its migration time was regarded as that of the micelle. Methanol can be considered to be insolubilized by the micelle, so its migration time will correspond to t_{eo}[11].

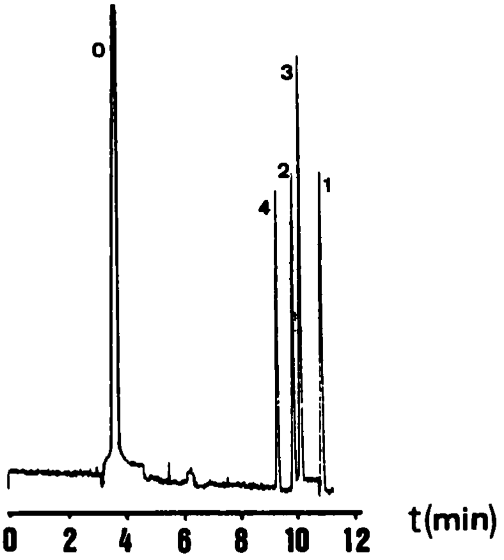


Figure 1 Electropherogram of the test mixture. Experimental conditions: buffer phosphate/borate 0.02M at pH 7.0, applied voltage 25 kV. For peak numbers see Table 1. Peak 0 corresponds to the neutral compounds.

Influence of buffer concentration

Three different borate-phosphate buffer concentrations (0.01, 0.02 and 0.04 M) with different concentrations of SDS have been studied in this work. By fixing the SDS concentration at 0.05 M, the influence of the buffer concentration on the separation was studied by observing the variation of two different parameters: the migration time (t_m) and the number of theoretical plates (N). It was observed that decreasing the buffer concentration, N increases and t_m decreases, so it seems that the better results will be obtained by the lowest buffer concentration. But for the lowest value investigated, a very poor separation was obtained. These results can be explained by the so-called elution range in MECC. When working by MECC, the range of elution times of solutes is limited between t_{eo} and t_{mc}[11]. Thus, the available peak capacity is clearly dependent on these parameters, and the increase of the ratio t_{mc}/t_{eo} should increase the separation efficiency of the system[15]. When following the variation of t_{mc} and t_{eo} with the buffer concentration, it was observed that the ratio between them increases when the buffer concentration increases. From these results, the best separation efficiency in the shortest analysis time was obtained by using a 0.02 M buffer concentration.

Influence of SDS concentration

By fixing the mentioned buffer concentration value, the influence of the SDS concentration on the separation was studied by observing the variation of the two separation parameters mentioned above, t_m and N.

As it can be observed in Figure 2, there was an increase on the t_m and N values when SDS concentration was increased. However, the magnitude of the variation is different for neutral and ionic compounds, being stronger for the neutral solutes.

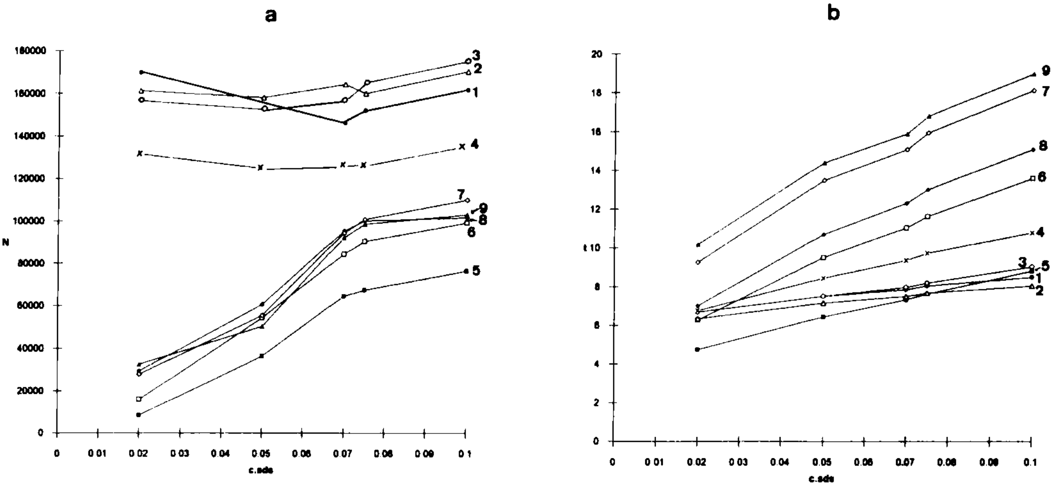


Figure 2 Dependence of number of theoretical plates (a) and migration times (b) on the concentration of SDS. Solutes are indicatedby the same numbers of those in Table 1. Applied voltage 15 kV. Other experimental conditions as in Figure 1.

These two different trends can be explained by the different interaction between the solutes and the micelle.

In the case of the neutral solutes, an increase on the SDS concentration will increase the probability of the interaction solute-micelle, so the migration time will increase. The possible explanation for the improvement in efficiency as micelle concentration is increased is that intermicelle resistance to mass transfer is a significant factor in the number of theoretical plates and it is reduced as intermicelle distance is reduced[14].

In the case of the acidic compounds, their interaction with the micelle is so low that the variation of SDS concentration practically do neither affect their migration velocity nor the efficiency of their separation.

Another interesting observation that can be made from results in Figure 2 is that for any value of the SDS concentration, the number of theoretical plates is higher for the acidic compounds than for the neutral solutes. So, the interaction solute-micelle will add new factors to the dispersion of the solute bands. From the obtained results, it seems than the better SDS concentration, that gives the best separation in the shorter migration time, is 0.07 M.

A sample electrokinetic chromatogram of the test mixture, which was obtained with the 0.02 M phosphate-borate buffer and a SDS concentration of 0.07 M, is shown in Figure 3. From this figure, two different trends for the elution order when using SDS micelles can be observed. On the one hand, the migration times of the neutral compounds increase with their

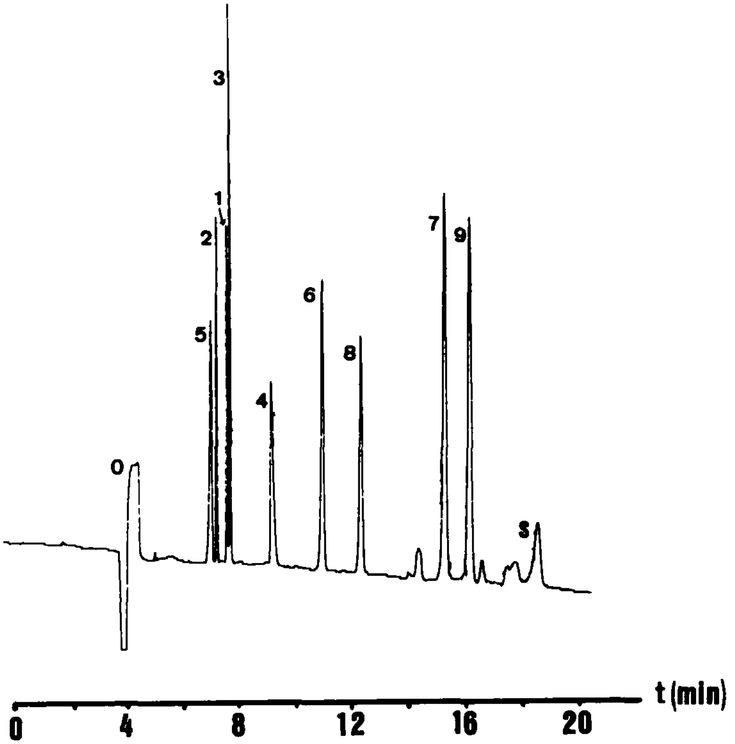


Figure 3 Electropherogram of the test mixture. SDS concentration 0.07M. Other experimental conditions as in Figure 2. Peak numbers as in Table 1. Peak 0: methanol. Peak s: Sudan III.

molecular size. On the other hand, the main factor affecting the velocity of the anionic solutes is their hydrophobicity (e.g. the migration time increases as the water solubility decreases).

A characteristic parameter in the MECC technique is the capacity factor k′ defined by the ratio n_{mc}/n_{aq} where n_{mc} and n_{aq} are the total moles of the solutes in the micelle and in the aqueous phase, respectively. k′ for an electrically neutral solute can be calculated from migration times[9] by

$$k' = (t_m - t_{eo})/t_{eo}(1 - t_m/t_{mc})$$

On the other hand, the negatively charged compounds could be subject to two different effects: the electrophoretic effect, which causes an increase in their migration time since the direction of electrophoretic migration of an anion is the reverse of that of electroosmotic flow, and the electrostatic repulsion toward the negatively charged SDS micelle that will suppress micellar solubilization. So, the capacity factor of an ionized solute can be calculated from[16]

$$k' = (t_m - t_0)/t_0(1 - t_m/t_{mc})$$

where t_0 is the migration time of the solute in the absence of SDS in the buffer.

In Table 2 it can be seen the migration times and the values calculated for the capacity factor at these experimental conditions. From results in Table 2 it can be observed that the negatively charged compounds have smaller capacity factors than the electrically neutral compounds. The reason is that compounds in the neutral form were solubilized more easily by the micelles and they reached the detector at relatively longer times than anionic solutes which are repulsed. The exception is Fenuron. Fenuron is a neutral compound but it has the lowest migration time of all the solutes. This fact can be due to its solubility in water, which is the highest of those of the studied compounds. So, Fenuron will be mainly in the aqueous phase than in the micellar phase and, because it is a neutral compound, it will migrate with the electroosmotic flow with a higher velocity than that of anionic solutes.

The capacity factor k′ can be related to the distribution coefficient K of a solute between micellar and aqueous phases by the equation

Table 2 Migration times (t_m in min.), water solubilities[18] (in g/l), calculated capacity factors (k′) and distribution coefficients (K) for the studied compounds. Experimental conditions for t_m and k′ values as in Figure 3.

COMPOUND	t_m	*WATER SOLUBITITY*	*k′*	*K*
Fenuron	7.33	3.85	1.09	69.98
2, 4-DP	7.51	2.20	0.31	22.46
2, 4-D	7.86	0.62	0.37	23.01
2, 4, 5-T	7.97	0.28	0.45	31.87
2, 4-DB	9.36	0.04	1.05	70.31
Monuron	11.04	0.23	3.80	237.61
Monolinuron	12.33	0.74	5.60	345.96
Diuron	15.09	0.042	14.3	878.11
Linuron	15.92	0.081	21.6	1301.02

$$k'=Kv(C_{SDS}-CMC)$$

where v is the partial specific volume, C_{SDS} the concentration and CMC the critical concentration of the micelle[9].

The plots of k' vs C_{SDS} are given in Figure 4. Intercepts of plots extrapolated to k'=0 are around 4 mM (averaged value 3.82 mM) and this concentration can be interpreted to be the CMC under the experimental conditions. This value is lower than the reported value of 8.1 mM in water because CMC is generally reduced with additions of electrolytes, and it is in agreement with the CMC value obtained by Khaledi and co-workers[16].

The linear relationships observed in Figure 4 implies that the distribution coefficients remain constant at least at the SDS concentrations below 0.1M.

Distribution coefficients calculated from slopes of these plots are listed in Table 2. The partial specific volume used for the calculations, 0.247 1 mol^{-1}, was taken from Shinoda[17]. It can be observed that the lowest K values correspond to the anionic compounds, the highest K values correspond to neutral compounds and Fenuron has a K value similar to the less water-soluble phenoxyacid (2,4-DB). There are no available data in the literature concerning micellar solubilization of the studied herbicides with SDS. The data obtained in the present work will be helpful in understanding this phenomenon, and the present method may provide a useful technique to determine K values as well as a high efficient separation method.

Acknowledgment

The authors are indebted to Prof. M. Aguilar for his interest in this work and to Dr. J. de Pablo for his helpful comments. Financial support provided by the CICYT (Project No. NAT89–1082), Ministry of Science and Education of Spain, is gratefully acknowledged.

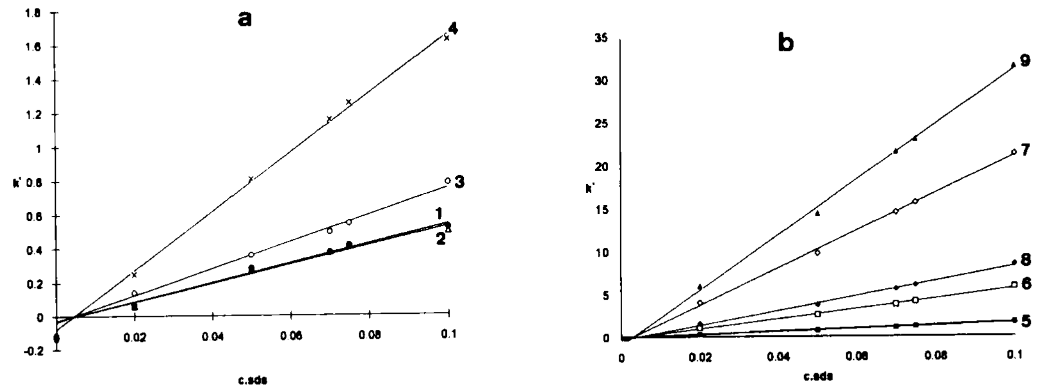


Figure 4 Dependence of k' on the concentration of SDS for (a) anionic compounds and (b) neutral compounds. Experimental conditions and solute numbers as in Figure 2.

References

1. *European Directory of Agrochemical Products. Part 2. Herbicides.* (Royal Soc. Chem., 1984).
2. D. Barceló, *Chromatographia*, **25,** 928–936 (1988).
3. R. Hamman, and A. Kettrup *Chemosphere*, **16,** 527–536 (1987).
4. S. K. Yeo, H. K. Lee, and S. F. Y. Li, *J. Chromatogr.*, **594,** 335–340 (1992).
5. M. Aguilar, A. Farran and V. Martí *Sci. Total Environ*, **132,** 133–140 (1993).
6. M. W. F. Nielen *J. Chromatogr.*, **637,** 81–90 (1993).
7. T. Tsuda, *J. High Resol. Chromatogr. Chromatogr. Commun.*, **10,** 622–624 (1987).
8. X. Huang, J. A. Luckey, M. J. Gordon, and R. N. Zare *Anal. Chem.*, **61,** 766–770 (1989).
9. S. Terabe, K. Otsuka, and T. Ando, *Anal. Chem.* **57,** 834–841 (1985).
10. H. Nishi, T. Fukuyama, M. Matsuo, and S. Terabe, *J. Chromatogr.*, **498,** 313–318 (1990).
11. S. Terabe, K. Otsuka, K. Ichikawa, A. Tsuchiya, and T. Ando, *Anal. Chem.*, **56,** 111–113 (1984).
12. K. Otsuka, S. Terabe, and T. Ando, *J. Chromatogr.*, **332,** 219–226 (1985).
13. K. Otsuka, S. Terabe, and T. Ando, *J. Chromatogr.*, **396,** 350–354 (1987).
14. M. J. Sepaniak, and R. O. Cole, *Anal. Chem.*, **59,** 472–476 (1987).
15. A. T. Balchunas, and M. J. Sepaniak, *Anal. Chem.* **59,** 1466–1470 (1987).
16. M. G. Khaledi, S. C. Smith, and J. K. Strasters, *Anal. Chem.* **63,** 1820–1830 (1991).
17. K. Shinoda, and T. Soda, *J. Phys. Chem.* **67,** 2072–2074 (1963).
18. *The Agrochemicals Handbook* (3rd edition) (Royal Soc. Chem. 1991).