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Analysis of basic antimalarial drugs by CZE and MEKC. Part 1 — critical factors affecting separation*

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Abstract: The separations of 11 antimalarial drugs and metabolites are shown by CZE at low pH and by MEKC at high pH. CZE is shown to be superior to MEKC in resolution capability for these compounds under the conditions examined. Both are shown to provide different selectivities to those obtained by ion-pair reversed-phase HPLC. The effect of sample injection solvent is examined in CZE and it is shown that field-amplified sample injection is effective for these compounds. In addition, it is shown that injection of sample in an organic solvent such as methanol augments the stacking of analytes resulting in lower detection limits. The limit of detection of some antimalarials in a urine matrix is reported.

Keywords: *CZE; MEKC; antimalarials; detection limits; sample injection.*

Introduction

In spite of the expanding literature appearing concerned with applications of capillary electrophoresis, relatively few papers have dealt with the separation and quantitation of basic drugs by the different modes of electrophoresis. In recent reviews [1, 2] less than 5% of papers quoted dealt with separation or analysis of basic drugs. This is unexpected since analysis of basic compounds by HPLC is often more difficult than that of acidic or neutral species because of the tailing often observed with these compounds. Several approaches using mobile phase additives [3] and dedicated stationary phases [4] have been used in attempts to overcome such tailing, which can result in poor resolution and inadequate quantitation. Also, as has been pointed out recently [5], there is an increasing need for multiple drug analysis to be carried out for clinical purposes and HPLC, with its limited peak capacity, is arguably not ideal for this purpose.

The high theoretical plate numbers achievable in capillary electrophoresis techniques, in contrast, are conducive to increased peak capacity. In addition, for basic compounds, the alternative modes of capillary electrophoresis under conditions of low or zero electroendosmotic flow at low pH or micellar electrokinetic chromatography at high pH when the ionization of such basic drugs is essentially suppressed are available.

It is intended in the present work to evaluate the utility of CZE and MEKC as alternative methods for the separation of a test group of commonly used antimalarial drugs, to compare the separations obtained by these alternative techniques and also to compare these with previous separations obtained using reversed-phase ion-pairing techniques in HPLC.

Quantitative detection limits attainable by CZE are usually regarded as being inferior to those using HPLC due to constraints of sample size and restricted path length when using UV absorbance detection. This is often regarded as a major disadvantage in the use of CZE for quantitative analysis. The effectiveness of field amplified sample injection will be evaluated for these drugs with a view to establishing their detection limits, using UV detection, in a biological matrix such as urine. This is an investigation into this critical analytical characteristic preliminary to a more complete investigation of quantitative aspects which will be reported in a future publication.

The overall purpose of the work is to assess the potential use of capillary electrophoresis techniques as alternative assay methods to HPLC in the analysis of antimalarial drugs for clinical and pharmacokinetic studies.

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Experimental

The electrophoresis equipment used was an Isco 3850 Capillary Electropherograph™ equipped with all injection modes. For this investigation the electrokinetic mode of injection was used with a standardized time of 10 s at 5 kV. The capillary (total length 65 cm) was untreated silica (50 μm i.d.) with an effective length between injection and detector of 50 cm. Detection was by UV at 254 nm and a constant applied voltage of 20 kV was used. The capillary was washed with 0.1 M sodium hydroxide solution between injections and rinsed with deionized water. Solid-phase extraction was by C_{18} Bond-Elut cartridges using a 10 sample vacuum manifold.

Antimalarial drug samples were obtained from a variety of sources. Proguanil (P), cycloguanil (CG) 4-chlorophenylbiguanide (CPB), cyclochlorproguanil (CCP), chlorproguanil (CP), primaquine (PQ) and pyrimethamine (PM) were supplied as gifts from Imperial Chemical Industries. Chloroquine (CQ) and desethylchloroquine (DCQ) were kindly supplied by The World Health Organisation. Mefloquine (M) was donated by Roche and quinine (Q) was obtained commercially from Aldrich. Sodium dodecylsulphate (SDS) was obtained from Fisons. Acetonitrile and methanol were supplied by Rathburn Chemicals. Water used for analysis was purified by distillation and treatment in a Millipore Milli-Q system. All other reagents used were of AnalaR or equivalent grade.

Results and Discussion

CZE separations

The optimum separation which was obtained using capillary zone electrophoresis from an aqueous solution of the 11 antimalarials in the test set is shown in Fig. 1. It can be seen that resolution >1 is obtained among all compounds with the exception of chloroquine and quinine. The resolution between this pair is estimated as 0.6. Theoretical plate numbers generated in this system ranged from 30,000 to 100,000 among the different solutes. The solvent for this optimum separation consisted of aqueous 25 mM disodium hydrogen phosphate adjusted to pH 2 by the addition of phosphoric acid. This composition was determined by examination of pH, and buffer concentration. The effect of acetonitrile concentration was also studied in view of the comparison to be made with micellar electrokinetic chromatography. Acetonitrile was found to decrease the migration times of all solutes with consequent loss of resolution. Of particular practical interest is the separation of CQ, P and their respective metabolites DCQ, CG and CPB by this technique since co-administration of P and CQ is a common prophylactic regimen. The separation obtained

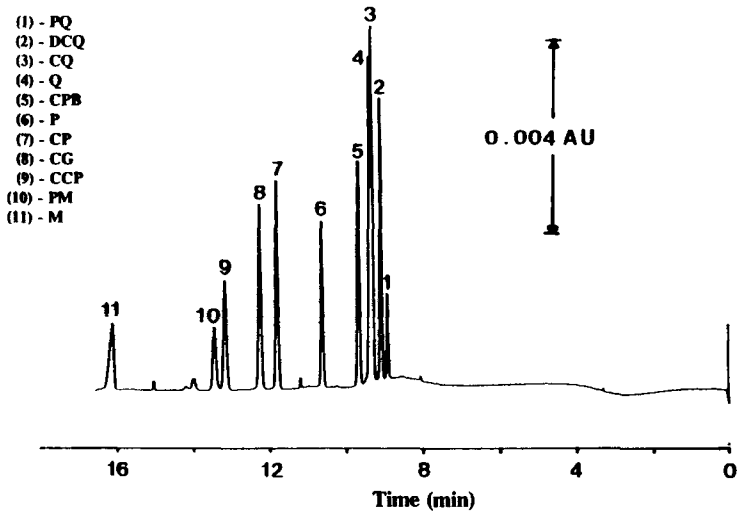

Figure 1
Electropherogram showing the separation for the group of antimalarials and metabolites. Conditions — solvent, 25 mM disodium hydrogen phosphate, pH 2.0 capillary, 50 μm i.d. × 65 cm (effective length 50 cm), applied voltage 20 kV, detection UV absorbance at 254 nm, injection 10 s at 5 kV electrokinetically in water. Concentration of antimalarials is 15 μg ml^{-1}.

for this limited group from urine is shown in Fig. 2(a) and is discussed further below.

MEKC separations

The behaviour of the test set of solutes under MEKC conditions was studied in a solvent of 25 mM disodium hydrogen phosphate adjusted to pH 10 with sodium hydroxide with 10% v/v acetonitrile and containing different concentrations of SDS. At pH 10 it is assumed that all of the basic solutes in the test set are essentially neutral so that separation should be as a result of preferential solubilization in the micelles formed by the SDS [6]. The effect of SDS concentration on migration time over the range 4–80 mM is shown in Fig. 3. It is seen that, as previously reported [6–8], there is a general increase of migration time with surfactant concentration. The resolution, however, was found to be markedly inferior to that obtained under CZE conditions and separation of all antimalarials was not possible. While it was not possible to separate chloroquine from its desethyl metabolite using low concentrations of SDS [Fig. 4(a)] it was, however, possible to separate proguanil and its metabolites [Fig. 4(b)]. It would also appear from Fig. 3 that solubilization is not the only retention process occurring since there are finite migration times for all the compounds below the accepted CMC for SDS [9]. This effect is apparent in other MEKC studies [6–8].

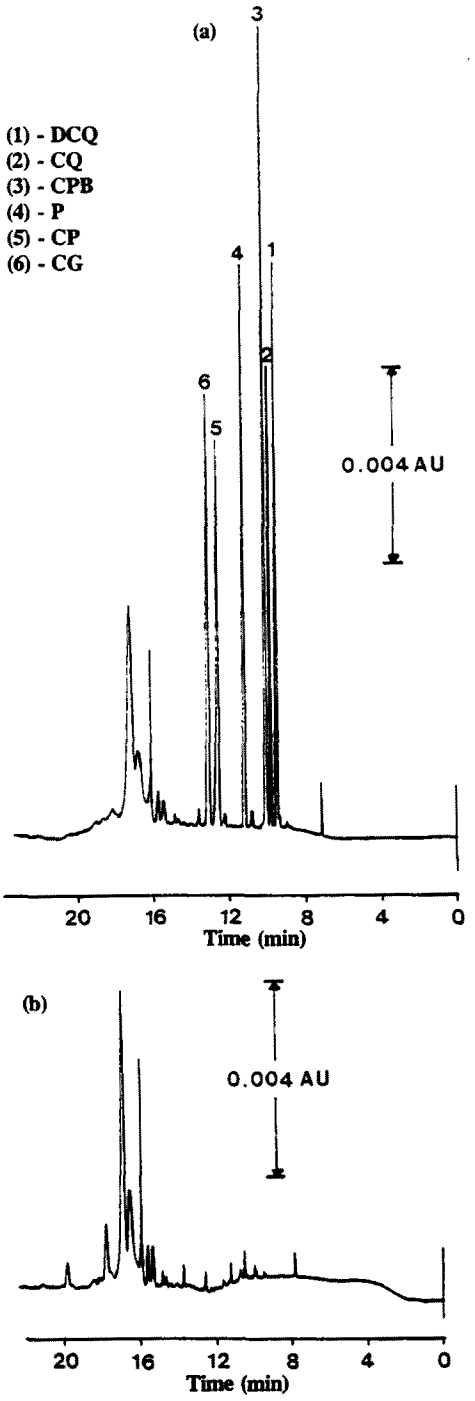

Figure 2
Electropherograms showing the separation of (a) P and CQ with their respective metabolites in urine with CP as internal standard and (b) blank urine. Conditions as for Fig. 1 except injection 10 s at 5 kV electrokinetically in methanol. Concentration of antimalarials is 1.5 μg ml^{-1}.

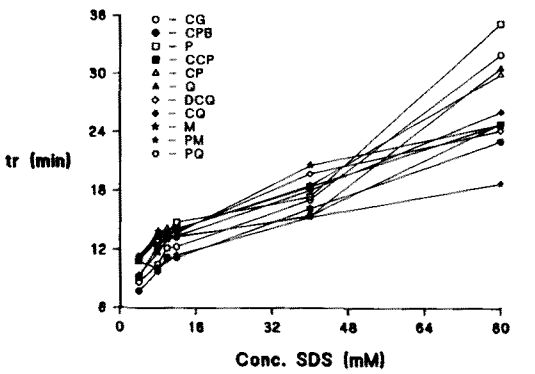

Figure 3
Variation of migration time (tr) for the antimalarials and metabolites as a function of surfactant [SDS] concentration. Solvent 25 mM disodium hydrogen orthophosphate, 10% acetonitrile, pH 10.0. Other conditions as for Fig. 1.

Comparison with ion-pair HPLC

It has been shown previously [10] that there is good correlation between retention times on a reversed-phase ion-pairing chromatographic system and log octanol–water coefficients for such antimalarials. Thus, confirmation that solubilization due to hydrophobicity is the prime retention factor in MEKC should be seen as a significant correlation between MEKC migration times and retention times in

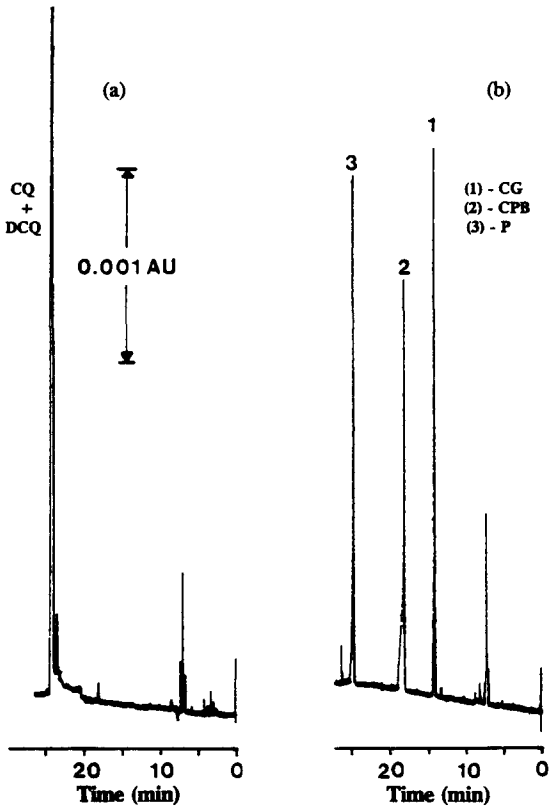

Figure 4
Electropherograms showing the separation of (a) CQ and metabolite (b) P and metabolites. Solvent, 25 mM disodium hydrogen phosphate, 10% acetonitrile, 10 mM SDS, pH 10.0. Other conditions as for Fig. 1. Concentration of antimalarials is 15 μg ml^{-1}.

ion-pair HPLC even though these measurements are made under different conditions and ionization. Likewise, lack of correlation between migration times in CZE and retention times in ion-pair HPLC should indicate the degree of orthogonality of the two techniques and this give an indication of their complementary use in drug analysis. Using reversed-phase ion-pair HPLC data from ref. 5 statistical analysis showed no significant correlation at the 5% level between the migration times in either of the electrophoresis methods with the HPLC retention times. While this might be expected for the CZE set it indicates that the mechanism of retention in MEKC may be more complex than partitioning with the micelles of SDS.

Effect of injection solvent

This is of relevance in separation of such compounds during analysis in biological matrices where there will normally be a pre-treatment stage involving liquid–liquid or solid-phase extraction resulting in the analytes being present in solvents of differing ionic composition to that of the original matrix and possibly containing organic solvents such as methanol.

Although the injection solvent used in CZE is normally the running buffer [2, 11], it has been shown previously [12, 13] that significant increases in on-column concentration can be achieved by the process of field-amplified sample injection (FASI). This stacking effect can be enhanced by the prior injection of a water plug into the capillary [13]. The applicability of this technique to the set of antimalarials was investigated, firstly because the magnitude of the stacking effect varies with individual analytes and also because the detection limits achievable using FASI will represent the optimum sensitivity which can be attained using purely aqueous solvents.

Table 1 shows the relative sensitivities, measured as peak height, relative to that obtained in running buffer, of injections in running buffer, diluted buffer and water with and without prior injection of a water plug. It is

Table 1
The effect of injection solvent on relative peak height for the set of antimalarial drugs

	Peak height relative to that in running buffer			
Compound	25 mM DSHP	2.5 mM DSHP	Water	Water with water plug
PQ	1	4.3	25.6	63.2
DCQ	1	4.8	36.6	88.6
CQ	1	4.5	34.5	79.4
Q	1	3.7	27.0	81.0
CPB	1	3.8	19.6	53.9
P	1	3.4	16.3	49.2
CP	1	3.3	16.0	50.1
CG	1	3.8	22.5	70.9
CCP	1	3.8	22.5	74.3
PM	1	2.0	12.0	40.7
M	1	3.5	17.5	40.4

evident that appreciable increases in sensitivity of detection for these compounds can be obtained by such stacking injection methods. It is estimated that the limit of detection in aqueous solution, employing FASI and including the injection of a water plug, is 40 ng ml^{-1}. Direct injection from aqueous solution in the absence of prior injection of a water plug yields a detection limit of 150 ng ml^{-1}. The possible alteration of sample composition by the essentially electromigration method required would not be significant for the present test set since they are all basic in nature.

In a separate series of experiments to examine the effect of injection in organic solvent on detection limits and also on the separation of analytes which could be obtained following solid-phase extraction from a urine matrix, urine was spiked with the antimalarials P and CQ and their corresponding metabolites at lower concentrations than those used for the aqueous solutions. Following solid-phase extraction of 1 ml of urine and washing with buffer (pH 2) followed by water, the analytes were eluted from the solid-phase cartridge in 1 ml methanol and a sample injected from this solution, thus involving no preconcentration of the analyte. The resulting electropherogram in CZE, together with that of a blank urine, obtained by injecting the methanol extract is shown in Fig. 2(a), (b). No sample matrix interference is present and the resolution among all compounds has been maintained. It was also observed that there was an appreciable increase in peak height for all compounds over that obtained by the injection of aqueous samples at the same concentration. We have not seen reports of such injections in organic solvents in the literature.

To verify the generality of this apparent increase in sensitivity, samples of this limited set of compounds were injected in solvents containing different concentrations of methanol. The results are shown graphically in Fig. 5 and indicate that the peak height increases with methanol concentration to a maximum of about 18–34 times that of the sample injected in water without the incorporation of a water plug, depending upon the compound. Using methanol as the injection solvent, the estimated detection limit for these compounds following solid-phase extraction, at a signal-to-noise ratio of 3 is approximately 5 ng ml^{-1} which represents an approximately 8-fold improvement in detection limit over that found

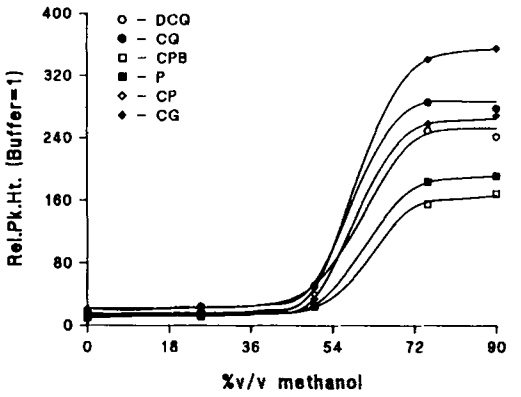

Figure 5
Variation of the relative peak height (running buffer = 1) with methanol concentration in the injection solvent for a set of antimalarials. Other conditions as for Fig. 1.

for field-amplified sample injection incorporating a separate water plug injection. In keeping with current practice in HPLC, appreciable preconcentration can be achieved subsequent to solid-phase extraction by evaporating the organic solvent and reconstituting in a smaller volume. This suggests that detection limits can be further reduced.

Conclusions

The above shows that direct CZE at low pH offers a useful alternative technique to ion-pair reversed-phase HPLC for the separation, in aqueous solution, of the major antimalarial drugs. It is also capable of resolving parent drug and major metabolites in a mixture of proguanil, chloroquine and their metabolites and shows different selectivities to those observed in HPLC. The increased peak efficiencies obtained will constitute a useful advance on chromatographic methods provided that adequate quantitation can be obtained. MEKC, under the conditions investigated, is not capable of comparable resolution.

It appears, given the general need to pretreat samples of drugs in biological fluids with the consequent preconcentration which is possible, that CZE has the required sensitivity to be useful in the quantitative detection of antimalarial drugs at least in urine. Development of assay procedures, evaluation of these and their validation will be the subject of Part 2 of this communication.

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