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Analysis of some pharmaceuticals by high voltage capillary zone electrophoresis*

K. D. ALTRIA† and C. F. SIMPSON

Analytical Science Group, Department of Chemistry, Birkbeck College, University of London, 20 Gordon St., London WC1H 0AJ, UK

Abstract: High voltage capillary zone electrophoresis (HVCZE) has been used to determine quinine, proflavine and other drugs. The technique may offer a useful alternative to chromatography in the analysis of pharmaceuticals.

Keywords: *Pharmaceutical analysis; high voltage capillary zone electrophoresis.*

Introduction

High voltage capillary zone electrophoresis (HVCZE) is a rapidly developing, highly efficient separation technique in which a large potential drop is applied across a narrow bore capillary. Charged species will electrophoretically migrate towards the appropriate electrode and are separated by differences in their electrophoretic mobilities. In addition, under the influence of the applied field, electroendosmotic (EEO) flow is generated in the capillary which transports solute molecules towards the detecting system independent of their individual charge. The characteristics of the EEO flow are such that minimal zone broadening occurs [1] as the solute travels along the capillary, which keeps zones sharp. Efficiencies of up to one million thereotical plates have been reported for protein separations [2].

The EEO flow direction and rate can be predictably controlled by a judicious choice of operating conditions [3, 4]. The mobility of a species is dependent upon its charge/mass ratio. The solute charge is related to both its pKa and the pH of the carrier electrolyte solution.

The HVCZE technique offers significant advantages, in terms of analysis time, when compared to conventional electrophoresis. The HVCZE method avoids labour-intensive procedures, such as gel formation and staining/destaining, involved in "normal" electrophoresis. Advantages over HPLC include limited capital expense (no expensive pumps or several different columns) and minimal running costs since solvent usage is in the order of 20 ml per day. However, the technique does suffer from the need for very sensitive detectors, no automated sampling devices and the lack of commercial apparatus.

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†To whom correspondence should be addressed.

To date, applications of HVCZE have largely been of a biological nature, e.g. the analysis of amino acids [1], proteins [2] and nucleotides [5]. However, the technique should be useful for the analysis of all ionic species. Neutral species may also be separated by electrokinetic chromatography [6] or solvophobic association [7].

The use of HVCZE for the analysis of some pharmaceuticals is described in the present paper.

Experimental

A dual polarity 0–60 kV power supply (Brandenburg, Croydon, Surrey, UK, Model No. 2928) was used. A fused silica capillary was supplied by Scientific Glass Engineering (Milton Keynes, Bedfordshire, UK). The system has previously been described [3], but the detection system used here comprised a modified Perkin–Elmer 2000 fluorimeter. Sample injection was by the "electromigration" technique as described elsewhere [1]. Data presented are the means of duplicate measurements.

All the reagents were analytical grade and supplied by British Drug Houses, (Poole, Dorset, UK). Solutions were prepared (by weight) in degassed, near conductivity water obtained from a Elgastat Spectrum Still, Model No. SC1 (High Wycombe, Bucks, UK).

A 100 cm × 75 μm i.d. capillary filled with 0.02 M phosphate buffer (81 cm to the optical centre of the detector) served as the separation tube operated at a working voltage of +30 kV.

Results and Discussion

Quinine is a widely used antimalarial drug which may, if taken in excess or by particularly sensitive people, have severe side-effects which include vomiting and hypotension. Many soft drinks and analgesic preparations contain quinine. Numerous publications have appeared on the analysis of quinine; methods include the use of HPLC [8] and another electrophoretic method, isotachophoresis [9].

The assay of quinine by HVCZE was typically completed within 10 min, with a precision relative standard deviation, (RSD) of 1.5% for 10 replicate migration time measurements. A calibration plot of quinine concentration, versus peak height, in the concentration range 1×10^{-3}–1×10^{-6}M, gave a linear relationship with a correlation coefficient of 0.99, the line passed throught the origin. The limit of detection was 1×10^{-7}M (3 mg l^{-1}).

The main source of zone broadening in HVCZE is molecular diffusion of the solute as it migrates along the capillary. Increasing the viscosity of the solvent reduces this broadening [4]. Figure 1a shows an electropherogram of 1×10^{-7}M quinine analysed with a 0.02 M phosphate buffer solvent. However, Fig. 1b shows the improvement upon increasing the viscosity by the addition of 30% (v/v) ethylene glycol to the phosphate buffer (Table 1).

Addition of ethylene glycol has been shown [10] to alter the EEO flow in a two-step process. Initially the EEO flow rate slows down, due to both an increase in solution viscosity and a deactivation of the capillary wall by adsorption of the ethylene glycol. After full-wall deactivation, the change in viscosity alone alters the EEO flow rate. Solute mobilities are also viscosity dependent and decrease in more viscous solutions. The migration time of a solute is proportional to both the electrophoretic mobility and electroendosmotic flow, and therefore sharply increases when the viscosity increases.

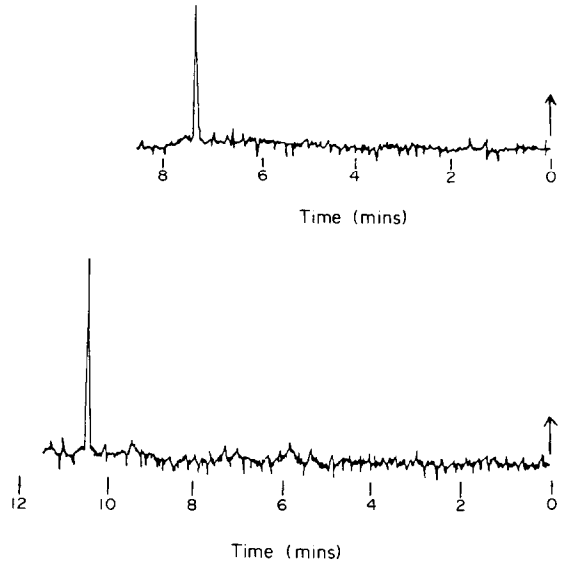

Figure 1
(a) Electropherogram of 10^{-7}M quinine (0.02 M phosphate buffer). (b) Electropherogram of 10^{-7}M quinine (0.02 M phosphate buffer with 30% ethylene glycol).

The increase in efficiency, as measured by number of thereotical plates, and migration times is shown below in Table 1.

The carrier electrolyte solution most widely used in HVCZE is 0.02 M phosphate buffer (pH 7). At this pH it is impossible to separate the geometrical isomers quinine and quinidine, as they have identical charge and mass. Quinine has two pKa values of 4.11 and 8.00, whereas quinidine has pKa values of 4.00 and 8.54. Distinct separation is possible when analysis is performed at pH 8.3 (Table 2). At this pH, quinine is a neutral species and is swept to the detector only by the EEO flow. In contrast, quinidine, which has a positive mobility at this pH, additionally migrates towards the detector, and hence is eluted first.

Table 1
Effect of viscosity change on migration time and peak efficiency

Solvent	Migration time (min)	Efficiency (No. of plates)
0.02 M Phosphate	7.2	50,000
0.02M M Phosphate with 30% (v/v) ethylene glycol	10.4	180,000

Table 2
Effect of pH change on the migration times of quinine and quinidine

pH	Solute	Retention time (min)
7.0	Quinine	7.2
7.0	Quinidine	7.2
8.3	Quinine	9.6
8.3	Quinidine	7.2

Analysis of proflavine, a wound disinfectant, was also shown to be possible by HVCZE. The limit of detection was found to be similar to that of quinine i.e. 1×10^{-7}M. A typical electropherogram is given in Fig. 2; the proflavine concentration was 5×10^{-6}M with a migration time of 5.4 min.

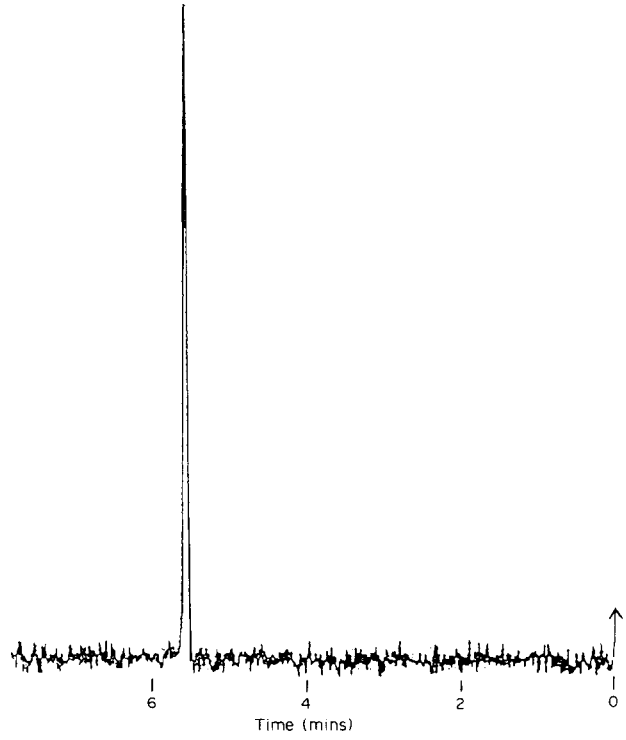

Figure 2
Electropherogram of 5×10^{-6}M proflavine.

Another interesting application of HVCZE is to monitor the degradation of a drug during storage. This was demonstrated by a study of the degradation of quinacrine, a drug which possesses antimalarial properties. Quinacrine hydrolyses to give a fluorescent degradation product, 6-chloro-2-methoxyacridone [11]. After two weeks of storage at 20°C the quinacrine had degraded by 44% (as measured by peak heights). Figure 3a and b show electropherograms before, and after the storage period, respectively.

The poor peak shapes and low efficiencies may be attributed to solute adsorption on to the surface of the capillary wall. This may be reduced by using a different carrier electrolyte solution. It may also be possible to monitor drug degradation in order to perform kinetic studies. The slight variation in migration times of the quinacrine is attributable to the use of two different working capillaries.

Sample introduction can be achieved by the electromigration method [1] in which the capillary is dipped into a sample reservoir and a voltage applied for a few seconds. A small aliquot of sample migrates into the capillary which is then analysed. The amount of sample migrating into the capillary is related to the magnitude and sign of both the solute mobility and EEO coefficient. Disproportionate sampling occurs as a more mobile

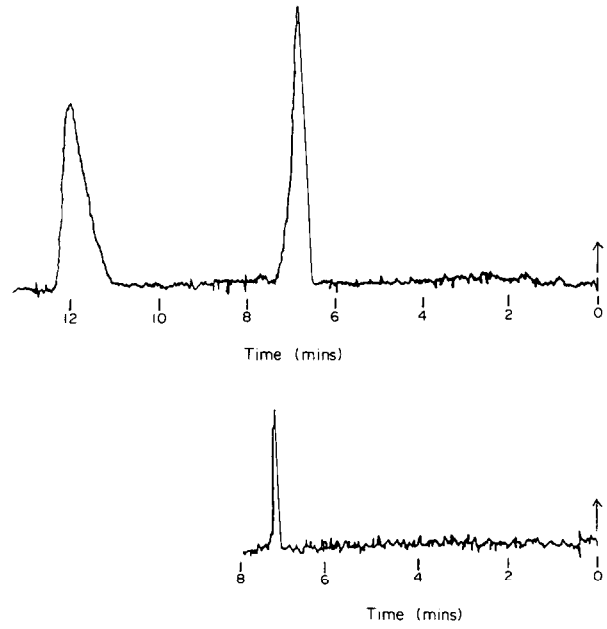

Figure 3
(a) Electropherogram of 10^{-6}M quinacrine. (b) Electropherogram of 10^{-6}M quinacrine after 14 days storage.

species appears more concentrated than a less mobile species of the same concentration. Use of an appropriate internal standard permits compensation.

Anthracene has been found to be a suitable internal standard for the analysis of either quinine or quinacrine, as this is a neutral species under the operating conditions used. Owing to limited anthracene water solubility a non-aqueous solvent was necessary; the solvent used was a mixture of acetonitrile — 0.02 M phosphate buffer (50:50, v/v). Use of non-aqueous solvents in HVCZE has been previously reported [12]. A typical electropherogram of a mixture of quinine, quinacrine and anthracene is shown in Fig. 4. The order of migration was quinacrine (A), quinine (B) and lastly the neutral species anthracene (C).

Peak heights were found to be a more reliable method of quantitation than peak area since the length of sampling time and applied voltage affects the volume of sample "injected". A precision of 1.4% (RSD) was obtained from 10 replicate peak-height measurements. An automated sample injection system would probably reduce some of this variation in sampling. This is currently under investigation.

It has also been shown that analysis of riboflavin-5-phosphate, a vitamin, is possible. Figure 5 shows an electropherogram of riboflavin-5-phosphate with a migration time of 10.2 min. Further work on the analysis of vitamins requires use of a UV detector; this is currently under development.

Conclusions

The HVCZE technique provides a fast and reliable method for the determination of some pharmaceuticals. Quantitation, use of internal standards, non-aqueous solvents, and monitoring of sample degradation are possible. Electrophoretic mobilities and EEO

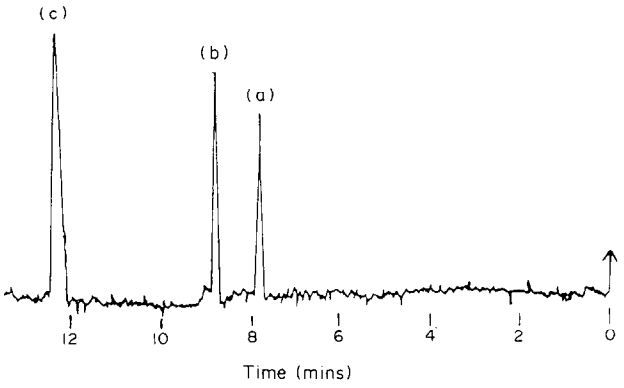

Figure 4
Electropherogram of a quinacrine, quinine and anthracene solution.

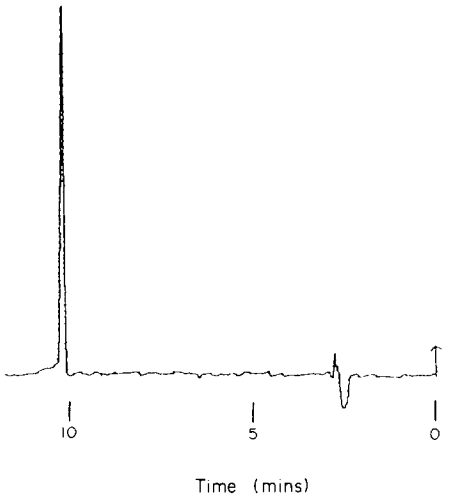

Figure 5
Electropherogram of 10^{-5}M riboflavin-5-phosphate.

flow rates can be predictably manipulated to facilitate difficult separations. The technique may offer a useful alternative to chromatography as performance characteristics are similar.

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