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Short communication

Separation of cimetidine and related materials by aqueous and non-aqueous capillary electrophoresis[1]

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Abstract

Capillary electrophoresis (CE) has been shown to be a highly efficient separation method well suited to the analysis of pharmaceutical products. The separation of impurities and degradation products from the compound of interest often requires the high resolving power of which CE techniques are capable. The majority of reported separations have been performed in aqueous media. However, the addition of small amounts of organic modifier may be used to effect changes in selectivity and improve resolution. Recent reports have suggested that the use of completely non-aqueous separation media allow improved selectivity and are beneficial for the analysis of hydrophobic materials. To investigate this we have undertaken the separation of a drug substance and a series of associated impurities and degradation products using both aqueous and non-aqueous media. The data obtained clearly indicates that highly efficient separations may be accomplished in non-aqueous systems and that analysis of hydrophobic materials becomes possible. Selectivities and efficiencies for the separations are described indicating that complementary information can be obtained.

Keywords: Buffer composition; Pharmaceutical analysis; Cimetidine

1. Introduction

The development and application of cimetidine for the relief and cure of gastric ulcers, and other disorders related to the production of excess stomach acid, was a major breakthrough in the treatment of such complaints. After many years of successful use, this and other similar drugs are now available without prescription. Control of the contents of commercially available medication is stringent and pharmaceutical companies have developed analytical methods to determine the purity of their products. Methods based on the use of HPLC have been generally employed but increasingly capillary electrophoretic separations [1] of drugs from associated impurities and degradation products have generated considerable interest.

The high efficiency, short migration times and rapid method development obtainable with capillary electrophoresis (CE) methods have many attractions in the area of pharmaceutical analysis. Standard CE procedures employ an electrolyte dissolved in water, often at high pH (>7) to generate the maximum electroosmotic flow for fast analysis. As many pharmaceutical products are basic separation would be best carried out at an acidic pH so as to ionise the

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materials. However, at low pH adsorption of cationic material onto the capillary wall may lead to peak tailing thus reducing efficiency. Procedures have been developed to overcome this, particularly the use of coated capillaries, which may either eliminate or considerably reduce the EOF but this may lead to increased analysis times.

In seeking to develop a CE method for the separation of cimetidine and its impurities an aqueous electrolyte media was employed but peak tailing was observed for some components at low pH and two compounds did not afford analysis due to their low solubility in the aqueous system. We therefore chose to investigate the possible use of non-aqueous media for the separation of cimetidine from its analogues, impurities and degradation products.

Addition of organic solvents, usually acetonitrile or methanol, to the aqueous separation media employed in CZE is well established [2,3] and may offer improved selectivity. The amount of solvent added is usually less than 20% by volume. Recently, literature reports [4–7] have appeared advocating the use of completely non-aqueous media for the separation of analytes and, in particular, hydrophobic materials. The criteria for choosing a suitable solvent for non-aqueous CE have been outlined by Korchemnaya et al. [8] with dielectric constant and viscosity being suggested as key parameters. A further consideration is the nature of the background electrolyte that may be used and the effective acidity/basicity of the final solution.

In this paper the separation of cimetidine and other related compounds in aqueous and non-aqueous media is described. Comparison of the efficiencies, selectivities and analysis times of the two different systems is made.

2. Experimental

2.1. Apparatus

All separations were performed using a Crystal CE Model 310 instrument (Thermo–Unicam, Cambridge, UK). On-column UV detection was performed at a wavelength of 230 nm using a Phillips model PU 4225 detector. Electropherograms were recorded using a Hewlett–Packard model 3395 integrator. Fused-silica capillaries of 50 μm I.D.×375 μm O.D. were purchased from Composite Metal Services (Hallow, UK). Separate capillaries were used for the aqueous and non-aqueous separations. The total length of each capillary was 60 cm and the effective length (i.e. distance from inlet to detector) was 44 cm. All samples and separation media were degassed prior to analysis using a Branson 1210 ultrasonic bath. All pH measurements were determined using a Hanna Instruments Checker which was calibrated using solutions of known pH.

2.2. Reagents

Cimetidine and related compounds were obtained from SmithKline Beecham (Harlow, UK) and their structures are shown in Fig. 1. Acetonitrile (ACN) and methanol of HPLC grade and ammonium acetate of reagent grade were purchased from Aldrich (Poole, UK). All other materials were of reagent grade or better. Borate and phosphate buffers were prepared using sodium tetraborate and sodium dihydrogenphosphate, respectively.

2.3. Procedures

2.3.1. Aqueous separations

Individual stock solutions (1000 mg l^{-1}) of each compound were prepared in water and diluted to a working concentration of 100 mg l^{-1} when required using the relevant buffer. The capillary was rinsed between separations with NaOH [0.1 *M*, 2000 mbar (1 mbar=100 Pa), 2 min], followed by water (2000 mbar, 0.8 min) and finally the relevant buffer (2000 mbar, 3 min). All separations were performed at an applied voltage of 30 kV and all injections were performed hydrodynamically at a pressure of 25 mbar for 0.2 min (corresponding to an injection volume of ~7.7 nl).

2.3.2. Non-aqueous separations

Individual stock solutions (1000 mg l^{-1}) of each compound were prepared in ACN–MeOH (50:50, v/v) containing 1% (*v*/*v*) acetic acid and diluted to a working concentration of 100 mg l^{-1} when required using the relevant separation media. At the start of each working day the capillary was rinsed with

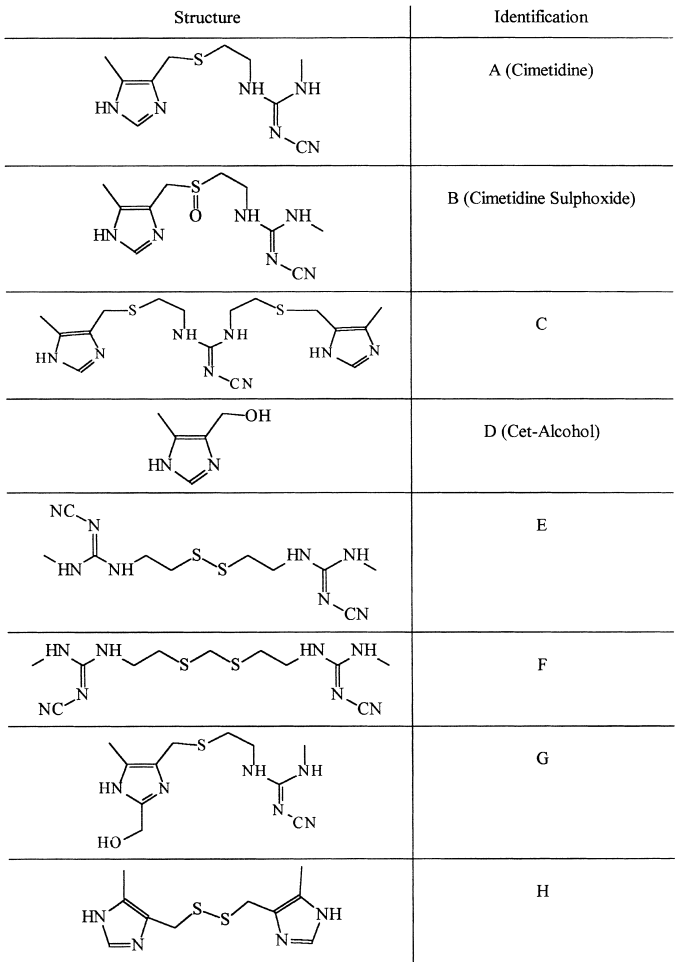

Fig. 1. Structures of cimetidine (A), associated impurities (C–H) and degradation product (B).

NaOH (0.1 *M*, 2000 mbar, 5 min), followed by ACN–MeOH (70:30, v/v) (2000 mbar, 5 min) and finally the relevant separation media (2000 mbar, 10 min). The capillary was only rinsed with the relevant separation media between separations for a period of 2 min. Initial method development was performed at an applied voltage of 20 kV. Subsequent separations were performed at an applied voltage of 30 kV. All injections were performed hydrodynamically at a pressure of 25 mbar for 0.2 min (injection volumes are viscosity dependent thus for the non-aqueous systems, where the viscosity is expected to be less than that of aqueous media, they will be in excess of 7.7 nl).

3. Results and discussion

3.1. Aqueous separation

Previous work [9,10] published on cimetidine and related compounds reported separations at different buffer pHs namely pH 7 and pH 2. Our initial development work, using cimetidine and two other compounds (A, B and C), was conducted over a range of pH values. As expected all three co-eluted at pH 9.15 (10 m*M* borate), with a migration time equal to that of the neutral marker (thiourea), thus confirming that they are uncharged at high pH and migrate at a rate equal to the electroosmatic flow

(EOF). At pH 7.14 (10 m*M* phosphate) only compound B was fully resolved from the other two components which again co-eluted with the EOF. Further successive reductions of pH led to improvement in resolution with full separation of all three components (Fig. 2) occurring at pH 3.14 (10 m*M* phosphate).

Using the same experimental conditions that allowed full separation of the three component mixture, a further five compounds were analysed. Two of these, namely compounds E and F, were of insufficient solubility in water to allow investigation and a further two components (C and D) were found to co-elute (Table 1). The separation of a five component mixture was achieved and the resulting electropherogram is shown in Fig. 3. Separation of all components is complete in under 5 min but there is some tailing for the final two peaks. Increasing the buffer concentration (10 m*M*, 15 m*M* and 20 m*M*) was found to improve the peak shape but did so at the expense of resolution. Addition of acetonitrile (10% and 20% by volume) offered no improvement in peak shape and proved detrimental to both resolution and analysis time. A combination of increased buffer concentration and the addition of organic solvent provided no improvement in either peak shape or resolution.

Fig. 2. Electropherogram of compounds A (cimetidine), B and C (migration times 3.99 min, 2.67 min and 3.02 min resp.). Conditions: electrolyte – 10 m*M* sodium dihydrogenphosphate, pH 3.14, separation voltage 30 kV.

Table 1
Migration times of compounds A–H

Compound	Migration time/min	
	Aqueous	Non-aqueous
A	3.94	5.21
B	2.66	3.60
C	3.00	5.07
D	3.00	4.74
E	–	5.47
F	–	5.46
G	4.17	5.26
H	2.52	4.79

Conditions: aqueous - 10 m*M* phosphate, pH 3.14, separation voltage 30 kV; non-aqueous - 25 m*M* ammonium acetate in acetonitrile–methanol (70:30, *v*/*v*)+1% acetic acid, pH* 6.7, separation voltage 20 kV.

The reproducibility of the separation was investigated and the R.S.D. was found to be under 1% for all components ($n=5$). However, to achieve this level of reproducibility regular replenishment (every five injections) of the buffer vials was required. Mean plate numbers (N) [calculated using $N=16(t_m/w_b)^2$ where t_m - migration time and w_b - peak width at base] ranged from 47 000 to 185 000 plates m^{-1} thus demonstrating the high efficiencies that can be achieved with CE techniques.

3.2. Non-aqueous separation

The maximum EOF observed was achieved employing 100% acetonitrile, however, we found that while the R.S.D. was satisfactory for repeat injections ($n=10$) the day to day reproducibility was poor. Addition of an electrolyte i.e. ammonium acetate (addition of glacial acetic acid was required to effect dissolution) offered an improvement in the reproducibility of the EOF but satisfactory separation of analytes was not obtainable.

Initial experiments indicated that a fast and reproducible EOF could be obtained using acetonitrile/

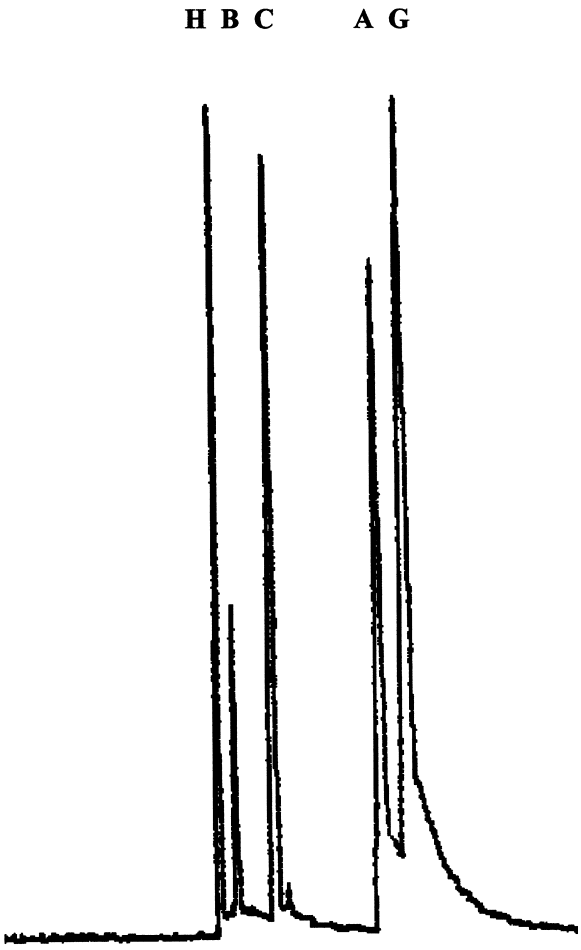

Fig. 3. Electropherogram of compounds A (cimetidine), B, C, H, and G (migration times 3.94 min, 2.66 min, 2.99 min, 2.52 min and 4.17 min, respectively). Conditions: electrolyte - 10 m*M* sodium dihydrogenphosphate, pH 3.14, separation voltage 30 kV.

methanol mixtures with an ammonium acetate concentration of 1 m*M*. Dissolution of ammonium acetate was aided by the addition of glacial acetic acid and 1% (*v*/*v*) was used throughout. (It was subsequently found that a much lower volume of acid was required and indeed on occasions no acid was required to effect dissolution.) Migration times of analytes are dependent on the composition of the organic media and this is illustrated in Fig. 4 with the velocity of the electroosmotic flow decreasing with increasing methanol composition. This was determined using thiourea as the 'neutral' marker. (We have subsequently observed that, depending on pH*, thiourea migrates behind other EOF markers, namely biphenyl and mesityl oxide, the implication being that it may carry a charge. This phenomena is currently under further investigation.) Optimum separation was achieved using a solvent composition of acetonitrile–methanol (70:30) and was employed in all subsequent work. Increasing the electrolyte concentration improved the separation with baseline resolution being achieved at a concentration of 25 m*M* but at the expense of slightly increased migration times. The observed order of migration remained constant regardless of solvent composition. Fig. 5 shows the separation of a three component (A, B and C) mixture, when compared to the aqueous separation of the same three compounds (cf. Fig. 2) the order of elution is the same but selectivity is clearly different. Efficiencies are high and experimental reproducibility excellent with R.S.D.$<$ 0.5% ($n=10$).

The apparent pH of the solutions (pH*) was measured using a standard pH meter and was found to decrease upon the addition of electrolyte and to further decrease upon the continued addition of acid. pK_a values of molecules in non-aqueous media are usually not known and may be substantially different to those determined in aqueous solutions. The degree of solvation will be dependent on the solvents employed and, where a mixed system is used, the mole fraction of each. Both of these effects will have considerable impact on the electrophoretic mobility of individual analytes and on the velocity of the electroosmotic flow.

Individual experiments on the eight compounds, including the two that could not be analysed under aqueous conditions, were carried out and the migration times are shown in Table 1. However, with the solvent media used, all of the components could not be separated in a single experiment i.e. compounds H and D, A and G, E and F co-eluted. This contrasts to the aqueous separation where the first two pairs were separable but E and F were insoluble. This demonstrates the change in selectivity that may occur in the different media.

An electropherogram of a five component mixture is shown in Fig. 6, the analysis time is under four min and shows much better peak shapes than were obtained under aqueous conditions. The greater peak width observed for component E may be due to a

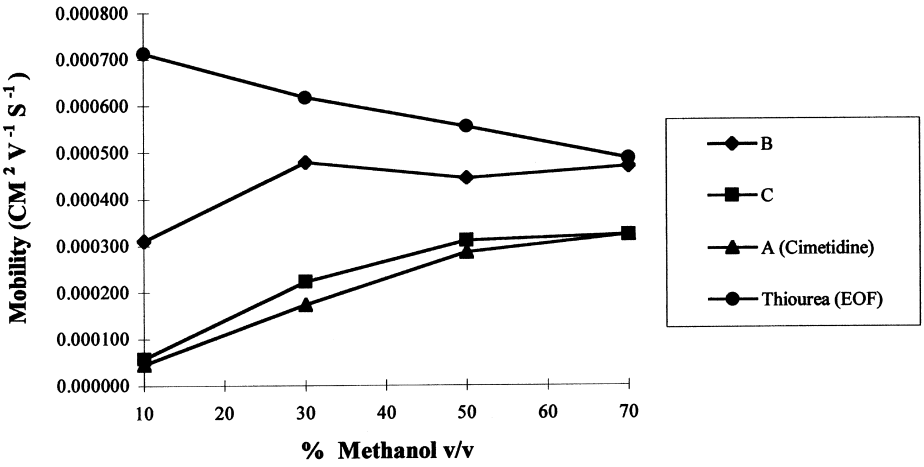

Fig. 4. Dependence of the electrophoretic mobilities of cimetidine (A) and compounds B and C on the composition of the organic medium [% (*v*/*v*) methanol in acetonitrile]. Conditions: electrolyte - 1 m*M* ammonium acetate+1% acetic acid, separation voltage 20 kV.

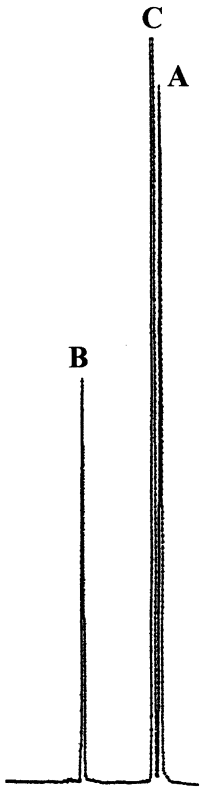

Fig. 5. Electropherogram of compounds A (cimetidine), B and C (migration times 5.02 min, 3.56 min and 4.87 min, respectively). Conditions: electrolyte - 25 m*M* ammonium acetate in acetonitrile–methanol (70:30, *v*/*v*)+1% acetic acid, pH* 6.7, separation voltage 20 kV.

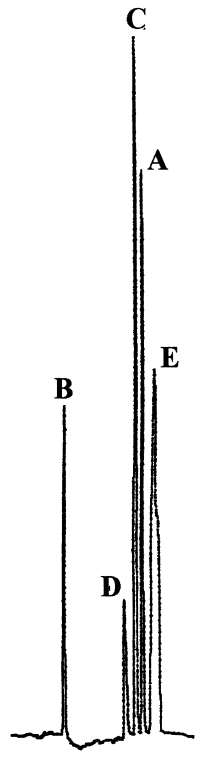

Fig. 6. Electropherogram of cimetidine (A) and compounds B, C, D and E (migration times 3.41 min, 2.41 min, 3.32 min, 3.10 min and 3.57 min, respectively). Conditions: electrolyte - 25 m*M* ammonium acetate in acetonitrile–methanol (70:30, *v*/*v*)+1% acetic acid, pH* 6.7, Separation voltage 30 kV.

co-eluting degradation product of one of the other components. Efficiencies calculated range from 60 000 to 700 000 plates m^{-1} and R.S.D. for each component $<0.5\%$ ($n=5$).

The R.S.D.s for the separations obtained in the non-aqueous media compare more than favourably to the R.S.D.s obtained in aqueous media. All experiments were conducted under ambient conditions and the greater rate of evaporation from non-aqueous solvents may have been expected to lead to variable migration behaviour, however, the large volume (~45 ml) of the outlet vial of the CE instrument may serve to minimise this effect. However, although we did not undertake a systematic study, the day to day reproducibility of the data obtained from non-aqueous work was observed to be less favourable than that of aqueous results.

4. Conclusion

Rapid and efficient separations of a drug substance from some of the associated impurities and degradation products has been accomplished by CE. This was achieved by using both aqueous and non-aqueous media. The combination of compounds that may be analysed was found to be different for each technique and therefore they may be considered complementary. Application of non-aqueous systems allows the analysis of hydrophobic materials that would otherwise pose problems for aqueous based separations.

The use of non-aqueous solvent systems clearly enhances the scope of CE methods but there remains much to understand before routine use.

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