Multivariate Optimization of a Sulfated-β-Cyclodextrin-Modified Capillary Zone Electrophoretic Method for the Separation of Chiral Arylalcohols

Yu-Ping Zhang, Hyun-Joo Noh, Seong-Ho Choi,[†] Jae Jeong Ryoo, Kwang-Pill Lee,[*] Kazutoku Ohta,[‡] Chuzo Fujimoto,[§] Ji-Ye Jin,[#] and Toyohide Takeuchi[$]

Department of Chemistry, Graduate School, Kyungpook National University, Daegu 702-701, Korea
[†]Department of Chemistry, Hannam University, Daejeon 306-701, Korea
[‡]Ceramic Research Institute, National Institute of Advanced Industrial Science and Technology, Nagoya 463-8560, Japan
[§]Department of Chemistry, Hamamatsu University, School of Medicine, Hamamatsu 431-3192, Japan
[#]Instrumental Analysis Center, Gifu University, Gifu 501-1193, Japan
[$]Department of Chemistry, Faculty of Engineering, Gifu University, Gifu 501-1193, Japan
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Chiral separation of aryalcohols such as 1-phenyl-propanol, 1-phenyl-2-proanol, and 2-phenyl-1-propanol by capillary electrophoresis has been optimized using the overlapping resolution mapping (ORM) scheme. Three critical parameters of the electrophoretic media, *i.e.* phosphate concentration, sulfated β-cyclodextrin (CD) concentration and pH, were chosen for optimization. The working ranges were initially presumed by 7 pre-experiments. Further optimization was carried out by another seven experiments within the narrow working ranges. From the final overlapping resolution mapping all peak pairs, the area of maximum separations were located. Using the conditions of a point in this area, we found that the target compounds were a baseline separated within 30 min. The maximum separation conditions of arylalcohols were a chiral selector concentration of 5.4%, a phosphate concentration of 28 mM, and a pH of 5.0.

Key Words : Capillary electrophoresis, Arylalcohols, Overlapping resolution mapping, Sulfated β-cyclodextrin, Optimization

Introduction

Enantiomerically pure arylalcohols are applied in many fields such as enantioselective synthesis and analysis. Enantiomerical separation of arylalcohols has been performed with chromatography including gas chromatography (GC)[1] and high-performance liquid chromatography (HPLC).[2] In recent years, the use of chiral capillary electrophoresis (CE)[3,4] has grown rapidly and has proven to be a useful tool for the resolution of arylalcohols enantiomers. Chiral CE offers a significant advantage over GC or HPLC in terms of a higher separation efficiency and lower sample reagent consumption.[5]

Cyclodextrins are extensively used in separations because of their unique property to form inclusion compounds with other smaller hydrophobic molecules.[6] A CE chiral separation can be achieved using chiral selectors, such as chiral crown ethers, chiral micelles, proteins and cyclodextrins (CDs) or their derivatives.[7,8] Cyclodextrins (CDs) are the most widely used chiral selectors. In particular, the shape and size selectivity of cyclodextrins provide an important parameter for separation because of the impact that stability constants of various magnitudes have on molecular discrimination.

Separations in CE are characterized by a large number of parameters where the interaction between the various parameters is hard to rationalize. For the separation of compounds with very similar mobilites, such as isomers and analogues, more than one parameter (*e.g.* modifier concentration, pH, electrolyte concentration, capillary temperature, electric field strength, etc.) need to be incorporated in the optimization strategy to achieve an adequate separation of such complex mixtures.

There are few strategies for the systematic optimization of CE and most of the optimum separation conditions have been achieved using simple univariate optimization procedures which are often ineffective in locating the true optimum and also time-consuming. The overlapping resolution mapping (ORM) scheme, as a statistical experimental model, has been widely used in HPLC,[9,10] to optimize the composition of the mobile phase through only seven experiments. A number of papers have reported the optimization of the separation by CE.[11-13]

Although the ORM scheme is simple, straightforward and rapid in itself, the choice of relevant parameters and their working range is very complicated, heavily affected by the analyst's experience and some preliminary experiments.

In the present study, we initially design seven pre-experiments according to the ORM scheme, and their working range should include the region where a basic separation be achieved.

Three main factors affecting the resolution were chosen the concentration of the selector, and the concentration of phosphate and pH during this process. Further optimization can be carried out by another seven experiments within narrower working range, and it was used to build a

[*]Author for correspondence. Tel: +82-53-950-5901, Fax: +82-53-952-8104, e-mail: kplee@knu.ac.kr

mathematical model in which three parameters including buffer concentration, selector concentration and pH were each regarded as the function of a minimum effective resolution. From the final overlapping resolution mapping, the different range of minimum resolution within the triangle was identified. The validity of the method was confirmed by an additional experiment using the optimum condition. The optimization programs were designed for the application of these approaches by which the baseline separation of three chiral compounds was obtained during a period of 30 min using the predicted optimum condition.

Experimental Procedures

Chemicals. The sulfated β-CD, 1-phenyl-1-propanol, 1-phenyl-2-propanol, and 2-phenyl-1-propanol were obtained from Aldrich Co., Inc. Wisconsin, USA. Other chemical reagents were used without any further purification. The pH of phosphate buffer solutions containing sulfated CD was adjusted with NaOH (0.1 M) or HCl (0.1 M) to a desired value. All solutions were degassed with ultrasonic equipment and filtered through a membrane (0.25 μM) before use. Doubly-distilled water was used throughout. All reagents used were of analytical reagent grade. A standard mixture was prepared at a concentration of about 500 ppm in ethanol (1 : 1 v/v).

Apparatus. CE separation was performed with a P/ACE 5500 system with a photodiode array detector (Beckman Instruments, Fullerton, CA,USA). An uncoated fused-silica capillary of 50 μm I.D and 57 cm (effective length 50 cm) was used. The capillary was conditioned before each analysis by flushing successively with 0.1 M NaOH, H_2O, and buffer for 3 min. Samples were injected with pressure at 0.5 p.s.i. for 3 s and separated at 15 kV (1 p.s.i. = 6894.76 Pa). Data in the range of 190-600 nm were acquired and processed through the P/ACE station version 1.2. Temperature was kept constant at 25 ^{o}C. The column dimension and injection volume were also fixed throughout the experiments. To avoid experimental complications from Joule heating, the applied voltage ($\leq$ 15 KV) to keep the current below 200 μA was limited. Compound recognition was performed by injection of each individual solute.

Software. Polynomial equations were obtained with Mathematica for students V2.2 (Wolfram Research). The triangular ORM program was edited by Fortran 4.0 (Microsoft Company).

Results and Discussion

An appropriate optimization strategy should be used to find good separation conditions in the shortest time with only a few experiments, which could assure the baseline-separation of all components. Basically, all optimization strategies consist of three distinct steps (i) the choice of the appropriate parameters and the parameter level or space, (ii) a model or algorithm to describe the migration behavior, and (iii) an experiment to obtain the optimum results or a criterion to evaluate the resulting electropherogram.

According to our experience, the concentration of a selector, the concentration of phosphate and pH are the principal factors that affect the resolution of alryalcohols, whereas in this study the temperature, electric field strength and etc play secondary roles.

Chiral separations by capillary electrophoresis (CE) depend on the selective interaction between a chiral selector and enantiomers in the buffer solution. An optimum CD concentration exists at which a maximum separation is reached. The type of CDs has a significant effect on separation selectivity. The application of charged CDs in a CE makes it possible to separate neutral enantiomers.[14,15] A sulfated-β CD seems to be quite effective in the separation of chiral arylalcohols, whereas neutral enantiomer-anionic CD complexes migrate in the opposite direction of the electro osmotic flow (EOF). This counter-EOF setup widens the separation window and resolution at the expense of longer analysis times. Apparently, the increase in CD concentration in the buffer system will improve the separation of arylalcohols. However, when the anionic CD is used for the counter-EOF setup, higher concentrations of the charged CD can cause a dramatic increase in the electric current. As a result, a weaker electric field strength has to be used to minimize Joule heating.

In general, increasing the ionic strength of the buffer facilitates separation as a result of the decrease in both the zeta potential and the wall adsorption of the solutes. As the phosphate concentration increases up to a limit, the number of theoretical plates increases having a better shape. More heat, however, is produced as the ionic strength is increases, so effective temperature control is necessary; smaller diameter capillaries allow the use of higher ionic strengths. In the present study, a range of 10-100 mM phosphate concentration was first selected for the optimization.

pH affects the EOF, the EOF increases as the pH becomes higher. The separation time decreases as this occurs. Due to neutral nature of arylalcohols, the buffer solutions of pH 5.0-9.0 were tested to determine the working pH range.

Although the increase in the selector and phosphate concentration in a buffer system with a low pH value could improve the separation of arylalcohol, a compromise must be considered between the separation time and resolution. On the other hand, we must consider the disadvantages incurred from the increased ionic strength due to Joule heating.

For this purpose, seven experiments were initially conducted at selected points in a triangle. The positions of these seven points are shown in Figure 1, indicating the percentages for the three parameters. In the present work, the resolution is used as a response function.

In the triangular ORM scheme in this work, seven experiments were conducted at selected points in a triangle (see Fig. 1) in three-dimensional space. In the initial experiment (see Table 1), the maximum and minimum concentration level of phosphate (x_1) tested were 10 and 100 mM, respectively, while those of the selector concentration

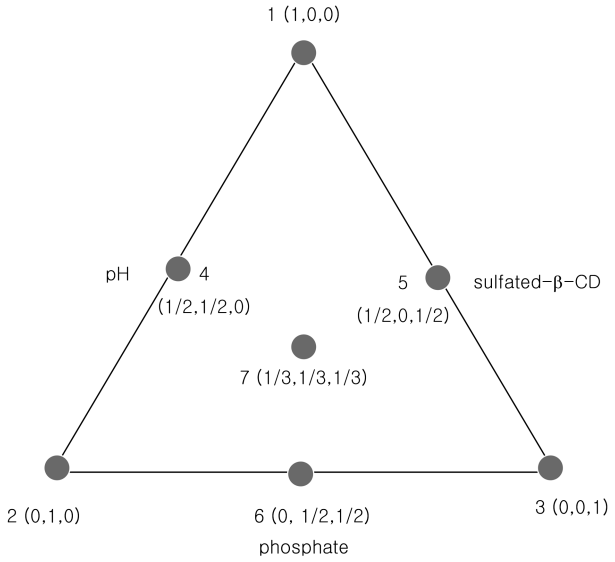

Figure 1. Plate location for the 7 pre-planned experiments. The coordinates at the points on the triangle (x_1, x_2, x_3) denote the percentages of phosphate, sulfated-β-CD, pH respectively.

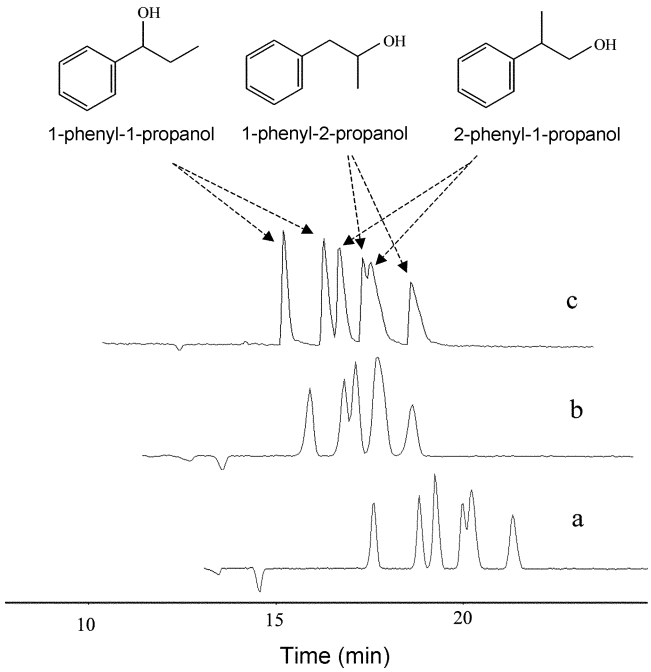

Figure 2. Electrophoregrams of the arylalcohols, a, b, c means the electorphoregrams of the first experiment No. 4, 6, 7. Experimental condition is the same as Table 1.

(x_2) and pH (x_3) were 0%-9% and 5-9, respectively. In any position of the triangle, the sum of the three variable values would always equal 1.

From the electrophoregrams obtained with the seven preplanned experiments, resolutions, R_s, between the adjacent peaks were calculated. Preliminary measurements showed that the largest value of all the minimum resolutions (R_{min}) among the seven experiments was very low ($R_{min} < 1.0$) and the R_s value of every peak pair for several experiments equaled zero. Using a computer program to predict the optimum resolution would be very difficult. All peaks overlapped in experiments No. 1, 3 and 5 which shows we cannot separate these compounds by capillary zone electrophoresis without adding a selector to the buffer. The addition of a high concentration CD widens the separation window and resolution at the expense of longer analysis times. In experiment No 2, no peak was detected during 60min, which shows we had added too much selector. In experiment No. 6, there was only one peak pair full overlap (see Fig. 2b). In experiments No. 4 and 7, we can find 6 peaks, and one peak pair was not baseline separated (see Fig. 2a, c). Therefore, chose a more narrow working range according to the condition of experiments No. 4, 6 and 7.

To further optimize the separation, seven experiments were again conducted at selected points according to Figure 1, with the parameter space becoming narrower compared with the first experiment. The minimum and maximum levels of phosphate concentration (x_1) tested were 20 and 60 mM, respectively, while those of the selector concentration (x_2) and pH (x_3) were 3.0%-6.0% and 5-7, respectively.

Table 2 shows the resolution values obtained for adjacent eluting species of further ORM experiments. The term ORM scheme used in this investigation implies that the optimum conditions can be achieved by overlapping all resolution plots. Measured resolutions were fitted to a special cubic polynomial model with the vertices of the triangular parameters as variables. The program models the response surface for each pair of peaks related to the percentage of the three parameters. These resolution values were then fitted into the polynomial equation according to the following equation:[16-18]

$$R = a_1x_1 + a_2x_2 + a_3x_3 + a_{12}x_1x_2 + a_{13}x_1x_3 + a_{23}x_2x_3 + a_{123}x_1x_2x_3$$

Table 1. The initial experiment condition to obtain the approximate parameter space for further optimization

Experiment Number	Phosphate (x_1, mmol/L)	Sulfated β-CD (x_2, mmol/L)	pH (x_3)	Percentage %	Peak Number
1	100	0	5.0	1, 0, 0	1
2	10	9.0	5.0	0, 1, 0	undetected
3	10	0	9.0	0, 0, 1	1
4	55	4.5	5.0	1/2, 1/2, 0	6
5	55	0	7.0	1/2, 0, 1/2	1
6	10	4.5	7.0	0, 1/2, 1/2	5
7	40	3.3	6.3	1/3, 1/3, 1/3	6

Table 2. Results of resolution between adjacent peaks according to the 7 pre-planned experiments

Experiment Number	1	2	3	4	5	6	7
R_{1-2}	4.53	8.67	2.11	7.98	2.55	5.36	3.68
R_{2-3}	1.61	2.94	0.61	2.80	0.71	1.73	1.28
R_{3-4}	2.87	5.23	1.12	4.60	0.98	2.81	1.97
R_{4-5}	0.92	1.54	0	1.48	0	0.88	0.68
R_{5-6}	4.35	6.98	1.44	6.07	1.56	3.78	2.46

Table 3. Coefficients of polynomial Equation for the 7 experiments

Peak pair	Coefficients						
	a_1	a_2	a_3	a_{12}	a_{13}	a_{23}	a_{123}
R_{1-2}	4.53	8.67	2.11	5.52	-3.08	-0.12	-45.35
R_{2-3}	1.61	2.94	0.61	2.1	-1.6	-0.18	-12.83
R_{3-4}	2.87	5.23	1.12	2.2	-4.06	-1.46	-19.93
R_{4-5}	0.92	1.54	0.00	1	-1.84	0.44	-2.58
R_{5-6}	4.35	6.98	1.44	1.62	-5.34	-1.72	-75.7

Where a_i is the coefficients and x_i is the percentages of each parameter as defined in Figure 1. With the aid of Mathematica software, the coefficients were determined (see Table 3). Once the coefficients were known, the resolution for every adjacent peak pair over the entire parameter space could be predicted and visualized as a resolution map within the triangle. (see Fig. 3). The R of the first peak pair is larger than 1.5 (denoted #) at any point within the triangle, but the forth peak pair is more complicated which optimum region is more narrow.

By subsequently overlapping all five peak pair resolution maps and plotting the lowest resolution amongst all the individual resolution maps, the region defining buffer compositions with which the minimum desired resolution can be achieved for all solutes in the mixture can be identified. The overlapping of the resolution values was performed according to two rules: (i) when overlapping five resolution values, the lower value of the resolution was retained; and (ii) the optimum point was represented by the highest resolution in the final overlapped diagram. The resolution value of every predicted point in the final ORM diagram was obtained after retaining the lowest resolution value of all peak pairs. In the final overlapped resolution mapping (see Fig. 4), the regions defining the composition of the experimental parameters was also established and presented with different symbols. In the final mapping, the regions marked by #, +, – and * represent four different predicted resolution levels namely, greater than 1.5, between 1.0 and 1.5, between 1.0 and 0.5, and less than 0.5. When the resolution is more than 1.5, the demand for quantified and quantitative analysis can both be met, which is shown in the region marked in the symbol (#). The minimum resolution of experiment number 2 is also within the optimum region (see

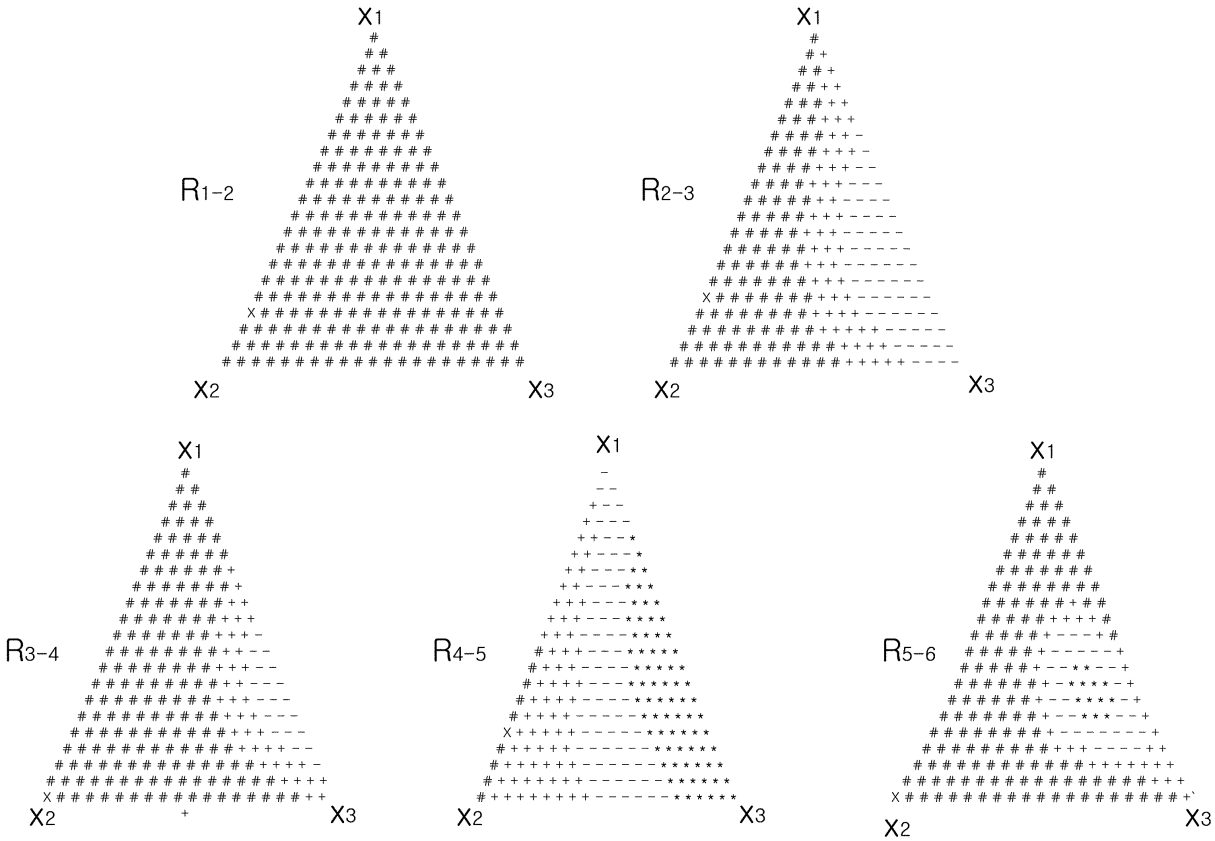

Figure 3. Resolution plot for the peak pairs. The coordinates at the points on the triangle (x_1, x_2, x_3) denote the percentages of phosphate, sulfated β-CD, pH respectively. (#), R > 1.5; (+) 1.0 ≤ R < 1.5; (–) 0.5 ≤ R < 1; (*) R < 0.5.

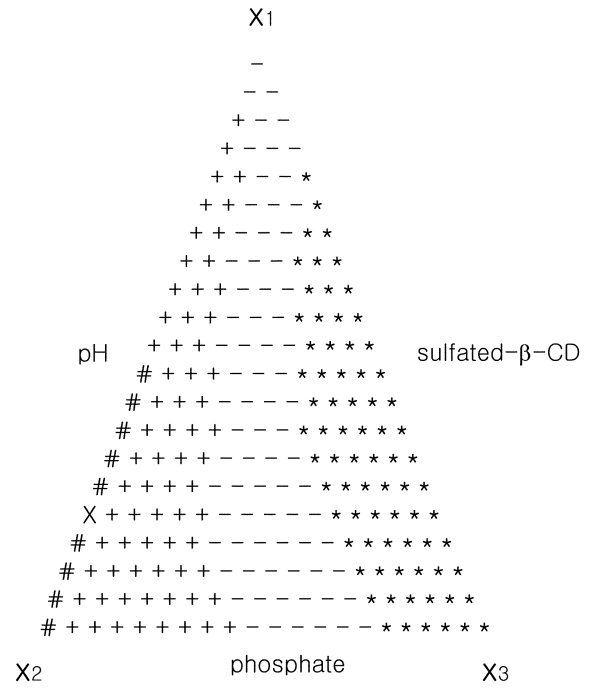

Figure 4. Final overlapped resolution mapping for the 5 peak pairs. The coordinates at the points on the triangle (x_1, x_2, x_3) denote the percentages of phosphate, sulfated *β*-CD, pH respectively. Notation: (#), R > 1.5; (+) 1.0 ≤ R < 1.5; (–) 0.5 ≤ R < 1; (*) R < 0.5, X stands for the optimum point.

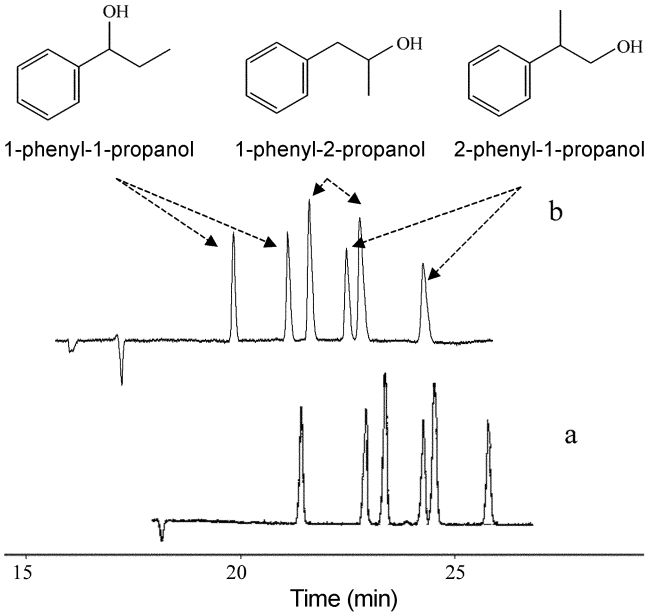

Figure 5. Electrophoregrams of the chiral arylalcohols. (a) means the electorphoregrams of the further experiment No. 2; pH=5.0; phosphate, 20 mM; selector, 6.0%. (b) means the electrophoregrams under the optimum condition; pH=5.0; phosphate, 28 mM; selector, 5.4%.

Fig. 5a). The maximum resolution is denoted by the symbol (x) in the final mapping, where the coordinates are x_1 = 20%, x_2 = 80% and x_3 = 0%, respectively. The values of the three parameters are a phosphate concentration of 28 mM, a chiral selector concentration of 5.4% (wt%), and a pH of 5.0, for which the predicted response value equals 1.58.

To test the validity of this optimization scheme and to obtain the optimum separation, point X in Figure 4 was selected from the final mapping to perform the experiments (see Fig. 5b). The minimum resolution of the experiment equals 1.62, which assures the baseline-separation of the arylalcohols.

Conclusions

The chiral separation of aryalcohols such as 1-phenyl-1-propanol, 1-phenyl-2-propanol, and 2-phenyl-1-propanol by capillary electrophoresis was studied using sulfated *β*-cyclodextrin (CD) as a chiral selector. Through the combination of ORM scheme, the optimum condition for the separation of three chiral compounds has been obtained which can maintain their separation with a chiral selector concentration of 5.4%, a phosphate concentration of 28 mM, an applied voltage of 15 kV, and a pH of 5.0.

The ORM scheme requires only a small set of preplanned experiments which are carried out systematically. The number of experiments for the ORM scheme is usually governed by the experimental design and the order of the polynomials chosen to relate the resolution to the experimental parameters. It should be noted that the selection of the range of parameters and the starting point for optimization are usually the key to success.

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