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Systematic optimization of capillary electrophoretic separation of sulphonamides

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ABSTRACT

The use of an optimization scheme for the separation of a group of sulphonamides is described. This scheme utilizes an interpretive optimization approach to predict the optimum conditions for the separation of a group of eight sulphonamides. By conducting nine preplanned experiments that span the maximum working range of the system, overall optimum conditions for separation can be obtained. To confirm the validity of the optimization procedure, additional experiments using the optimum conditions predicted by the scheme were performed. The results demonstrated that satisfactory separation could be achieved by using the optimum separation conditions predicted by the scheme.

INTRODUCTION

Sulphonamides are drugs which are extensively used in the treatment of bacterial infections in both animals and man, and there is currently great interest in their separation and assay [1–3]. High-performance liquid chromatography (HPLC) is commonly used for this purpose but, as most of these compounds are ionic, it is expected that capillary electrophoresis (CE) may be a valuable alternative method.

CE is a highly efficient and rapid technique that is being increasingly used in the biological and pharmaceutical fields [4–6]. Extremely high separation efficiencies have been demonstrated using this technique. However, optimum separation conditions using CE are normally obtained by trial-and-error methods and these approaches result only in local optimum conditions rather than overall optima. A systematic approach for CE separation was recently reported by Vindevogel and Sandra [7], in which a Plackett–Burman statistical design was used. The method required only eight experiments for the optimization of five factors, all related to buffer composition. However, no fixed rules existed for the selection of low and high levels of the parameters used in the optimization procedure and further optimization may need to be attempted based on the conclusion of the optimization experiments [7].

In this investigation, CE with β-cyclodextrin as modifier was used for the assay of eight sulphonamides. A systematic optimization scheme was utilized. This scheme, known as the overlapping resolution mapping (ORM) scheme, had been previously applied for the optimization of HPLC separations [8,9]. In this paper, the use of a modified ORM scheme for the optimization of the CE separation of sulphonamides is described.

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. Fused-silica capillary tubing of 50 cm × 50 μm I.D. with an optically transparent coating was obtained from Polymicro Technologies (Phoenix, AZ, USA). A Shimadzu

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(Kyoto, Japan) Model SPD-6A UV spectrophotometric detector and a microUVIS20 UV detector (Carlo Erba, Milan, Italy) were 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 obtained by mixing appropriate portions of sodium dihydrogenphosphate and sodium tetraborate solutions. β-Cyclodextrin, which was used as a modifier in the electrophoretic medium, was purchased from Fluka (Buchs, Switzerland). The eight sulphonamide standards used (Table I) were purchased from Sigma (St. Louis, MO, USA). The sulphonamide standards were each dissolved in HPLC-grade methanol (BDH, Poole, UK) at a concentration of 1000 ppm.

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 level 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.

RESULTS AND DISCUSSION

Initial attempts to separate the sulphonamides using CZE conditions in which the pH of the electrophoretic media was adjusted to 6, 7 and 8 proved unsuccessful. Owing to the ionic nature of the sulphonamides, slight changes in pH was found to affect the separation adversely. In fact, it was found that the resolution of the peaks could be affected by as small as 0.05 unit change in the pH of the electrophoretic medium. In view of the susceptibility of resolution to pH changes for this group of compounds, optimization through pH variation by the trial-and-error method was considered to be unsatisfactory. The addition of a suitable modifier to the buffer, which would serve to stabilize the system in response to slight changes in pH and to enhance the separation efficiency, was therefore considered. As the sulfonamides are relatively polar, we would not expect that the use of sodium dodecyl sulphate (SDS) in the electrophoretic medium, as is normally employed in micellar electrokinetic chromatography (MEKC) [10–12], would be effective in solvating the compounds. This is because the sulphonamides would prefer to remain in the aqueous buffer medium rather than partition with the micelles. A better choice would be β-cyclodextrin, as it is neutral and yet possesses polar hydroxy groups. β-Cyclodextrin was therefore chosen as a modifier in the electrophoretic medium.

TABLE I

SULFONAMIDES INVESTIGATED

Compound	Abbreviation
Sulphamethoxypyridazine	SMP
Sulphachloropyridazine	SCP
Sulphasalazine	SS
Sulphamerazine	SM
Sulphaguanidine	SG
Sulphadiazine	SD
Sulphaquinoxaline	SQX
Sulphamethazine	SMZ

An ORM scheme was used to determine the optimum conditions for the separation of sulphonamides. The ORM scheme is a statistical experimental model used to define the region of interest in which the optimum conditions reside. The term ORM used in this investigation implies that the optimum conditions were achieved by overlapping all resolution plots. The scheme predicts the optimum operating pH and β-cyclodextrin composition of the electrophoretic medium from nine preliminary experiments.

A schematic flow chart for the optimization scheme is shown in Fig. 1. The initial step for this scheme involved setting the criteria for the separation. Two criteria were considered: first, the peaks should be baseline separated, and second, the overall migration time should be less than 15 min. For this scheme, there is great flexibility in choosing the migration range for the system. This depends on the type of compounds under investigation and the nature of the investigation. As the sulphonamides would be expected to be ionized in the electrophoretic medium, the migration times for this group of compounds under CZE conditions would be relatively short. In addition, it is desirable not to choose too long a migration range for the separation. In this instance, an overall migration time of 15 min was chosen.

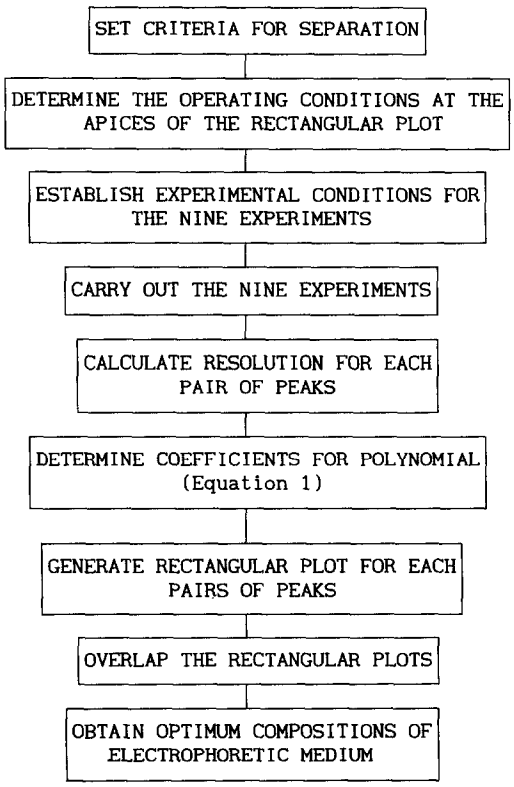

Fig. 1. Schematic representation of the optimization scheme.

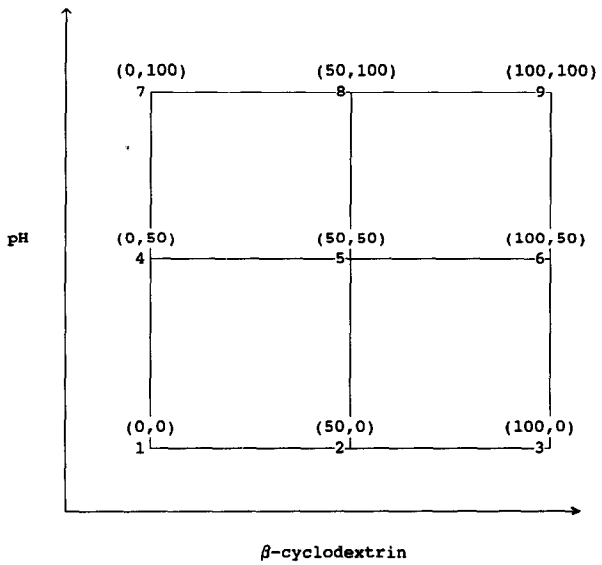

Fig. 2. Locations of the nine experiments chosen from the rectangular plot. The composition at each point is represented as a percentage at the respective apices.

After the criteria had been set, the operating conditions at the four apices of the rectangular plot were determined. The nine experiments were strategically chosen at appropriate positions in the rectangular plot as shown in Fig. 2. The ORM scheme is flexible in that it allows the four experiments at the vertices of the rectangular plot to cover as much as possible the whole working range of the system. These four experiments are only subjected to the preset criteria mentioned earlier and the type of compounds under investigation. In determining the working range for the pH of the buffer, it was considered undesirable to use extreme pH conditions because they were known to be detrimental to the untreated column. Therefore, a moderate pH of 8 was chosen. Otsuka and Terabe [13], in investigations on the effect of pH on electroosmotic flow, showed that the electroosmotic flow, v_{eo}, decreased as pH decreased. The use of a low-pH buffer would obviously lead to an increase in analysis time. In view of the preset criteria which stipulated that the total analysis time should not be greater than 15 min, the lower limit of pH was set at 6.

β-Cyclodextrin as modifier in the electrophoretic medium has been shown to effect separation through a host–guest relationship [14] with the analytes. Small amounts of β-cyclodextrin in the separation media have been found to be sufficient for optimizing the separation of water- and fat-soluble vitamins [15]. Therefore, concentrations higher than 10 m*M* were considered unnecessary. In addition, higher concentrations of β-cyclodextrin in the buffer solution would pose a solubility problem. Consequently, minimum and maximum β-cyclodextrin concentrations of 0 and 10 m*M* were chosen.

After setting the operational conditions at the four apices of the rectangular plot, the other five experiments as shown in Fig. 2 would then be easily defined. For example, the pH of the buffer and the amount of β-cyclodextrin in the electrophoretic media for point 5 (5 m*M* β-cyclodextrin and pH 7.0) shown in Fig. 2 are calculated as follows: the amount of β-cyclodextrin is the sum of the amount of β-cyclodextrin at point 4 (0 m*M* β-cyclodextrin) and the mean of the difference between point 4 (0 m*M* β-cyclodextrin) and point 6 (10 m*M* β-cyclodextrin). Similarly, the pH of the buffer can be obtained from point 2 (pH 6) and point 8 (pH 8). The detailed

TABLE II

EXPERIMENTAL CONDITIONS USED FOR THE NINE EXPERIMENTS

Experiment No.	pH	β-CD concentration (m*M*)
1	6.0	0
2	6.0	5
3	6.0	10
4	7.0	0
5	7.0	5
6	7.0	10
7	8.0	0
8	8.0	5
9	8.0	10

conditions at the various points of the rectangular plot are listed in Table II.

Once all the conditions for the nine experiments had been determined, the experiments were conducted. The resolutions, R, between adjacent peak pairs in each of the electropherograms were then calculated from the migration times obtained from the nine experiments using the equation

$$R = \frac{2(t_2 - t_1)}{W_1 + W_2} \quad (1)$$

where t_1 and t_2 are the migration times of two adjacent peak pairs and W_1 and W_2 are the widths of the peak pairs. These values were fitted into a polynomial equation:

$$R = a_0 + a_1x_1 + a_2x_2 + a_{12}x_1x_2 + a_{11}x_1^2 + a_{22}x_2^2 + a_{112}x_1^2x_2 + a_{122}x_1x_2^2 + a_{1122}x_1^2x_2^2 \quad (2)$$

where a_i are the polynomial coefficients and x_i are the proportions of each variable in percentage as defined in Fig. 2. A BASIC program was used to determine the coefficients of the polynomial. Once the coefficients had been determined, the resolution between peak pairs for conditions other than those in the nine experiments could be calculated using eqn. 2. As there are eight compounds, there would be seven pairs of adjacent peaks. The rectangular plots for the seven pairs of peaks were then generated. Fig. 3 shows a typical rectangular plot for one of the peak pairs, where the different symbols correspond to different ranges of resolution between peak pairs. By overlapping the seven rectangular plots, the final

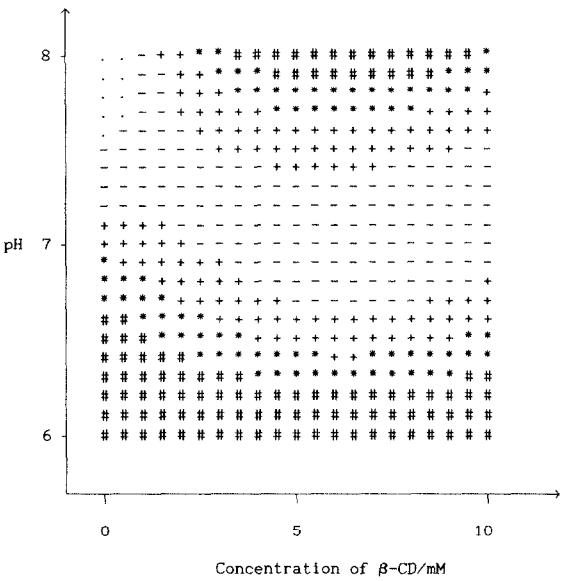

Fig. 3. Typical rectangular resolution plot of a pair of peaks. Notation: ·, $R < 0.5$; –, $0.5 \leqslant R < 1.0$; +, $1.0 \leqslant R < 1.5$; ∗, $1.5 \leqslant R < 2.0$; #, $R \geqslant 2.0$.

overlapped rectangular plot was obtained and is shown in Fig. 4. Fig. 5 is a three-dimensional representation of such a plot. The final overlapped resolution plot represents the minimum values of the

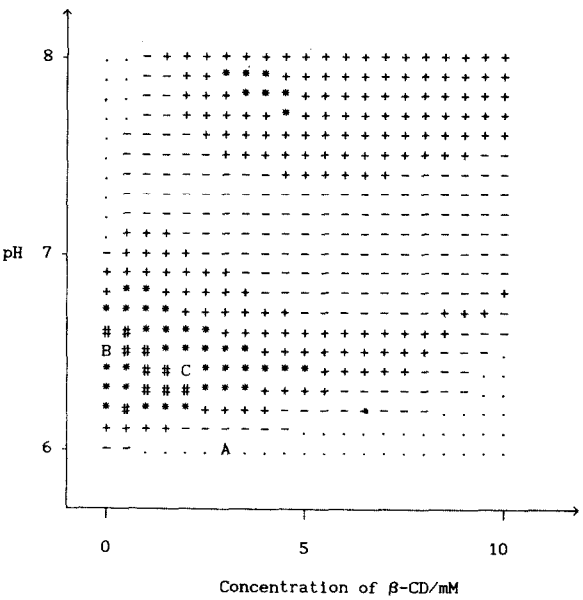

Fig. 4. Final overlapped resolution plot for all seven pairs of peaks. Notation as in Fig. 3.

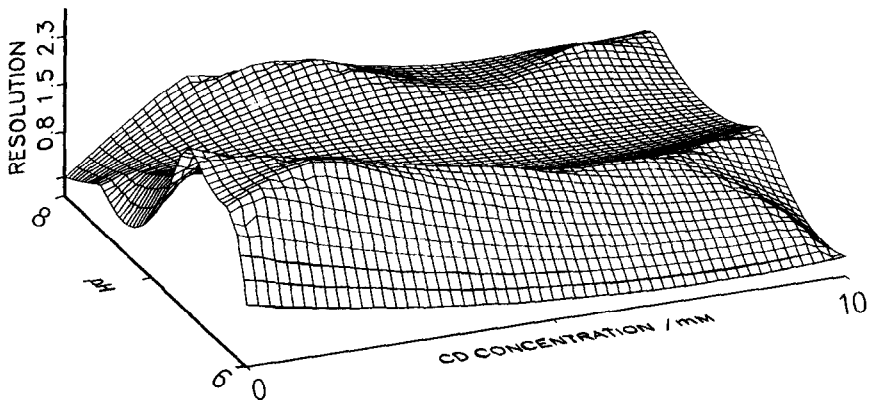

Fig. 5. Three-dimensional representation of the final overlapped resolution plot.

resolution between all peak pairs under the conditions bounded by the rectangular plot. The region marked with # indicates the highest resolution between all peak pairs and this is where the optimum conditions are expected to be found. It is noted in Figs. 4 and 5 that the overall optimum corresponds to pH 6.4 and 2 m*M* β-cyclodextrin (R = 2.24). Although satisfactory separation can also be obtained at pH 6.5 or 6.6 without β-cyclodextrin (R = 2.03 and 2.10, respectively), the use of β-cyclodextrin as a modifier served to stabilize the system and resulted in enhanced separation.

In order to evaluate the validity of the ORM scheme, experimental conditions corresponding to points A, B and C in Fig. 4 (the final overlapped resolution plot) were chosen from the regions represented by the symbols · and #, respectively. Poor resolution ($R < 0.5$) would be expected for the condition represented by point A whereas high resolution ($R \geqslant 2.0$) would be expected for conditions represented by points B and C. Point B represented the optimum conditions when no modifier is added. Point C represented the overall optimum conditions, *i.e.*, the conditions with the highest resolution within the range of experimental conditions. Typical electropherograms using these conditions (represented by points A, B and C) are shown in Figs. 6, 7 and 8, respectively. As can be seen from Fig. 6, the first two peaks (SG and SMZ) co-elute for experimental conditions corresponding to point A, which lies outside the optimum region. From Figs. 7 and 8, it can be observed that for experimental

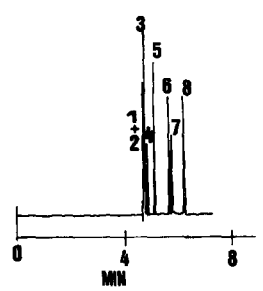

Fig. 6. Electropherogram for the eight sulphonamides with electrophoretic conditions corresponding to point A in Fig. 4: 0.05 *M* phosphate–0.05 *M* borate (pH 6.0) and 3 m*M* β-cyclodextrin; Separation tube, 50 cm × 50 μm I.D.; detection wavelength, 210 nm; voltage, 15 kV. Peaks: 1 and 2 = methanol, SG and SMZ; 3 = SMP; 4 = SM; 5 = SD; 6 = SQX; 7 = SS; 8 = SCP.

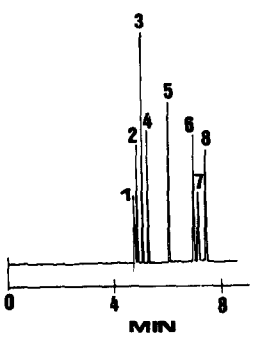

Fig. 7. Electropherogram for the eight sulphonamides with electrophoretic conditions corresponding to point B in Fig. 4: 0.05 *M* phosphate–0.05 *M* borate (pH 6.5); separation tube, 50 cm × 50 μm I.D.; detection wavelength, 210 nm; voltage, 15 kV. Peaks: 1 = methanol and SG; 2 = SMZ; 3 = SMP; 4 = SM; 5 = SD; 6 = SQX; 7 = SS; 8 = SCP.

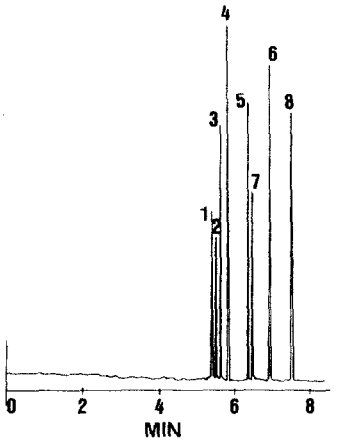

Fig. 8. Electropherogram for the eight sulphonamides with electrophoretic conditions corresponding to point C in Fig. 4: 0.05 *M* phosphate–0.05 *M* borate (pH 6.4) and 2 m*M* β-cyclodextrin; separation tube, 50 cm × 50 μm I.D.; detection wavelength, 210 nm; voltage, 15 kV. Peaks: 1 = methanol and SG; 2 = SMZ; 3 = SMP; 4 = SM; 5 = SD; 6 = SQX; 7 = SS; 8 = SCP.

conditions corresponding to points B and C selected from the optimum region, all the peaks are baseline separated and the values of the resolution between all the pairs of peaks are more than the preset value of 2. The total analysis time was less than 8 min, which is considerably less than the preset criterion of a maximum analysis time of 15 min.

The results clearly demonstrated the versatility of the ORM scheme for CE separations. The most important feature of the scheme is that it could be used to determine the overall optimum conditions for the separation, which involved optimization of both pH and β-cyclodextrin concentration. Further, it could be used to determine the optimum conditions for separation by varying pH alone (corresponding to pH 6.5 or 6.6 and 0 m*M* β-CD). From the results obtained, it is concluded that overall optimum conditions for CE separation can be obtained much more readily using the systematic optimization scheme than by trial-and-error approaches.

CONCLUSIONS

An ORM scheme was employed for the optimization of CE separation of eight sulphonamides. The scheme provided a systematic procedure which could be used to obtain the overall optima within the range of experimental conditions investigated. To confirm the validity of the optimization procedure, additional experiments were performed which demonstrated that satisfactory separation could be obtained using experimental conditions selected from the optimum region of the resolution plot. In view of the promising results obtained, it is believed that the scheme can be readily extended to obtain optimum experimental conditions for other CE separations.

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