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Separation of cardiac glycosides by micellar electrokinetic chromatography and microemulsion electrokinetic chromatography

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Abstract

The interest of micellar electrokinetic chromatography (MEKC) and microemulsion electrokinetic chromatography (MEEKC) for the resolution of four cardiac glycosides is demonstrated. First, the influence of some parameters on the resolution of the solutes in MEKC such as the concentration of the surfactant, pH, addition of organic modifiers and urea is discussed. Then, results are compared with those obtained in MEEKC using different microemulsion compositions. Results indicate that MEEKC possesses several advantages over MEKC for the separation of relatively hydrophobic compounds such as digitalic compounds. First, microemulsions allow a better manipulation of the migration time window and of the retention of the solutes. Moreover, efficiency is improved with shorter analysis time. © 1997 Elsevier Science B.V.

Keywords: Cardiac glycosides; Digoxin; Acetyldigoxin; Acetyldigitoxin; Deslanoside

1. Introduction

Cardiac glycosides such as digoxin, acetyldigoxin, acetyldigitoxin and deslanoside are pharmaceutically important drugs used for the treatment of congestive heart failure and atrial fibrillation. These cardiac glycosides are administrated in very low doses and there is a narrow margin between the therapeutic and the toxic dose. Therefore, it is very important to be able to analyze the amounts of such cardiac glycosides present in several drugs.

Several analytical methods have been reported to analyze such compounds. Previously described methods include colorimetric [1], fluorimetric [2], thin layer chromatographic [3] and gas–liquid chromatographic techniques [4]. High-performance liquid chromatography has also been demonstrated to be an effective analytical method for the determination of these cardiac glycosides in various media. Several systems have been described including normal-phase chromatography [5–7], reversed-phase chromatography [8–10], using classical or miniaturized systems. However the separations are generally time consuming (30 min to 1 h) and satisfactory resolution is not always achieved. More recently, high-performance capillary electrophoresis (HPCE) has been recognized as a powerful analytical separation technique. The use of high electrical fields results in short analysis times, high efficiency and resolution. Initially devoted to the analysis of charged com-

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pounds, HPCE and its related techniques have proved their effectiveness for the separation of a great variety of compounds including neutral ones. In addition, the many different separation modes offer different separation mechanisms and selectivities. HPCE mechanisms of separation are different from chromatographic ones, making the HPCE technique a complementary tool in the field of separation methods.

The aim of this study is to demonstrate the usefulness of capillary electrokinetic chromatography (EKC) for the separation of the cardiac glycosides. Since such compounds are neutral solutes possessing close chemical structure and poor water solubility it is necessary to separate them in the presence of a pseudo-stationary phase possessing its own electrophoretic mobility and solubilization properties. Ionic micelles are the most widely accepted pseudo-stationary phases and in this case, the technique is called micellar electrokinetic chromatography (MEKC). Its main advantage is the availability of various surfactants offering different selectivity and allowing the resolution of a wide variety of compounds. More recently, Watarai [11] introduced the use of microemulsion as a separation medium in electrokinetic chromatography (MEEKC). Microemulsions are transparent solutions that consist of a surfactant, a co-surfactant such as medium alkyl-chain alcohol, oil and water. The structure of an oil in water emulsion is similar to that of the micelle except that the microemulsion has an oil droplet as a core. The surfactant and cosurfactant are located onto the surface to stabilize it. Microemulsions are thermodynamically stable if the composition is properly chosen. To date only a few applications using MEEKC have been reported in the literature [12–16].

In this study, we demonstrate the advantages of MEEKC over MEKC, in terms of separation ability and efficiency, in the case of the separation of several cardiac glycosides.

2. Experimental

2.1. Apparatus

MEKC experiments were carried out with a SpectraPhoresis 1000 (Thermo Separation Products) using PC 1000 software. The capillaries were untreated fused-silica capillaries (internal diameter: 50 μm, length 44 cm) with the detection window located at 8 cm from the capillary extremity.

MEEKC experiments were carried out with a PACE 2100 (Beckman) using Gold software. The capillaries were untreated fused-silica capillaries (internal diameter: 50 μm, length 47 cm) with the detection window located at 7 cm from the capillary extremity.

With both systems, the UV detection was achieved at 220 nm and the run temperature was 25°C.

2.2. Preparation of the migration electrolytes

In MEKC the background electrolyte consisted of 0.02 or 0.03 mol l^{-1} lithium tetraborate ($Li_2B_4O_7$) adjusted to the desired pH value with orthophosphoric acid. Various amounts of sodium or lithium dodecylsulfate (SDS or LiDS) and urea were added to the background electrolyte.

In MEEKC, the microemulsions (100 g for each batch) used were prepared by mixing (in the following order) the desired amounts of surfactant (SDS), co-surfactant (1-butanol), oil (heptane) and buffer (sodium tetraborate 0.05 mol l^{-1}, pH 9.3). Heptane has to be added after 1-butanol in order to avoid any heptane evaporation. The solutions were mixed by ultrasonification for 10 min and the transparent solutions were then left to stand for 30 min at room temperature prior to use. These microemulsions remained stable for at least several weeks when prepared in the appropriate manner, and when the solutions were stocked hermetically to avoid any change in composition consecutive to the volatilization of heptane.

2.3. Capillary conditioning procedures

Each new fused-silica capillary was rinsed with NaOH 1 mol l^{-1} for 30 min and then with deionised water for 15 min.

In MEKC, the capillary was washed with the running buffer for 2 min prior to each experiment. At the end of the day, the capillary was rinsed for 2 min with NaOH 1 mol l^{-1}, and for 5 min with deionised water.

In MEEKC, the capillary was washed with the running microemulsion for 5 min prior to each experiment. Every two runs, the capillary was rinsed for 10 min with the sodium tetraborate buffer ($Na_2B_4O_7$ 0.05 mol l^{-1}, pH 9.3). At the end of the day, the capillary was rinsed for 2 min with NaOH 0.1 mol l^{-1}, and for 5 min with deionised water.

2.4. Tracers for electroosmotic flow and pseudo-stationary phases

Methanol was used as a tracer for the electroosmotic flow in both MEKC and MEEKC.

As usual, Sudan III was used as a marker for the micelles in MEKC. In MEEKC, as recommended by

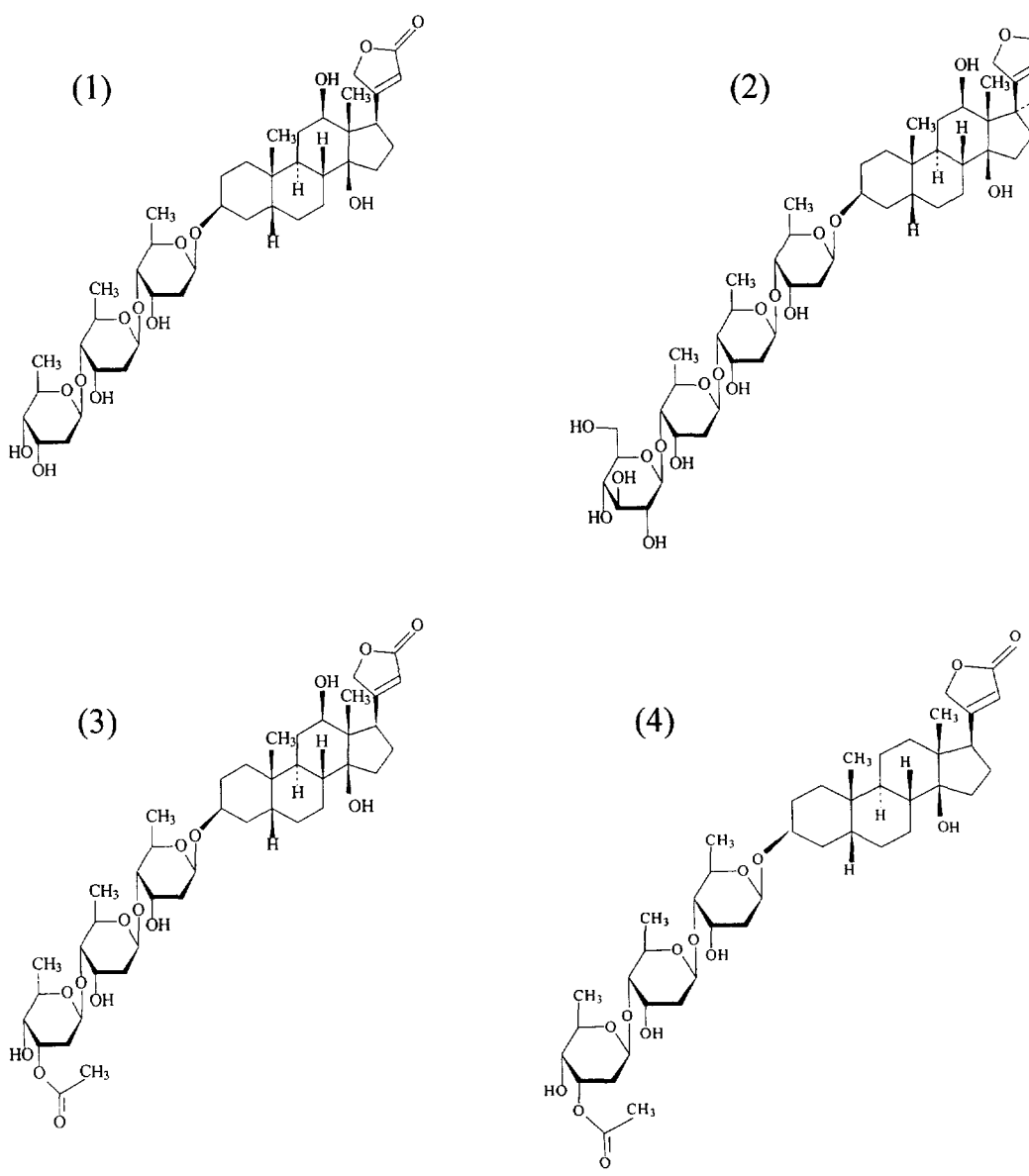

Fig. 1. Formula of the cardiac glycosides. (1) Digoxin, (2) deslanoside, (3) acetyldigoxin, (4) acetyldigitoxin.

Ishihama et al. [14], dodecylbenzene was used as a tracer for the microemulsion.

All these tracers were dissolved in the running electrolyte (micellar phase or microemulsion). In the case of the microemulsion, a very small amount of methanol was used in order to avoid any turbidity of the solution.

2.5. Sample preparation

Cardiac glycosides (acetyldigoxin, digoxin, acetyldigitoxin and deslanoside) the formula of which are given in Fig. 1, were a gift from Professeur Lachatre (University Teaching Hospital, Limoges, France). Stock solutions of these compounds were prepared in methanol and diluted with the running electrolyte prior to use in MEKC. In MEEKC, owing to the detrimental effect of methanol on the stability of the microemulsion, the cardiac glycosides were directly dissolved in the separation medium.

3. Results and discussion

3.1. Micellar electrokinetic chromatography (MEKC)

No separation of the four compounds was observed in a 0.02 mol l^{-1} lithium tetraborate background electrolyte (pH 9.3) at a SDS concentration equal to 0.025 mol l^{-1}. An increase of the SDS concentration up to 0.05 mol l^{-1} allowed partial resolution of the digitalic compounds: deslanoside and digoxin (coeluting peaks) were separated from acetyldigoxin and acetyldigitoxin (coeluting peaks). A further increase of the SDS concentration did not allow any improvement.

The influence of the pH of the background electrolyte (ranging from 6.0 to 9.3) was also studied in a 0.03 mol l^{-1} lithium tetraborate electrolyte containing SDS at a 0.05 mol l^{-1} concentration. Despite an extended migration time window at pH 6.0, no baseline separation of the four compounds could be observed (Fig. 2a).

The influence of the dodecyl sulfate counterion (Li^+ or Na^+) was also investigated, since the use of LiDS has been shown to improve efficiency for the most hydrophobic compounds [17]. No significant improvement was observed, and SDS was used for further work owing to its lower cost. Moreover, the

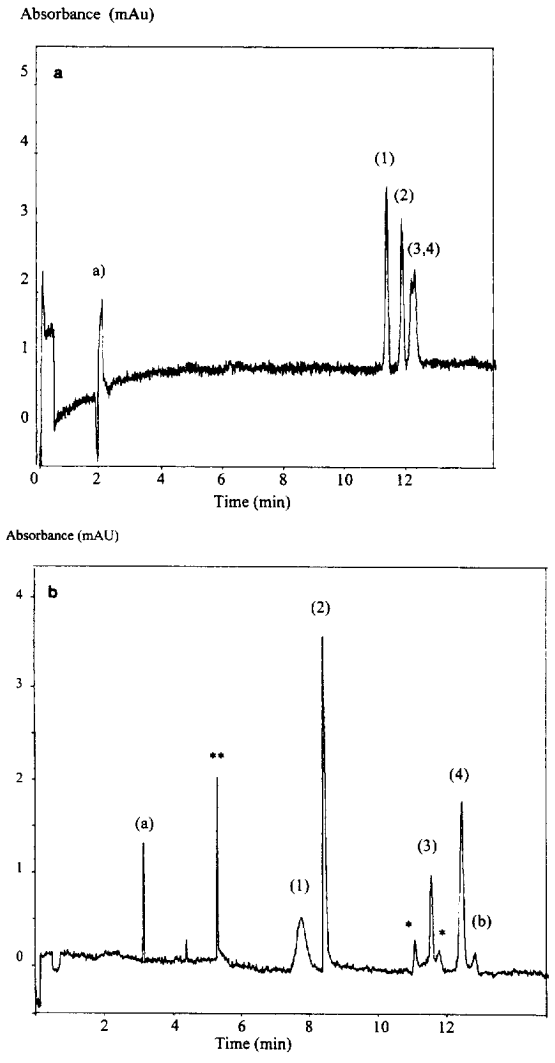

Fig. 2. (a) Separation of four cardiac glycosides in MEKC. Electrolyte: $Li_2B_4O_7$ 0.03 mol l^{-1}, pH=6.0, SDS 0.05 mol l^{-1}. Fused-silica capillary: L=44 cm, l=38 cm, I.D.=50mm. V=25 kV, T=25°C, UV detection at 220 nm. (1) Deslanoside, (2) digoxin, (3) acetyldigoxin, (4) acetyldigitoxin. (b) Separation of four cardiac glycosides by MEKC in presence of urea. Electrolyte: $Li_2B_4O_7$ 0.02 mol l^{-1}, pH=6.0, SDS 0.035 mol l^{-1}, urea 7 mol l^{-1}. Fused-silica capillary: L=44 cm, l=36 cm, I.D.=50 μm. V=30 kV, T=25°C, UV detection at 220 nm. (a) Methanol: electroosmotic flow marker; (b) Sudan III: micelle marker. (1) Digoxin, (2) deslanoside, (3) acetyldigoxin, (4) acetyldigitoxin. *=impurities, **=Sudan III impurity.

addition of organic modifier (methanol or acetonitrile) in order to reduce the distribution equilibrium of the digitalic compounds between the background electrolyte and the pseudo-stationary phase remained unsuccessful.

Finally, the influence of the addition of urea on the separation of the cardiac glycosides was investigated. As demonstrated in the literature [18], the addition of urea in micellar electrokinetic chromatography allowed the reduction of the distribution of the solutes between the background electrolyte and the pseudo-stationary phase by increasing the solubility of the hydrophobic compounds in aqueous media. It may also be noticed that the elution order of deslanoside and digoxin was reversed when urea was added to the background electrolyte.

The four cardiac glycosides were baseline resolved for an urea concentration of 7 mol l^{-1} (Fig. 2b). However, such a concentration lead to a dramatic loss of efficiency for digoxin (3500 theoretical plates for a 7 mol l^{-1} urea concentration to be compared with the 80 000 plates obtained under classical MEKC conditions). Meanwhile, this effect is much less pronounced with other species (≠15% to 30% loss of efficiency).

3.2. Microemulsion electrokinetic chromatography (MEEKC)

Microemulsions used in this study consisted of sodium tetraborate buffer at pH 9.3/sodium dodecylsulfate (SDS)/1-butanol/heptane (see compositions in Table 1). Owing to the relative proportions of each constituent and to the negative charge of the surfactant (SDS), such microemulsions consisted of negatively charged microdroplets of oil in water, stabilized in the aqueous medium by the surfactant and cosurfactant located at their surface. Under the alkaline conditions (pH 9.3), the electroosmotic mobility of the bulk solution (ranging from 3.90×10^{-4} to 3.63×10^{-4} $cm^2\ s^{-1}\ V^{-1}$) was slightly higher than the electrophoretic mobility of the microemulsion (ranging from -2.58×10^{-4} to -2.87×10^{-4} $cm^2\ s^{-1}\ V^{1}$). Consequently, the microemulsion and the neutral solutes migrated towards the cathodic end at which detection took place, the migration order being governed by the relative hydrophobicity of the solutes. The four compounds of interest were baselined resolved in an analysis time ranging from 8 to 12 min. The corresponding electropherograms are represented in Fig. 3. Whatever the composition of the microemulsion, the electroosmotic mobility and the electrophoretic mobility of the microemulsion were not strongly affected (see Table 2). The influence of the microemulsion composition on the capacity factors and selectivities is hard to ascertain since it was necessary to balance the variation of the composition of one component in the microemulsion by the variation of another. Moreover the composition range studied was limited by the stability of the microemulsion; for example, a decrease of the SDS concentration implies a reduction of the heptane proportion to avoid demixing of the microemulsion. Although, the results indicate that the variation of the microemulsion composition, inside a very narrow range, allows the manipulation of the retention and the selectivity. Another advantage of MEEKC over MEKC in the presence of urea lies in the high efficiencies that can be obtained (see Table 2).

4. Conclusion

These results show that MEKC, in the presence of urea, allows the separation of the cardiac glycosides of interest. The addition of urea to the micellar

Table 1
Composition (relative percentages by weights) of the microemulsions A to D. Borate buffer: $Na_2B_4O_7$ 0.05 mol l^{-1}, pH 9.2

	Microemulsion A	Microemulsion B	Microemulsion C	Microemulsion D
SDS (%)	1.66	0.83	1.20	0.83
Heptane (%)	0.81	0.42	0.60	0.20
1-Butanol (%)	6.61	6.61	6.61	6.61
Borate buffer (%)	90.92	92.14	91.59	92.36

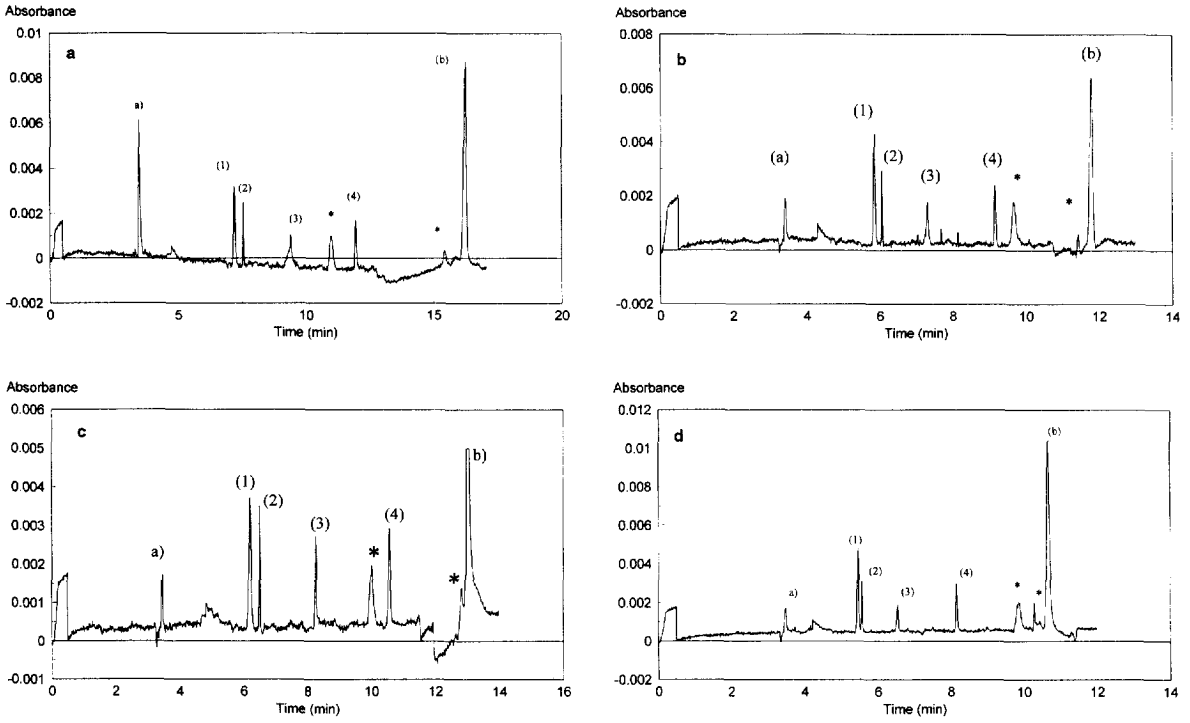

Fig. 3. Separation of four cardiac glycosides by MEEKC. (a) Microemulsion A, (b) microemulsion B, (c) microemulsion C, (d) microemulsion D. Fused-silica capillary: L=47cm, l=40 cm, I.D.=50 μm. V=25 kV, T=25°C, UV detection at 220 nm. (a) Methanol, (1) deslanoside, (2) digoxin, (3) acetyldigoxin, (4) acetyldigitoxin, (*) impurity, (b) dodecylbenzene.

medium is very useful for the separation of such hydrophobic compounds from the standpoint of increasing their solubility in aqueous media and extending the migration window. However, this improved resolution of the digitalic compounds is obtained at the expense of migration times and efficiencies.

MEEKC allows the resolution of the four compounds of interest whatever the composition of the microemulsion, in a short analysis time and with

Table 2
Electroosmotic mobilities, electrophoretic mobilities of the microemulsions, capacity factors of the digitalic compounds, efficiencies (N) with microemulsions A, B, C and D

Micro-emulsion	Mobilities $cm^2 s^{-1} V^{-1}$	Deslanoside	Digoxin	Acetyldigoxin	Acetyldigitoxine
A	$m_{eo}=3.63\times10^{-4}$	$k'=1.93$	$k'=2.23$	$k'=4.08$	$k'=9.26$
	$m_{ep}=-2.86\times10^{-4}$	$N \# 50\,000$	$N \# 400\,000$	$N \# 55\,000$	$N \# 158\,000$
B	$m_{eo}=3.90\times10^{-4}$	$k'=1.61$	$k'=1.81$	$k'=3.32$	$k'=8.25$
	$m_{ep}=-2.83\times10^{-4}$	$N \# 77\,000$	$N \# 450\,000$	$N \# 132\,000$	$N \# 166\,000$
C	$m_{eo}=3.83\times10^{-4}$	$k'=1.71$	$k'=1.97$	$k'=4.17$	$k'=11.90$
	$m_{ep}=-2.87\times10^{-4}$				
D	$m_{eo}=3.75\times10^{-4}$	$k'=1.30$	$k'=1.39$	$k'=2.45$	$k'=6.09$
	$m_{ep}=-2.58\times10^{-4}$				

efficiencies higher than those obtained with MEKC in urea.

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