Determination of antihistamines in pharmaceuticals by capillary electrophoresis

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ABSTRACT

Capillary electrophoresis with on-column UV detection at 214 nm was used to separate a group of nine antihistamines. All these compounds were satisfactorily separated within *ca.* 6 min using a mixed carrier system containing sodium dodecyl sulphate with β-cyclodextrin and tetrabutylammonium hydrogensulphate as modifiers. The application of the method to the determination of the amount of antihistamines present in commercial pharmaceutical samples was demonstrated. In addition, the migration behaviour of the antihistamines in the mixed carrier system was examined.

INTRODUCTION

Micellar electrokinetic chromatography (MEKC), since first initiated by Terabe *et al.* [1], has attracted much attention as an efficient separation technique in recent years [2–6]. This technique possesses both the advantages of capillary zone electrophoresis (CZE) in the separation of charged species and the selectivity required for the separation of neutral compounds [4–6]. This method is based on micellar solubilization and electrophoretic migration of the micelle. Solutes are separated by their differential distribution between the micelle and the surrounding aqueous phase and the differential migration of the two phases. To date MEKC has been successfully employed for the separation of environmental pollutants [7,8], biological compounds [3,5] and pharmaceutical products [9–13].

Most of these applications involve the use of a single surfactant, namely sodium dodecyl sulphate. To enhance further the selectivity of the system, the use of modifiers has been reported. Bushey and Jorgenson [14] found in their separation of dansylated methylamine and dansylated methyl-d_3-amine that the addition of methanol increases the elution range of the system. The introduction of cyclodextrin in

the buffer system has been reported to provide additional selectivity for chiral separation via host–guest-type complexation [15].

In this work, the separation of nine antihistamines using MEKC with both β-cyclodextrin and tetrabutylammonium hydrogensulphate (TBA) as modifiers was examined. The method was applied to the determination of antihistamines in two commercial drug samples. In addition, the migration behaviour of the antihistamines in the mixed carrier system was investigated.

EXPERIMENTAL

Equipment

Capillary electrophoresis was performed using a P/ACE 2000 system (Beckman, Palo Alto, CA, USA). The system is equipped with a capillary cartridge containing a 47 cm × 50 μm I.D. (effective length 40 cm) capillary tubing (Polymicro Technologies, Phoenix, AZ, USA). The column temperature was maintained at 25°C throughout the investigation. On-column UV detection with the wavelength set at 214 nm was employed. Chromatographic data were collected and analysed using a Shimadzu (Kyoto, Japan) Chromatopac C-R6A data processor.

Chemicals

All chemicals were of analytical-reagent grade or better. The buffer solution was prepared by dissolving sodium dihydrogenphosphate dihydrate and sodium tetraborate in water purified by a Millipore Milli-Q system. The electrophoretic media containing sodium dodecyl sulphate (SDS) and the modifiers were prepared as described previously [6]. SDS, β-cyclodextrin and TBA of the purest grade were purchased from Fluka (Buchs, Switzerland). The nine antihistamines used as test standards are listed in Table I. Standard solutions at a concentration of 500 ppm were prepared in high-performance liquid chromatographic-grade methanol (J. T. Baker, Phillipsburg, NJ, USA). All nine antihistamines were supplied by Sigma (St. Louis, MO, USA). Sample solutions were introduced using pressure injection with a pressure of 0.5 p.s.i. (3.5 kPa) and an injection time of 1 s. The amount injected is estimated to be 5.2 nl.

Sample preparation

The procedure for the determination of the active component in commercial antihistamine tablets is as follows. Each tablet was first ground and then extracted twice with a known amount of methanol in an ultrasonic bath kept at low temperature throughout the extraction. The extracted samples were then subjected to centrifugation followed by filtration through a 0.45-μm membrane before injection into the system for analysis.

RESULTS AND DISCUSSION

MEKC and CZE

Preliminary experiments carried out to separate the antihistamines using CZE and MEKC with SDS failed to provide satisfactory results. Because of the similar structural groups present in most of the antihistamines investigated, these methods did not provide adequate selectivity for satisfactory separation of this group of compounds. As a result, even for MEKC, only two broad distorted peaks were observed.

MEKC with β-cyclodextrin

The introduction of β-cyclodextrin to the micellar electrophoretic media resulted in a slight improvement in the overall resolution of the antihista-

mines. At the same time, it was found that the migration times decreased with increasing β-cyclodextrin concentration. This is consistent with the fact that the β-cyclodextrin molecules would compete with the micelles for the solutes. As the β-cyclodextrin moiety carries no charges, its mobility would not be influenced by electrophoretic attraction and hence its migration velocity would be exclusively governed by the bulk movement of the aqueous phase. Therefore species that were originally affected by the presence fo SDS either through Van der Waals solubilization or by electrostatic ion-pair formation with SDS would now be experiencing an additional partition mechanism via a host–guest type of interaction with the β-cyclodextrin moiety. This additional interaction present in the system would subsequently lead to an increase in selectivity and a considerable reduction in the migration times. However, in spite of the improvement in the resolution with the inclusion of β-cyclodextrin, the selectivity provided in this system was still insufficient to resolve all the peaks completely.

MEKC with tetrabutylammonium salts

Nishi *et al.* [17] in an investigation of some charged species observed an improvement in the resolution and the shape of the peaks using tetraalkylammonium salts in the MEKC mode. Unfortunately, our attempts to use this system again failed to achieve satisfactory results for the separation of the antihistamines, despite a marked improvement in the peak shape for most of the peaks. As all our attempts to separate this group of antihistamines with the known mixed carrier systems were not very successful, a new approach was subsequently adopted to overcome the problem of poor selectivity for this group of compounds.

MEKC with β-cyclodextrin and tetrabutylammonium salts

The new scheme involved the use of a mixed carrier system which includes SDS, β-cyclodextrin and TBA in the electrophoretic medium. With this new carrier system, enhanced selectivity was observed for the separation of the antihistamines. A typical electropherogram for the nine antihistamines is shown in Fig. 1. It can be seen that all peaks were satisfactorily separated within an extremely short analysis time of *ca.* 6 min, which is much shorter

than the time required for most of the systems used previously in this study. At the same time, the pair of enantiomers included in the mixture, (±)-chlorpheniramine (peak 10 in Fig. 1), were also satisfactorily separated using this mixed carrier system.

An unusual observation noted was that the methanol peak (i.e., the insolubilized marker normally used in MEKC work) migrated out much later than some of the antihistamine peaks (peaks 1, 2 and 3). Under normal circumstances, in MEKC and other MEKC applications involving the use of modifiers such as cyclodextrin, the methanol peak would be the first solute to migrate out. In fact, the work carried out by Nishi et al. [17] employing tetraalkyammonium salts did not reveal the peculiar trend observed in this work. In order to explain this anomalous behaviour, the separation mechanism for this mixed carrier system needs to be considered.

It is known that the addition of the tetraalkylammonium salts (TAA) would increase the ionic strength of the electrophoretic media, which in turn would result in an increase in current, I (current due to transport of charge by the fluid). Consequently, an increase in the electric field strength (E) could be observed. As a result, the electroosmotic velocity, v_{eo}, of the system would also increase (v_{eo} is proportional to E). Therefore, this increase would contrib-

Fig. 1. Electropherogram of antihistamines. Electrophoretic solution, 10 mM SDS in 0.05 M borate–0.05 M phosphate buffer with 10 mM TBA and 10 mM β-cyclodextrin (pH 7.5); separation tube, 47 cm × 50 μm I.D. capillary tube kept at 25°C; voltage, 21.5 kV; detection wavelength, 214 nm. Peak numbers as in Table I; M = methanol.

ute to the short analysis time observed in Fig. 1. At the same time, the cationic TAA would tend to combine with the anionic surfactant via ion pairing. As a result, the properties of the anionic surfactant would be considerably altered. The extent of surface binding of the TAA on the anionic site of the surfactant molecules would be largely governed by the size and nature of the alkyl chain. It has been reported that TAA salts with longer alkyl chains, such as the TBA salt used in this investigation, would tend to bind strongly to the negative sites of the micelle [17]. Subsequently, the negative charges present on the SDS micelle would be greatly reduced and therefore the modified SDS would now be experiencing a weaker electrophoretic pull towards the anode. As a result, the micellar velocity towards the cathode would be increased. This effect would also contribute to the increase in the bulk movement, thus increasing the current through the electrophoretic medium. Hence an overall decrease in the migration times of the solutes was observed.

A more important consequence of this modification is that ion-pair formation between the positively charged antihistamines and the SDS micelles would be inhibited. Therefore, the ion pairing of the positively charged antihistamines with the SDS molecules would be excluded. Further, these positively charged species would also experience electrophoretic repulsion from the anode. Hence they would migrate out much earlier than the remainder of the neutral species such as methanol. A point to note is that such an observation was not seen in the previous investigation involving the use of TAA [17]. The reason could be that in the previous study the ratio of TBA salt to the SDS concentration was much lower than that used in this work. As TBA salts are reported to bind strongly to SDS, it seems that it is only at higher concentrations of TBA salt that this anomalous behaviour can be observed.

In general, the results obtained showed that in spite of the slight modification, the mixed carrier system still possessed the characteristics of both CZE and MEKC. The separation of the charged species was found to be governed by the nature of the charges as in CZE separations, while the migration behaviour of the neutral species was found to be governed by the differential partition mechanism as in MEKC. This is evident from the results shown in Fig. 1. The earlier migrating species are the anti-
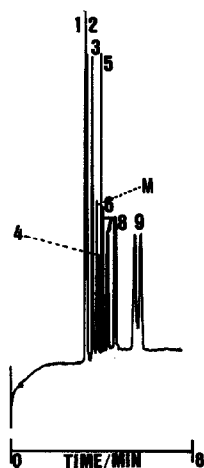
TABLE I

ANTIHISTAMINES INVESTIGATED, ABBREVIATIONS AND DETECTION LIMITS

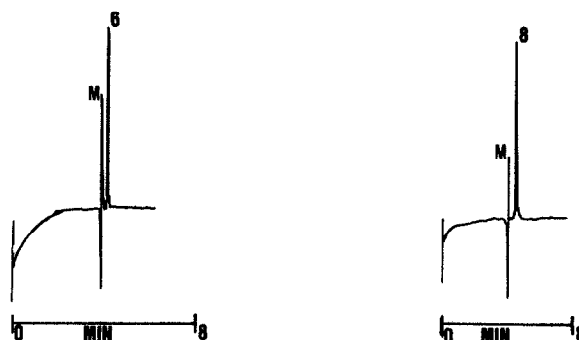| No.	Compound	Abbrevia-tion	Detection limit (pg)
1	Pheniramine	Phen	53.3
2	Doxylamine	Doxyl	55.9
3	Methapyrilene	Meth	57.2
4	Thonylamine	Thon	152.0
5	Triprolidine · HCl	Trip	76.1
6	Dimenhydrinate	Dimen	133.3
7	Cyclizine	Cycl	130.0
8	Promethazine	Prom	139.8
9	(±)-Chlorpheniramine	Chlo	143.0

Fig. 2. Electropherogram of dimenhydrinate drug samples. Electrophoretic conditions as in Fig. 1. Peaks: M = methanol; 6 = Dimen.

Fig. 3. Electropherogram of promethazine drug samples. Electrophoretic conditions as in Fig. 1. Peaks: M = methanol; 8 = Prom.

histamines, which normally possess basic groups which favour protonation. The migration order of these positively charged species would, as in many CZE separations, be influenced by the extent of protonation (*i.e.* highly positively charged species would migrate out faster). On the other hand, the neutral antihistamines, the species migrating out later than methanol, would be separated by differential partition between the β-cyclodextrin and the modified SDS. This group of compounds usually consists of the species which contained substituent groups that inhibit protonation. For example, with chlorpheniramine and promethazine, owing to the presence of electron-withdrawing groups in these compounds (*i.e.*, chloride and sulphur, respectively), protonation would be hindered. As a result, they were found to migrate slower than most of the other species. The additional partition mechanism would enhance the selectivity of the system. As in this instance the migration of the modified SDS would not be directly affected by the electrophoretic attraction of the high potential end, the overall mi-

gration times of the neutral species would be reduced. Even though negatively charged species were not investigated, it would be reasonable to expect that ion-pair formation between these species and the TBA salt would be likely to occur. As a result, faster analysis would also be expected.

Therefore, the major advantage of the use of such a mixed carrier system over other reported systems used in previous investigations is that the combined advantages of CZE and MEKC for the separation of charged and neutral species are present in this system. At the same time, by simply varying the TAA salt and SDS concentrations, in addition to the type of TAA salt used, the extent of modification of the SDS surface can be manipulated. As a result, higher selectivity could be readily achieved within a short analysis time.

TABLE II

AMOUNTS OF ANTIHISTAMINES FOUND IN PHARMACEUTICALS

Type of tablet	Amount indicated per tablet (mg)	Amount found ± S.D. (mg) (n = 5)		
		Tablet 1	Tablet 2	Tablet 3
Dimenhydrinate	50	45.00 ± 0.04	46.53 ± 0.03	46.01 ± 0.03
Promethazine	25	24.50 ± 0.03	25.03 ± 0.02	25.05 ± 0.01

Determination of antihistamines in pharmaceutical samples

The method developed was subsequently used for the determination of antihistamines present in drugs. Linear calibration graphs in the range 100–500 ppm were obtained. The correlation coefficients obtained were better than 0.99 for the nine antihistamines. The detection limits for these compounds are given in Table I. Typical electropherograms of the extracted samples are shown in Figs. 2 and 3 and the results of quantitation are given in Table II. The results obtained suggested that the proposed method can be a useful procedure for the routine determination of antihistamines in pharmaceuticals.

CONCLUSIONS

The usefulness of a mixed carrier system in capillary electrophoresis for the separation of antihistamines has been demonstrated. A major advantage of such a system is its high selectivity, which permits the separation of the antihistamines in a short analysis time with no significant losses in resolution. We believe that this is the first use of a mixed carrier system in capillary electrophoresis for the separation of antihistamines. From the promising results obtained, it can be expected that there will be great potential in the use of this type of technique for the separation of other pharmaceutical products and biological compounds.

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