Determination of sulphonamides in pharmaceuticals by capillary electrophoresis

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ABSTRACT

A mixture of seven sulphonamides was separated using capillary electrophoresis with β-cyclodextrin as modifier. The separation was carried out with on-column UV detection at 210 nm. The effects of the pH of the electrophoretic media and β-cyclodextrin concentration on the selectivity and migration times of the sulphonamides were investigated. In addition, the migration behaviour of this group of compounds was examined. This technique was then applied to the determination of sulphonamides in pharmaceuticals.

INTRODUCTION

Sulphonamide drugs are used for the treatment of susceptible bacterial infections, such as those related to the respiratory, digestive and urinary tracts. This group of compounds has been analysed successfully by thin-layer chromatography [1] and high-performance liquid chromatography (HPLC) using isocratic and gradient elution [2–5], but these methods are time consuming [2–3]. Interest in sulphonamides mainly lies in the determination of residues in animal tissues. However, sulphonamides can be toxic when they are used at therapeutic doses [6]. Pharmaceutical assays on these drugs have also been published [7]. As sulphonamides are mostly polar compounds, capillary electrophoresis (CE) might be expected to be a good alternative for their analysis.

CE has become popular in recent years mainly because of its ability to separate charged and neutral species with high efficiency. Micellar electrokinetic chromatography (MEKC), one of the modes of CE, involves the use of surfactant solutions such as sodium dodecyl sulphate (SDS). Selectivity in MEKC separations can be enhanced by adding modifiers to the system. The introduction of cyclodextrin into the micellar solution has been reported to provide additional selectivity for chiral separations via host-guest-type complexation [8]. To date, CE has been successfully employed in analyses of environmental pollutants [9,10], biological compounds [11,121 and pharmaceutical products [13,14].
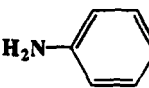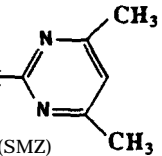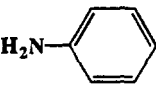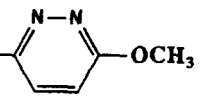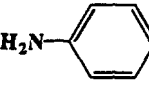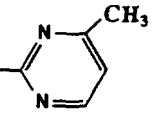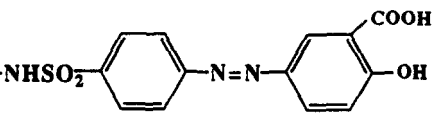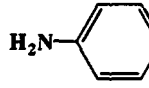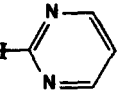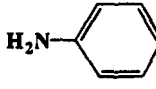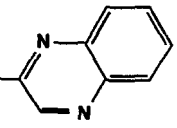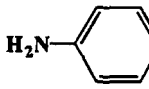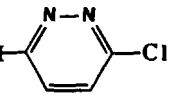
In this work, the separation of seven sulphonamides using capillary zone electrophoresis (CZE) with β-cyclodextrin (β-CD) as modifier was examined. This technique was applied to the determination of sulphonamides in commercial drug tablets. In addition, the effects of pH and β-CD concentrations on the migration behaviour of the sulphonamides in the system are discussed.

EXPERIMENTAL

Experiments were conducted on a laboratory-built CE system. A Spellman (Plainview, NY, USA) Model RM15P10KD power supply capable of delivering up to 15 kV was used. A fused-silica capillary tube of 50 μm I.D. (Polymicro Technologies, Phoenix, AZ, USA) and 50 cm effective length with an optically transparent coating was employed. A

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R	R'	pKa

R **R'** **pKa**

SULFAMETHAZINE (SMZ) — 7.5

SULFAMETHOXYPYRIDAZINE (SMP) — NA**

SULFAMERAZINE (SM) — 7.0

SULFASALAZINE (SS) — NA

SULFADIAZINE (SD) — 6.4

SULFAQUINOXALINE (SQX) — 5.5

SULFACHLOROPYRIDAZINE (SCP) — NA

**: NOT AVAILABLE

Fig. 1. Structures of sulphonamides, their abbreviations and some pK_a values. The pK_a value refers to the ionization of the hydrogen attached to N-1.

Shimadzu (Kyoto, Japan) Model SPD-6A UV spectrophotometric detector was used for the detection of peaks. Chromatographic data were collected and analysed using a Shimadzu Chromatopac C-R6A data processor.

The pH of the buffer solutions used in the CE system was adjusted by mixing appropriate portions of sodium dihydrogenphosphate and sodium tetraborate solutions. β-Cyclodextrin was purchased from Fluka (Buchs, Switzerland). The seven sulphonamide standards used were purchased from Sigma (St. Louis, MO, USA). The structures of the seven sulphonamides, their abbreviations and some of the pK_a values are shown in Fig. 1. The sulphonamide standards were dissolved in HPLC-grade methanol (BDH, Poole, UK), each at a concentration of 1000 μg/ml.

Sample solutions were introduced manually. One end of the capillary was placed in a sample vial containing the sample solution and the sample was introduced by siphoning from the sample solution at a height of 9 cm higher than the electrophoretic solution in which the other end of the tube was immersed. The injection time was about 4 s. Each injection was estimated to be 1 nl.

Sample preparations

The method for the extraction of sulphonamides from pharmaceuticals is as follows. The sample was first ground into powder in a mortar, 100 mg of the powder were accurately weighed into a 100-ml volumetric flask, 75 ml of 0.025 M NaOH solution were added and the solution was shaken for 30 min. The solution was then diluted to volume with 0.025 M NaOH, mixed and filtered. The solution was then diluted to half its concentration with 0.025 M NaOH solution before injection into the CE system for analysis.

For the determination of the percentage recovery of the extraction procedure, 100 mg of standard sulphonamide were weighed into a 100-ml volumetric flask. Then the extraction procedure described was followed. A 50-ml volume of 0.025 M NaOH solution was used to dilute the solution before injection into the system.

RESULTS AND DISCUSSION

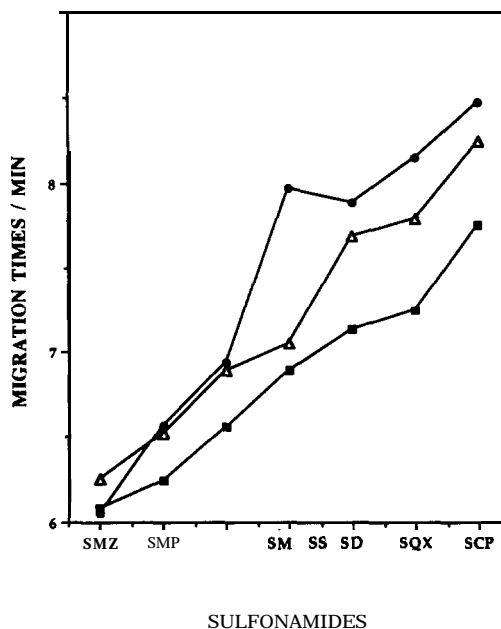Preliminary experiments were conducted at a

Fig. 2. Variation of migration times of sulphonamides with β-CD concentration. ● = 0; △ = 5; ■ = 10 mM β-CD. Electrophoretic medium, 0.05 M phosphate-0.05 M borate buffer (pH 7); separation tube, 50 cm × 50 μm I.D. fused-silica capillary; voltage, 15 kV; detection wavelength, 210 nm.

constant pH of 7 and varying the concentration of β-CD at 0, 5 and 10 mM. The effect of the concentration of β-CD on the migration times of the sulphonamides is shown in Fig. 2. As can be seen, there is a decrease in the migration times of the solutes with increase in β-CD concentration. This could be attributed to the fact that with an increase in β-CD concentration, the solutes would have a higher propability of being incorporated into the neutral cavity of β-CD. Terabe *et al.* [15] successfully separated neutral polycyclic aromatic hydrocarbons (PAHs) using cyclodextrin-modified micellar electrokinetic chromatography. These compounds have at least two benzene rings that can be effectively incorporated into the cavity of CD, which consequently resulted in separation. The same could be said of the sulphonamides. These compounds have at least one benzene ring each and a heterocyclic ring in their structures, as shown in Fig. 1, which could easily be incorporated into the cavity of β-CD. In addition, Fig. 2 shows that the migration order does not change with increase in β-CD concentration. This indicates that the addition of β-CD

to the electrophoretic medium does not significantly affect the overall ionization state of the sulphonamides. Nevertheless, the resolution between the peaks was observed to improve with higher concentrations of β-CD because of the increase in the formation of inclusion complexes.

This observation of decreasing migration times of the sulphonamides with increase in /?-CD concentration is in contrast to the observations of Fanali [16], who found that the migration times increased with increase in β-CD concentration for chiral compounds. This is not surprising because at this pH (7), some of the sulphonamides would be partially ionized or totally deprotonated, depending on their pK_a values (given in Fig. 1). The partially ionized solutes (SMZ and SM, p$K_a \geqslant 7$) were also found to elute earlier than solutes that were totally ionised (p$K_a < 7$). As the experiments were conducted at a constant pH, we would expect the change in the electroosmotic flow velocity with increase in β-CD concentration to be minimal. In the absence of β-CD, the partially negatively charged sulphonamides would be experiencing an electrophoretic pull towards the positive electrode. These partially ionized solutes would be incorporated into the β-CD on addition of β-CD, which is moving at a speed equal to the electroosmotic velocity. Under CE conditions, the neutral β-CD would travel faster than the anionic solutes towards the cathode. In addition, inclusion complexation of the solutes with β-CD would increase with increase in β-CD concentration. Hence the electrophoretic pull towards the anode would be diminished. Subsequently, an increase in the apparent velocity and thus a decrease in the migration times for the neutral or partially ionized compounds was observed. The migration times for the anionic solutes was also observed to decrease even though there was no effective complexation, as incorporation of the negatively charged compounds into the neutral β-CD cavity would be hindered. The observed shorter migration migration time of the negatively charged sulphonamides could be attributed to hydrogen bonding between the OH groups on the glucose ring of β-CD and the nitrogen moiety of the sulphonamides. As a result, the anions would be dragged along with the β-CD towards the negative electrode. Consequently, a decrease in migration times was observed for the sulphonamides with increase in β-CD concentration.
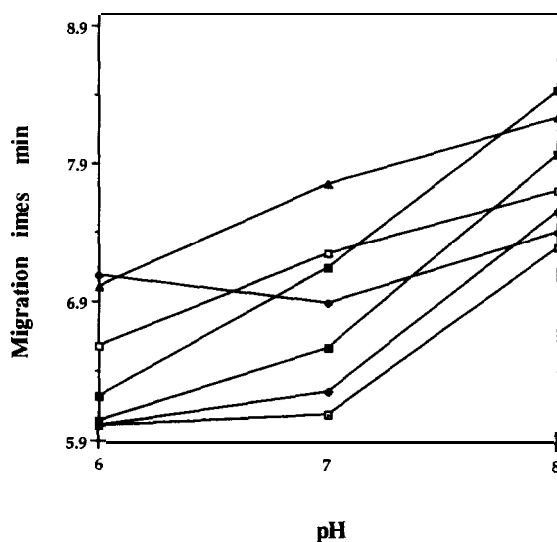

Fig. 3. Variation of migration times of sulphonamides with pH of the buffer. Electrophoretic medium, 0.05 *M* phosphate–0.05 *M* borate buffer-10 m*M* β-CD; separation tube. 50 cm × 50 μm I.D. fused-silica capillary; voltage, 15 kV; detection wavelength. 210 nm. 1 = SD; 2 = SCP; 3 = SM; 4 = SQX; 5 = SMP; 6 = SS; 7 = SMZ.

The effect of pH on the migration behaviour of sulphonamides was also investigated. Experiments were carried out at different pH values at a constant /?-CD concentration of 10 m*M*. The effect of pH on the migration times of the sulphonamides is illustrated in Fig. 3. There is a general increase in migration times of the analytes with increase in pH. From the structures of the sulphonamides, it was noted that they can act as weak acids in dilute alkaline solutions or weak bases in dilute acidic solutions. Therefore, at high pH (8), the sulphonamides tend to be deprotonated at N-1, the nitrogen bonded next to the sulphur moiety. Consequently, these compounds would be negatively charged at this pH and would not be effectively incorporated into the neutral cavity of the β-CD. Instead, they would experience a stronger attractive pull towards the anode. It is known that electroosmotic flow velocity increases with increase in pH [17]. In the present instance, as the increase in electroosmotic flow is less than the electrophoretic velocity of the analytes, the overall effect is a net decrease in the apparent velocity, which accounts for the increase in migration times of the sulphonamides. It was found that at a pH of 7 and with 10 m*M* β-CD, satis-
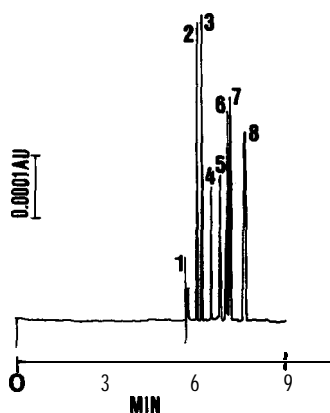
Fig. 4. Electropherogram of the seven sulphonamides. Electrophoretic medium, 0.05 M phosphate-0.05 M borate buffer (pH 7)– 10 mM β-CD; separation tube, 50 cm $\times$ 50 μm I.D. fused-silica capillary; voltage, 15 kV; detection wavelength, 210 nm. Peaks: 1 = MeOH; 2 = SMZ; 3 = SMP; 4 = SM; 5 = SS; 6 = SD; 7 = SQX; 8 = SCP.

factory separation for the group of seven sulphonamides was achieved. A typical electropherogram is shown in Fig. 4. It was observed that there was no general trend in the migration orders of the sulphonamides. It is not possible to explain the trend solely by considering the structures alone because the R and R' substituents vary in shape, sizes and polarity in this instance. However, it was found that the migration order could be related to the pK_a values, *i.e.,* compounds with higher pK_a values (higher than the pH of the buffer) would migrate faster than those with pK_a values less than the pH of the electrophoretic medium.

TABLE I

SULPHONAMIDES INVESTIGATED, CORRELATION COEFFICIENTS AND DETECTION LIMITS OBTAINED AT A SIGNAL-TO-NOISE RATIO OF 2

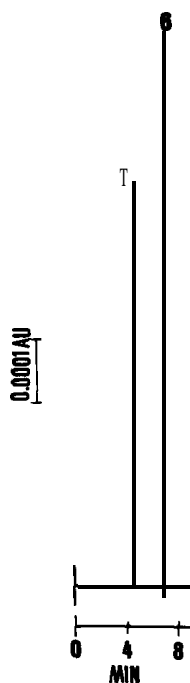| Sulphonamide[a]	Correlation coefficient. *r*	Detection limit (pg)
SMZ	0.97674	18
SMP	0.9667	19
SM	0.9907	26
SS	0.9937	43
SD	0.9986	31
SQX	0.9838	25
SCP	0.9969	25

[a] See Fig. 1 for full names.

Fig. 5. Electropherogram of the extracted sample in 150 ml of 0.025 M NaOH. Electrophoretic conditions as in Fig. 4. Peaks: T = trimethoprim; 6 = SD.

Determination of sulphonamides in pharmaceuticals

Once a satisfactory separation had been obtained, the technique was applied to the determination of sulphonamides in pharmaceuticals. Linear calibration graphs in the range 100–1000 μg/ml were obtained for the seven sulphonamides. Low detection limits in the picogram range was obtained. The correlation coefficient, *r,* and the detection limits at a signal-to-noise ratio of 2 are given in Table I.

Combination drug formulations in the form of tablets or suspensions containing sulphonamides are most frequently used to administer oral doses to human patients. In this investigation, drug tablets containing the active component (sulphadiazine) and trimethoprim (inhibitors of folic acid synthetase) were used. Good linearity was obtained between the amount injected and peak area. The recovery using the extraction method [18] was found to be 85.97 $\pm$ 0.03%. A typical electropherogram of the extracted samples is shown in Fig. 5. For two sulphadiazineetrimethoprin tablets with an indicated content of 0.410 g, the amounts found, based in a

recovery of 85.97%, were 0.412 ± 0.0051 and 0.407 ± 0.0048 g (mean $\pm$ S.D., $n = 4$). Hence the amount was within the limits of 95–100% of the amount indicated. The results obtained suggested that CE is a useful method for the routine determination of sulphonamides in pharmaceuticals.

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