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Multivariate approach for the enantioselective analysis in MEKC-MS: II. Optimization of 1,1'-binaphthyl-2,2'-diamine in positive ion mode

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Abstract

Enantiomeric separation and detection of 1,1'-binaphthyl-2,2'-diamine (BNA) has been successfully optimized by micellar electrokinetic chromatography coupled to electrospray ionization mass spectrometry (MEKC-ESI-MS) using a polymeric surfactant polysodium *N*-undecenoxy carbonyl-L-leucinate (poly-L-SUCL) as a pseudostationary phase. In the first step, MEKC conditions were optimized by a five-factor three-level central composite design (CCD) of experiment. All five MEKC factors (buffer pH, percentage of acetonitrile in the running buffer, concentration of surfactant, concentration of NH₄OAc, and voltage) were found significant to the responses (measured as the chiral resolution and analysis time). The interactions between MEKC factors were further evaluated using a quadratic model equation which allowed the generation of 3-D response surface image to reach the optimum conditions. To obtain the best signal to noise (*S/N*) ratio, sheath liquid composition and spray chamber parameters were successfully optimized using the same strategy. Baseline enantiomeric resolution in less than 20 min and optimum mass spectrometry signal of BNA enantiomers (*S/N* = 45 at 0.4 mg/mL) were ultimately achieved at the optimized conditions. The adequacy of the model was validated by experimental runs at the optimal predicted conditions. The predicted results were found to be in good agreement with the experimental data.

Keywords

1,1'-binaphthyl-2,2'-diamine; chiral separation; central composite design; MEKC-MS; polymeric surfactant

1 Introduction

Although the second dimension offered by the MS detection is very attractive choice to obtain molecular mass (*m/z*) and structural information of co-eluting analytes in HPLC or CE, separation is very critical for optical isomers with identical *m/z*. Micellar electrokinetic chromatography (MEKC) coupled to electrospray ionization mass spectrometry (ESI-MS) using molecular micelle has been increasingly used for chiral analysis since its first report in 2001 [1]. Molecular micelles (also known as polymeric surfactants) are typically formed via covalent bonds of the vinyl terminated surfactant monomers. Some of the properties of molecular micelles include zero critical micelle concentration, structural rigidity, and less competitive interactions between surfactant and other chiral selectors (e.g., cyclodextrins) in the running buffer [2,3]. In addition, molecular micelles are very difficult to ionize under normal ESI-MS conditions, which makes them compatible with MS detection. Such

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capabilities of molecular micelles consequently enhance the signal-to-noise ratio (S/N) and electrospray stability [2,4-8] in MEKC-MS. Another practical advantage of molecular micelles includes compatibility with higher concentration of organic solvents in the running buffer [3, 9]. These aforementioned features of molecular micelle make MEKC-ESI-MS an effective hyphenation methodology for analysis of both chiral and achiral compounds. Several chiral molecular micelles derived from amino acids or small peptides head groups have been utilized in a univariate approach to optimize separation of various classes of enantiomers and employed in MEKC over the past decade [10-13]. However, the detector used in majority of chiral MEKC studies is a UV detector, which is inherently less sensitive than ESI-MS.

The multivariate statistical experimental designs may provide important benefits in the development of chiral MEKC-MS methods because they are considered superior to sequential design due to their ability to simultaneously screen all critical variables and determine their interactions with a relatively smaller number of experiments. In addition, they also provides a better global optimum condition [14]. The most commonly used multivariate method is a factorial design, which is useful in screening out the critical factors that could affect both separation and detection.. However, the interactions among the various separation parameters or detection parameters are neglected in a factorial design. If these interactions are not negligible, more complex design (such as central composite, Box-Behnken, D-optimal, etc) should be used. [14,15] Central composite design (CCD) is one of the most normally used higher order designs, which provides data for the fitting of a linear polynomial model to a set of data. The most critical points of this design are: (a) the combinations of the extreme values for all the factors within their ranges (often coded as -1 and $+1$ level); (b) axial points that are outside the original factor ranges (often coded as $-\alpha$ and $+\alpha$ level); (c) center point which includes all factors at their mean values (coded as 0 level and are usually run multiple times to conduct lack of fit test). Quadratic response surface models are usually constructed from the CCD, based on which maximum or minimum values can be predicted [15-18]. The response in the case of chiral MEKC-MS can be resolution of the peak pair, migration times and signal-to-noise (S/N) of the two enantiomers.

As a further extension to our earlier works on the analysis of chiral compound using MEKC-MS, we present here the first report for the chiral MEKC separation and ESI-MS detection of 1,1'-binaphthyl-2,2'-diamine (BNA). The BNA is one of the 2,2'-substituted 1,1'-binaphthyl derivatives that have been extensively used as asymmetric ligands in the synthesis of chiral catalysts [19,20]. Due to their highly stable chiral configuration, binaphthyl derivatives are also widely used as model test compound in chiral recognition [21,22] as well as chiral separation [23-26]. A polymeric chiral surfactant polysodium *N*-undecenoxy carbonyl-L-leucinate (poly-L-SUCL) was chosen as a pseudostationary phase. A five-factor (acetonitrile (ACN) concentration, background electrolyte (BGE) concentration, poly-L-SUCL concentration, buffer pH, and voltage) full factorial CCD was carried out to obtain the actual (experimental) responses. The experimental MEKC responses for chiral resolution and migration time were compared to the predicted responses. Next, three factors (% methanol (MeOH), pH, and ammonium acetate (NH_4OAc) concentration) were considered in the sheath liquid condition optimization to predict the best possible S/N for the MS detection. The drying gas temperature (DGT) and drying gas flow rate (DGF) were studied using the same multivariate approach to evaluate the significance of spray chamber parameters. Finally, the adequacy of the developed MEKC-MS method was validated by experimental runs at the predicted conditions.

2 Materials and Methods

2.1 Chemicals and reagents

The analyte ($\pm$) BNA was obtained from Aldrich (Milwaukee, WI). Both MeOH and ACN (HPLC grade), glacial acetic acid (99.7+% ACS reagent) were obtained from Caledon Laboratories Ltd (Georgetown, ON, Canada). A 7.5 M NH_4OAc aqueous solution was purchased from Sigma-Aldrich (St. Louis, MO). Ammonium hydroxide (NH_4OH , 28%-30% ammonia solution) was purchased from EM Science (Gibbstown, NJ). Triply deionized water (18 M Ω cm) was generated in the laboratory using Barnstead Nanopure II Water System (Dubuque, IA). Chemicals used to synthesize L-SUCL such as ω -undecylenyl alcohol, pyridine, triphosgene, L-leucine, dichloromethane, sodium bicarbonate, sodium hydroxide, hydrochloric acid, and ethyl acetate were purchased from Sigma-Aldrich (St. Louis, MO). All the chemicals have the purity of 98% or higher if not stated otherwise and were used as received.

2.2 Synthesis of poly-L-SUCL

The surfactant monomer of L-SUCL was synthesized using the procedure developed by Rizvi *et al.* [27]. The monomers were polymerized using a total dose of 20 Mrad of ^{60}Co radiation by Phoenix Memorial Laboratory (University of Michigan, Ann Arbor, MI).

2.3 Preparation of running buffer and analyte solutions

The BGE was NH_4OAc , which was prepared at different concentrations with different percentage of ACN. The pH of the NH_4OAc BGE was adjusted as needed by NH_4OH (1 M) or acetic acid (1 M). ACN was then added to this buffer to obtain the desired final NH_4OAc concentration. The buffer was then filtered by 0.45 μm PTFE syringe filter (Fisher Scientific, Pittsburgh, PA) and ultrasonicated for 15 min. Next, poly-L-SUCL surfactant was added into the buffer. The molar concentration of poly-L-SUCL was calculated using the molecular weight of its monomer and is expressed as equivalent monomer concentration (EMC). The final running buffer was vortexed and ultrasonicated for another 15-20 min before usage. Stock analyte solution of ($\pm$) BNA (1 mg/mL) was prepared in ACN and stored at 4 °C. The working analyte solution was prepared by diluting the standard stock solution with deionized water to a final concentration of 0.40 mg/mL.

2.4 MEKC-ESI-MS instrumentation

An Agilent capillary electrophoresis system (3D-CE system, Palo Alto, CA) interfaced to an Agilent 1100 series quadrupole mass spectrometer (Palo Alto, CA) was used to carry out the MEKC-ESI-MS experiments. The sheath liquid was delivered using an Agilent 1100 series HPLC pump with a 1:100 splitter. The raw data was collected and analyzed by the Agilent 3D-CE/MSD ChemStation software (Rev. A.08.04). A 60 cm long fused silica capillary (50 μm i.d., 360 μm o.d., purchased from Polymicro Technologies, Phoenix, AZ) was used for the MEKC-MS experiments.

2.5 MEKC-ESI-MS conditions

A new capillary was flushed with 1 M NH_4OH for 40 min followed by deionized water for 15 min before usage. Prior to each run, the capillary was rinsed with actual running buffer for 5 min. After each run, the capillary was flushed with deionized water for 1 min, 1 M NH_4OH for 2 min, and deionized water for 2 min, respectively, as post-conditioning protocol. The column cassette temperature was set to 20°C. Positive voltage was applied for all the CE runs (voltage varied according to experimental design) keeping the sprayer on ground potential. Analytes were kept at 15°C temperature in the autosampler and injected hydrodynamically at the pressure of 5 mbar for 3 sec. The sheath liquid delivered at 5 $\mu\text{L}/\text{min}$ was MeOH/ H_2O in different ratios containing various concentration of NH_4OAc and pH. ESI-MS detection was

carried out in the selected ion monitoring (SIM) mode for protonated molecular ion of BNA $[M+H]^+$ ($m/z = 285$). Other MS parameters were set as follows: nebulizer pressure, 2 psi; fragmentor voltage, 90 V; capillary voltage, +3 kV; gain setting, 3.

2.6 Experimental design and data analysis

The Design-Expert (version 7.0.3, Stat-Ease, Inc. Minneapolis, MN) software was employed to perform the experimental design and response surface methodology (RSM) data analysis. Five factors were chosen for the MEKC optimization: buffer pH (F_1), ACN percentage in running buffer (F_2), concentration of polymeric surfactant (F_3), concentration of NH_4OAc (F_4), and voltage (F_5). Three factors were chosen for sheath liquid optimization: MeOH percentage (F_1), pH (F_2), and NH_4OAc concentration (F_3). Two factors were chosen in spray chamber parameter optimization: DGF (F_1) and DGT (F_2). Three levels were set for each of the factors. The detailed values for each level are shown in Table 1. For the full factorial CCD, the α value, which is defined as the distance from the center of the design space to a star point, used in MEKC optimization was 2.38. For sheath liquid and spray chamber optimization, this value was 1.68 and 1.41, respectively. Nebulizer pressure was not studied in the MEKC optimization because its influence to enantiomeric resolution is very clear. That is, the higher the nebulizer pressure, the bigger the suction effect the nebulizer creates at the outlet end of the capillary, which increase the laminar flow in the capillary and consequently deteriorate the resolution and decrease the run time [28]. Therefore, throughout all the experiments, we simply kept the nebulizer pressure at 2 psi, which is the lowest level that could maintain a stable current. As to capillary cassette temperature, we found in the previous study that this factor is difficult to control in our instrument because about half of the capillary needs to be exposed to room temperature when interfacing to ESI chamber.

Table 2 shows the detailed design and response (enantiomeric resolution and migration time measured as the elution time of the last peak) for all 50 runs generated by the CCD. The full factorial CCD covers all the combinations of factors at their ± 1 levels and by comparing the errors of the repetitive center points with those of the $\pm \alpha$ level, the significance of each factor is determined. Data of the experimental responses were input into the Design-Expert software and were fitted into different models for further analysis and optimization. Quadratic model was chosen for both responses of the MEKC optimization and linear and two-factor interaction (2FI) model were chosen for sheath liquid and spray chamber optimization. Models were then validated by the process of analysis of variance (ANOVA). The interactions between all significant factors were demonstrated by the 3-D RSM surface plots generated by the software. Finally, the optimum combination of all variables was calculated from the model. To guarantee baseline separation, maximum enantiomeric resolution was set as the only goal for the MEKC condition optimization experiment. In sheath liquid composition and spray chamber parameters study, maximizing the S/N was chosen as the goal of the optimization.

3 Results and Discussion

To achieve the optimum MEKC-ESI-MS separation (best enantioseparation and highest S/N for the analyte) three sets of experiments were carried out. First, the multivariate CCD optimization of MEKC condition including five factors (buffer pH, ACN percentage in running buffer, concentration of surfactant, concentration of NH_4OAc , and voltage) were chosen and tested at these levels in a CCD design. The second set of experiments involved the optimization of sheath liquid parameters. Three factors were considered: MeOH/ H_2O ratio, NH_4OAc concentration, and pH. The third set of experiments was carried out to optimize the spray chamber condition, which includes drying gas flow rate and drying gas temperature.

3.1 Preliminary experiments

Before using the multivariate CCD experiment for the optimization of MEKC conditions, a series of preliminary sequential studies on all the factors that could affect the MEKC separation were performed to determine the most important factors which need to be further optimized using multivariate experiments. In addition, their range (i.e. the levels) required for later experiments was also determined. A fairly wide range was explored for the following factors: the buffer pH, NH₄OAc concentration, surfactant concentration, voltage, temperature, organic modifier (MeOH and ACN), and nebulizer pressure. In contrast to our previous studies on negatively charged binaphthyl derivatives (i.e. 1,1'-bi-2-naphthol (BOH) and 1,1'-binaphthyl-2,2'-diyl-hydrogen-phosphate (BNP)) [29], the use of ACN was found to be very effective for enantioselectivity of positively charged BNA. The optimum conditions determined by univariate approach for enantioseparation of BNA were as follows: pH 10.5, 35 mM poly-L-SUCL, 20 mM NH₄OAc, 20% (v/v) ACN, voltage +20 kV, and nebulizer pressure 2 psi. A brief preliminary study was also carried out to evaluate and determine the range of sheath liquid (%MeOH: 20-80% (v/v), pH: 6.00-8.50, and [NH₄OAc]: 5-40 mM) and spray chamber (drying gas flow rate: 4-6 L/min and drying gas temperature: 150-250 °C) conditions.

3.2 Multivariate approach

Table 1 shows the detailed design levels for all five factors to be studied in MEKC optimization. Level 0 of each factor was determined based on the optimum value from the preliminary study. Next, a reasonable value was created both at the high and low levels (level +1 and -1). The detailed justifications for the selected range can be found in our recent MEKC-MS studies on (±) BNP and (±) BOH [29].

The design matrix including both experimental and model predicted responses of the CCD for MEKC optimization are presented in Table 2. Chiral resolution for (±) BNA and the retention time of the last eluting peak were considered as quality responses. A total of 50 experiments were carried out according to the CCD, 8 of which (experiment 2, 9, 10, 17, 19, 26, 28, and 49) are repetitive runs with all the factors at their mean values. The errors of these repetitive runs were compared with the excess design points by *F*-test to determine the significance of the critical factors [14] (data not shown). As shown in Table 2, enantiomeric resolution varies from 0.03 to 1.42 and analysis time from 5.8 to 22.6 min.

Shown in Table 3 are all the coefficients for the terms of the predicted multifactor models calculated by the Design-Expert software. These coefficients represent the influence of the corresponding factor to the final response. For example, positive coefficient means the factor is directly proportional to the response and vice versa. Enantiomeric resolution and analysis time (i.e. retention of the last eluted enantiomer) are both considered as response in this table. The probabilities of each term having no effect on the response (i.e., Prob>*F*) are also summarized in Table 3. A coefficient is not considered significantly different to zero if its Prob>*F* value is bigger than 0.05, and the corresponding factor is regarded as non-critical. Other results of ANOVA such as *R*², adjusted *R*², predicted *R*² and *Q*² are tabulated in Table 3 (the last four rows). These values are all close to 1, suggesting a very good fitness for both models.

3.2.1 Model validation—Models obtained from the CCD experiment were further validated by ANOVA as shown in Table 4. Each model was first tested by lack of fit using the ANOVA results shown in the fourth and ninth row of Table 4. The *F* value for lack of fit test was calculated by the mean square of lack of fit divided by the mean square of pure error. As shown in Table 4, the *F* values are 1.4 and 1.5 for chiral resolution and migration time, respectively. Both are smaller than the tabulated value at the 95% confidence level. The probability of the

null hypothesis being true (i.e., the difference between lack of fit and pure error is caused by random error) is 0.33 and 0.31 for both responses. Thus, we can not reject the null hypothesis and there is no evidence of lack of fit at 95% level for both models. Further tests for the significance of the models were carried out by calculating the model F value as shown in the second and seventh row of Table 4. The F values were calculated as the mean square of the model divided by the mean square of residual. The results are 30 and 100, respectively, and the $\text{Prob}>F$ values are <0.0001 for both models, which indicate that there is at least one significant factor for each model.

3.2.2 Evaluation of MEKC parameters on enantioseparation and migration time

—As is shown in Table 3, eight terms in the quadratic model for the chiral resolution are significant at the level of 0.05 (F_1 - F_5 , F_2F_4 and F_5^2) with $\text{Prob}>F$ values less than 0.05. Interestingly, only %ACN when combined with voltage has some interactive effect ($\text{Prob}>F = 0.041$). Among them, buffer pH (F_1), surfactant concentration (F_3), and BGE concentration (F_4) are directly related to the response due to positive coefficients. The coefficient of surfactant concentration is the highest, which means this factor has the biggest effect on the response. This suggests that high concentration of surfactant provides more chiral binding sites and thus enhance the chiral interaction between the analyte and molecular micelles. Voltage (F_5) and the %ACN (F_2) are inversely related to the resolution in this model due to negative coefficients. Higher voltage decreases the retention time and does not give the analyte enough time to interact with the polymeric surfactant. The addition of ACN might change the micellar structure and intervene the interaction between the micelle and the analyte [30,31]. This might be able to explain its negative effect on the resolution.

Figure 1 shows how the response of resolution changes in accordance with any two factors and the relationship between them. Fig. 1 (A) is a three dimensional view of response surface plot which shows the impact of the combination of pH and %ACN. The plot indicates that pH is positively correlated to the response (i.e. resolution), while %ACN is inversely correlated. This comparison suggests that the %ACN has a bigger effect as the slope of the surface plot along the %ACN axis is bigger than that of pH axis. The best resolution is obtained when both factors are at their extremes (i.e., the highest for pH and the lowest for %ACN). Fig. 1 (B) shows the combination of $[\text{NH}_4\text{OAc}]$ and pH. The surface plot goes from the point where both factors are at their lowest value (pH 10.00 and 15.00 mM NH_4OAc , respectively) to its maximum when both factors are at their highest value (pH 11.00 and 25.00 mM NH_4OAc , respectively). Note that the plot is pretty steep at lower pH and lower NH_4OAc concentration, which means that the response is very sensitive to the change of the two factors in this region. However, the top region of the plot is fairly flat, indicating a comparably robust zone at the combination of higher pH and higher NH_4OAc concentration. The plot shown in Fig. 1 (C) is a fairly planar plot almost goes straight up from the corner of low [poly-SUCL] and low pH to the corner of high [poly-SUCL] and high pH. This plot is in accordance with the direct relationship of the response and the two factors. In addition, the slope of the surface is bigger along [poly-SUCL] axis than pH axis, suggesting that the effect of surfactant concentration is bigger than that of pH. In Fig. 1 (D), pH still shows the same trend as shown in Fig. 1 (A) while voltage has a bigger (yet negative) effect to the response. The highest resolution in this plot occurs at the combination of the highest pH and lowest voltage.

The interaction of ACN when combined with either poly-L-SUCL, NH_4OAc or voltage allowed the generation of response surface images shown in Figs. 1 (E-G). Fig. 1 (E) displays the relationship between [poly-L-SUCL] and %ACN. At the combination of the highest [poly-L-SUCL] and lowest %ACN, the plot reaches its peak. In this figure, %ACN shows bigger effect than [poly-SUCL] because the plot is steeper along the %ACN axis. This is not surprising since the absolute value of coefficient for %ACN is bigger than that of [poly-SUCL] (0.28 vs. 0.070 as shown in Table 3) in the model. Fig. 1 (F) shows a similar resolution trend for the

interactive effects between $[\text{NH}_4\text{OAc}]$ and %ACN as Fig. 1 (E). The highest resolution is reached at the highest $[\text{NH}_4\text{OAc}]$ and lowest %ACN. The effect of %ACN is again much bigger than that of $[\text{NH}_4\text{OAc}]$. In Fig. 1 (G), the plot suggests that the two interactive factors (voltage and %ACN) are inversely correlated to the response and the maximum resolution is achieved at the minimums of the two factors.

Figs. 1 (H-I) illustrates the interaction between voltage vs. $[\text{NH}_4\text{OAc}]$ or [poly-L-SUCL]. As suggested in the plot, voltage is inversely related to the response while $[\text{NH}_4\text{OAc}]$ or [poly-L-SUCL] is positively related. Again, voltage has a much bigger effect (similar to the plot shown in Fig. 1D) because the slope is much steeper along the voltage axis. Finally, Fig. 1 (J) shows the relationship of the two positive factors: $[\text{NH}_4\text{OAc}]$ and [poly-L-SUCL]. As indicated in the plot, both factors have very comparable effect and provide the highest response at their highest values.

The coefficients of the migration time listed in Table 3 shows that 8 terms (F_1 - F_5 , F_3F_5 , F_2F_5 , and F_5^2) in the quadratic model are significant to the response. The factors such as buffer pH (F_1), surfactant concentration (F_3), and NH_4OAc concentration (F_4) have positive coefficients suggesting increasing migration time upon increasing any of these factors. On the other hand, voltage (F_5) and %ACN (F_2) are inversely related to the migration time as evident by the negative coefficients. Higher concentration of surfactant retains the analyte more in the micellar phase and hence would increase the elution time. Increasing NH_4OAc concentration causes higher ionic strength of the running buffer as well as thinner electric double layer, consequently decreasing the EOF [8,32]. In particular, note that the voltage has the biggest effect on the migration time among all factors (i.e., has the highest absolute value of coefficient).

3.2.3 Evaluation of sheath liquid parameters on S/N and peak area—MeOH

percentage, pH, and NH_4OAc concentration were chosen as the three factors for the sheath liquid optimization (Table 1). Sheath liquid flow rate was not examined in this study because several of our previous studies have shown that there is only a very narrow range (e.g., 4-6 $\mu\text{L}/\text{min}$) where this parameter is significant. Hence, in most cases 5 $\mu\text{L}/\text{min}$ provided effective ionization. Therefore, a total of three factors were studied at three levels, which resulted in experimental matrix consisting of 20 experiments (Table 5) with 6 replicate runs (labeled as experiment 3, 5, 7, 11, 12, and 18). The range of all the selected factors was justified based on our earlier MEKC-ESI-MS study [9]. Peak area and S/N were chosen as responses in this study. Acceptable repeatability was obtained for the replicate runs. The %RSD values for the peak area was 27% and 31% for peak area and S/N, respectively. From Table 5, we can see that some of the experiments at extreme sheath liquids conditions were not successful due to current breakdowns. These conditions include very low percentage of MeOH (experiment 9), very low NH_4OAc concentration (experiment 14), or a combination of both (experiment 10 and 16). Figure 2 (A-B) shows the runs which gave the worst (experiment 6) and best (experiment 19) S/N, respectively at the MEKC conditions of pH: 11.5, 20% ACN, $[\text{NH}_4\text{OAc}]$: 25 mM, [poly-L-SUCL]: 40 mM, and voltage: +15 kV. ANOVA was again used to further validate the models. The ANOVA data suggest no evidence of lack of fit for both models (data not shown). The top half of Table 6 shows the regression coefficients for the sheath liquid factors. A close examination of $\text{Prob}>F$ shows that concentration of NH_4OAc in the sheath liquid had significant single effects on both S/N and peak area with $\text{Prob}>F$ values of 0.017 and 0.0010, respectively. However, this factor is inversely related to the peak area and S/N mainly because NH_4OAc in sheath liquid suppresses the ESI-MS signal at high concentrations. Interestingly, % (v/v) MeOH in the studied range did not have a significant single effect ($\text{Prob}>F = 0.33$) on S/N but was significant ($\text{Prob}>F = 0.015$) as an interactive effect when combined with NH_4OAc .

3.2.4 Spray chamber parameter optimization on S/N and peak area—Two factors, DGF and DGT, were included in the evaluation of spray chamber parameter study using a three level CCD with eight runs and five central points (experiment 2, 4, 5, 6, and 10), which result in a total 13 runs. Peak area and *S/N* are again chosen as responses. The detailed design and results are shown in the bottom half of Table 5. The %RSD for all the replicate runs was 27% for peak area and 8.2% for *S/N*. These %RSD values indicate acceptable experimental errors. Figure 2 (C-D) shows the electropherograms of the runs which gave the worst (experiment 13) and best (experiment 7) *S/N*, respectively. The two models for both peak area and *S/N* are also validated by ANOVA, which suggest that the model for peak area shows no sign of lack of fit but the model for *S/N* does. Finally, judging from Table 6, we can see that DGT is a significant factor and inversely related to both peak area and *S/N* with the Prob>F values of 0.013 and 0.027, respectively. This could be due to the possibility of BNA being less stable at higher temperature, consequently decomposing in the spray chamber.

3.3 Final optimum conditions

The optimum MEKC separation condition was determined by calculating the maximum values of the responses and the corresponding variables in the model generated from the CCD. To guarantee baseline chiral separation, highest resolution for ($\pm$) BNA was chosen as the only optimization criterion. The best MEKC conditions determined by the model were as follows: F_1 (buffer pH): 11.5, F_2 (%ACN): 20%, F_3 (concentration of surfactants): 40 mM, F_4 (concentration of NH_4OAc): 25 mM, F_5 (voltage): +15 kV. Compared to the optimum conditions obtained from the univariate approach discussed in section 3.1, the buffer pH, concentration of surfactant and concentration of NH_4OAc from multivariate experiment are all higher (11.5 vs. 10.5, 40 mM vs. 35 mM, and 25 mM vs. 20 mM, respectively); while voltage is lower (+15 kV vs. +20 kV). However, %ACN remains the same (20% v/v). Similar strategies were also applied to optimize the best sheath liquid conditions and spray chamber conditions. For both optimizations, highest *S/N* was chosen as the criterion. The optimum sheath liquid conditions were determined as: MeOH/ H_2O (80:20, v/v), 5 mM NH_4OAc , pH 6.0. The optimum spray chamber conditions are determined to be: drying gas flow rate: 6 L/min; drying gas temperature: 150 °C. Electropherograms of MEKC-MS under the final optimum MEKC-MS conditions is shown in Figure 3. The enantioresolution of BNA is 1.61. The average *S/N* of both enantiomers is 45. As shown in the inset of Fig. 3, the experimental values are close to what were predicted by the response surface models and are better than what were obtained from the preliminary sequential experiment (data not shown). Under the optimized MEKC-MS condition, the experimental and model predicted had a percent discrepancy difference of only 3.3%, 12%, and 12% for resolution, analysis time and *S/N*, respectively.

4 Concluding remarks

The enantiomers of ($\pm$) BNA was separated and detected by MEKC-ESI-MS using a polymeric surfactant poly-L-SUCL in a positive ion mode. A three-level five-factor full factorial CCD experiment was employed to optimize the MEKC separation parameters. Response surface models were built base on the CCD experiment and all factors were analyzed and optimized. It was found from the experiment that all five factors of MEKC are significant to both chiral resolution and analysis time of ($\pm$) BNA. The most significant factors that affect chiral resolution were identified as %ACN and voltage (both have negative effect to the resolution); while the most significant factor that affect the run time is voltage (inversely correlated to run time). Their relationship and interactions were further explored by the response surface plots generated from the model. Using maximum enantioresolution as criterion, all the factors were optimized by the models created in the analysis and the response also predicted. Actual running data of the optimum condition agreed with the predicted value and provided baseline resolution for ($\pm$) BNA. Sheath liquid composition and spray chamber conditions were also optimized

using the same methodology. Models for the peak area and the *S/N* for the ESI-MS signal was established and NH_4OAc concentration and drying gas temperature were found significant in these studies and were both inversely related to the *S/N* of the analyte. Running under the optimized MEKC-MS condition, the final experimental results matched adequately with the predicted value (Fig. 3 inset). This study not only provides an optimized MEKC-ESI-MS method for the chiral analysis of ($\pm$) BNA, but also suggests that multivariate experimental design methods such as CCD are effective tools for optimization of chiral separation and detection in MEKC-ESI-MS.

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List of abbreviations

ANOVA, analysis of variance
 BNA, 1,1'-binaphthyl-2,2'-diamine
 BNP, 1,1'-binaphthyl-2,2'-diyl-hydrogenphosphate
 BOH, 1,1'-bi-2-naphthol
 CCD, central composite design
 EMC, equivalent monomer concentration
 MeOH, methanol
 NH_4OAc , ammonium acetate
 NH_4OH , ammonium hydroxide
 poly-L-SUCL, polysodium *N*-undecenoxycarbonyl-L-leucinate
 RSM, response surface methodology

References

- Shamsi SA. Anal. Chem 2001;73:5103–5108. [PubMed: 11721906]
- Valle BC, Billiot FH, Shamsi SA, Zhu X, Powe AM, Warner IM. Electrophoresis 2004;25:743–752. [PubMed: 14981703]
- Palmer CP. J. Sep. Sci 2008;31:783–793. [PubMed: 18348324]
- Palmer CP, Terabe S. Anal. Chem 1997;69:1852–1860.
- Rizvi SAA, Zheng J, Shamsi SA. Electrophoresis 2007;28:1762–1778. [PubMed: 17480038]
- Akbar C, Rizvi SAA, Shamsi SA. Anal. Chem 2005;77:1672–1683. [PubMed: 15762571]
- Shamsi SA, Miller BE. Electrophoresis 2004;25:3927–3961. [PubMed: 15597424]
- Shamsi SA. Electrophoresis 2002;23:4036–4051. [PubMed: 12481292]
- Hou J, Zheng J, Rizvi SAA, Shamsi SA. Electrophoresis 2007;28:1352–1363. [PubMed: 17465416]
- Mohanty A, Dey J. J. Chromatogr. A 2005;1070:185–192. [PubMed: 15861803]
- Williams AA, Fakayode SO, Huang X, Warner I. Electrophoresis 2006;27:4127–4140. [PubMed: 17075942]
- Rizvi SAA, Simons DN, Shamsi SA. Electrophoresis 2004;25:712–722. [PubMed: 14981700]
- Rizvi SAA, Shamsi SA. Electrophoresis 2005;26:4172–4186. [PubMed: 16217829]
- Anderson, MJ.; Whitcomb, PJ. DOE Simplified Practical Tools for Effective Experimentation. Vol. 2nd ed.. Productivity Press; New York: 2007.
- Ferreira SLC, Bruns RE, da Silva EGP, dos Santos WNL, Quintella CM, David JM, de Andrade JB, Breitenkreitz MC, Jardim ICSF, Neto BB. J. Chromatogr. A 2007;1158:2–14. [PubMed: 17416377]
- Anderson, MJ.; Whitcomb, PJ. RSM Simplified: Optimizing Processes Using Response Surface Methods for Design of Experiments. Productivity Press; New York: 2005.
- Schappeler J, Guillaume D, Prat J, Veuthey J-L, Rudaz S. Electrophoresis 2007;28:3078–3087. [PubMed: 17724698]

18. Varesio E, Gauvrit J-Y, Longerey R, Lantéri P, Veuthey J-L. Electrophoresis 1997;18:931–937. [PubMed: 9221880]
19. Wan KT, Davis ME. Nature 1994;370:449–450.
20. Setnička V, Urbanová M, Bouř P, Král V, Volka K. J. Phys. Chem. A 2001;105:8931–8938.
21. Pu L. Chem. Rev 1998;98:2405–2494. [PubMed: 11848968]
22. Billiot FH, McCarroll MC, Billiot EJ, Warner IM. Electrophoresis 2004;25:753–757. [PubMed: 14981704]
23. Kano K, Minami K, Horiguchi K, Ishimura T, Kodera M. J. Chromatogr. A 1995;694:307–313. [PubMed: 7719468]
24. Nakamura H, Sano A, Sumii H. Anal. Sci 1998;14:375–378.
25. Pais LS, Loureiro JM, Rodrigues AE. Chem. Eng. Sci 1997;52:245–257.
26. Yarabe HH, Rugutt JK, McCarroll ME, Warner IM. Electrophoresis 2000;21:2025–2032. [PubMed: 10879962]
27. Rizvi SAA, Shamsi SA. Electrophoresis 2003;24:2514–2526. [PubMed: 12900863]
28. Hou J, Zheng J, Shamsi SA. J. Chromatogr. A 2007;1159:208–216. [PubMed: 17499757]
29. He J, Shamsi SA. J. Chromatogr. A 2009;1216:845–856. [PubMed: 19110258]
30. Thorsteinsdóttir M, Ringbom C, Westerlund D, Andersson G, Kaufmann P. J. Chromatogr. A 1999;831:293–309.
31. Mikaeli S, Thorsén G, Karlberg B. J. Chromatogr. A 2001;907:267–277. [PubMed: 11217034]
32. Cikaló MG, Bartle KD, Myers P. J. Chromatogr. A 1999;836:35–51.

Figure 1.

Response surface graphs for the enantiomeric resolution of (±) BNA involving all significant factors in separation optimization using CCD experiment. Factors which are not analyzed in the plots are held at their mean values (i.e. level 0 in Table 1). Experimental conditions: 60 cm × 50 μm id fused silica capillary; analytes: 0.4 mg/mL BNA in ACN/H₂O (40:60, v/v); injection: 5 mbar, 3 seconds; sheath liquid: MeOH/H₂O (50:50, v/v), 5 mM NH₄OAc, flow rate: 5 μL/min; ESI-MS parameters: nebulizer pressure: 2 psi; drying gas flow rate: 5 L/min; drying gas temperature: 200 °C; capillary voltage: 3000 V, m/z = 285; fragmentor: 90; gain: 3. MEKC separation conditions are shown in Table 2.

Figure 2.

Selected electropherograms of MEKC-MS runs from the CCD experiments for the optimization of sheath liquid composition (A-B) and spray chamber parameters (C-D). Experimental conditions for (A-B) are: 60 cm $\times$ 50 μ m id fused silica capillary; 25 mM NH_4OAc in 20% ACN (v/v) buffer, pH 11.5; 40 mM poly-L-SUCL; CE voltage +15 kV, column temperature 20 $^\circ\text{C}$; analytes: 0.4 mg/mL BNA in ACN/ H_2O (40:60, v/v); injection 5 mbar, 3 sec; sheath liquid flow rate: 5 $\mu\text{L}/\text{min}$; Spray chamber parameters are same as those in Fig. 1. Detailed sheath liquid conditions can be found in Table 5 (experiment 6 and 19 of the sheath liquid parameters). Separation conditions for (C-D) are the same to that of (A-B) except that the sheath liquid conditions are: MeOH/ H_2O (80:20, v/v), 5 mM NH_4OAc , pH 6.0, delivered at a flow rate of 5 $\mu\text{L}/\text{min}$. Detailed spray chamber conditions can be found in Table 5 (experiment 13 and 7 of the spray chamber parameters).

Figure 3.

Experimental electropherograms of MEKC-MS enantioseparation of (±) BNA under final optimized conditions: 60 cm × 50 μm id fused silica capillary; 25 mM NH₄OAc in 20% ACN (v/v) buffer, pH 11.5; 40 mM poly-L-SUCL; +15 kV, 20 °C; analytes: 0.4 mg/mL BNA in ACN/H₂O (40:60, v/v); injection size: 5 mbar, 3 seconds; sheath liquid: MeOH/H₂O (80:20, v/v), 5 mM NH₄OAc, pH 6.0, flow rate: 5 μL/min; ESI-MS parameters: nebulizer pressure: 2 psi; drying gas flow rate: 6 L/min; drying gas temperature: 150 °C; capillary voltage: 3000 V. m/z = 285; fragmentor: 90; gain: 3.

Table 1
Level of factors in the CCD used for the optimization of separation parameters, sheath liquid parameters, sheath liquid parameters, and spray chamber parameters in MEKC-MS of BNA

MEKC parameters					
Level	F ₁ : pH	F ₂ : %ACN	F ₃ : [poly-L-SUCL] (mM)	F ₄ : [NH ₄ OAc] (mM)	F ₅ : Voltage (kV)
-1	10.00	25	30.0	15.0	15
0	10.50	30	35.0	20.0	20
+1	11.00	35	40.0	25.0	25
Spray chamber parameters					
Level	F ₁ : %MeOH (v/v)	F ₂ : pH	F ₃ : [NH ₄ OAc] (mM)	F ₄ : DGF ^a (L/min)	F ₅ : DGT ^b (°C)
-1	20	6.00	5.0	4.0	150
0	50	7.25	22.5	5.0	200
+1	80	8.50	40.0	6.0	250

^a DGF: drying gas flow rate

^b DGT: drying gas temperature

Table 2
Resolution and migration time data gathered from the CCD experiment and model predicted responses of BNA enantiomers

Exp. #	Experimental parameters				Experimental/Model predicted responses		
	pH	%ACN	[poly-L-SUCL] (mM)	[NH ₄ OAc] (mM)	Voltage (kV)	Resolution	Migration time ^c (min)
1	11.00	35	40.0	25.0	25	0.36	7.8
2	10.50	30	35.0	20.0	20	0.89	10.0
3	10.00	25	30.0	15.0	25	0.66	7.1
4	11.00	35	40.0	15.0	25	0.44	7.3
5	10.00	25	30.0	25.0	25	0.81	7.8
6	10.00	35	40.0	25.0	25	0.51	7.4
7	11.00	35	40.0	15.0	15	0.72	13.1
8	11.69	30	35.0	20.0	20	0.89	10.3
9	10.50	30	35.0	20.0	20	0.97	10.0
10	10.50	30	35.0	20.0	20	0.96	10.2
11	11.00	35	30.0	15.0	25	0.36	6.9
12	11.00	35	30.0	25.0	25	0.05	6.6
13	11.00	25	30.0	25.0	15	1.36	14.9
14	11.00	25	30.0	25.0	25	0.89	7.8
15	10.00	25	30.0	15.0	15	1.19	12.5
16	10.00	35	30.0	25.0	15	0.48	11.6
17	10.50	30	35.0	20.0	20	0.84	9.5
18	10.50	30	35.0	20.0	30	0.33	5.5
19	10.50	30	35.0	20.0	20	0.73	9.0
20	11.00	25	40.0	25.0	25	0.98	8.7
21	11.00	35	40.0	25.0	15	0.69	14.6
22	10.00	35	40.0	15.0	25	0.12	6.9
23	10.00	35	30.0	25.0	25	0.11	6.8
24	10.50	30	35.0	31.9	20	0.80	10.8
25	10.00	35	40.0	25.0	15	0.74	14.8
26	10.50	30	35.0	20.0	20	0.81	10.1
27	11.00	25	30.0	15.0	15	1.03	13.7

Exp. #	Experimental parameters				Experimental/ Model predicted responses				
	pH	%ACN	[poly-L-SUCL] (mM)	[NH ₄ OAc] (mM)	Voltage (kV)	Resolution	Migration time ^c (min)		
28	10.50	30	35.0	20.0	20	0.84	0.85	10.2	10.3
29	9.31	30	35.0	20.0	20	0.62	0.67	8.4	9.0
30	10.50	30	35.0	20.0	8	0.86	0.97	22.6	21.9
31	11.00	25	40.0	25.0	15	1.42	1.38	15.4	16.5
32	10.50	30	35.0	8.1	20	0.61	0.61	8.0	8.7
33	10.00	25	40.0	15.0	15	1.16	1.09	13.7	14.0
34	10.00	35	40.0	15.0	15	0.60	0.58	11.5	12.7
35	10.00	25	30.0	25.0	15	1.19	1.22	13.3	14.2
36	11.00	35	30.0	25.0	15	0.59	0.50	12.7	13.4
37	10.00	35	30.0	15.0	15	0.49	0.45	11.0	11.2
38	10.50	42	35.0	20.0	20	0.04	-0.04	8.7	9.6
39	10.50	30	23.1	20.0	20	0.63	0.64	8.6	8.8
40	11.00	35	30.0	15.0	15	0.59	0.58	11.7	12.4
41	10.50	18	35.0	20.0	20	1.20	1.32	11.9	12.0
42	11.00	25	40.0	15.0	15	1.35	1.22	14.9	15.1
43	10.00	25	40.0	15.0	25	0.66	0.64	7.3	7.6
44	10.00	35	30.0	15.0	25	0.03	0.10	5.8	6.7
45	11.00	25	40.0	15.0	25	0.79	0.80	7.6	8.2
46	10.00	25	40.0	25.0	25	0.88	0.91	7.7	8.6
47	10.50	30	46.9	20.0	20	0.93	0.96	10.4	11.1
48	11.00	25	30.0	15.0	25	0.69	0.74	7.1	8.2
49	10.50	30	35.0	20.0	20	0.77	0.85	9.4	10.3
50	10.00	25	40.0	25.0	15	1.38	1.35	15.3	15.7

^c Migration time of the last peak of BNA.

Table 3

Regression coefficient of the coded factors and analysis of variance for the response surface models of chiral resolution and migration time (quadratic model) for the optimization of MEKC factors for BNA

Term	Resolution		Migration time ^c	
	Coefficient	Prob> <i>F</i> ^d	Coefficient	Prob> <i>F</i>
Intercept	0.86		9.7	
F ₁ : pH	0.046	0.049	0.34	0.0001
F ₂ : ACN%	-0.28	< 0.0001	-0.60	< 0.0001
F ₃ : [surfactant]	0.070	< 0.0001	0.48	< 0.0001
F ₄ : [NH ₄ OAc]	0.046	0.0046	0.50	< 0.0001
F ₅ : Voltage	-0.19	< 0.0001	-3.2	< 0.0001
F ₁ F ₂	0.0044	0.80	-0.018	0.84
F ₁ F ₃	0.0031	0.86	-0.021	0.82
F ₁ F ₄	-0.025	0.16	-0.089	0.33
F ₁ F ₅	0.0072	0.68	-0.14	0.14
F ₂ F ₃	0.021	0.24	0.12	0.18
F ₂ F ₄	-0.038	0.041	0.033	0.71
F ₂ F ₅	0.026	0.15	0.22	0.019
F ₃ F ₄	0.022	0.22	0.12	0.18
F ₃ F ₅	0.00063	0.97	-0.23	0.018
F ₄ F ₅	0.0034	0.85	-0.17	0.065
F ₁ ²	-0.013	0.32	-0.085	0.22
F ₂ ²	-0.038	0.0082	0.085	0.22
F ₃ ²	-0.0089	0.51	-0.056	0.42
F ₄ ²	-0.023	0.10	-0.078	0.26
F ₅ ²	-0.059	0.0005	0.78	< 0.0001
<i>R</i> ²		0.95		0.99
Adjusted <i>R</i> ² ^e		0.92		0.98
Predicted <i>R</i> ² ^f		0.84		0.95
<i>Q</i> ²		0.95		0.96

^c Migration time of the last peak.

^d Probability of the null hypothesis being true (the factor has no significant effect on the response) based on the F-test for comparing model variance with residual variance. Any term with *P* < 0.05 is considered significant, and call for rejection of null hypothesis.

^e Coefficient of determination adjusted for the number of terms in the model.

^f A measure of the amount of variation around the mean explained by the model, coefficient of determination is based on the predicted residuals from the model.

Table 4
ANOVA table for models used in the optimization of MEKC parameters

Responses	Source	Sum of squares	Degrees of freedom	Mean square	F-ratio	Prob>F
Resolution	Model	5.7	20	2.9×10^{-1}	30	<0.0001
	Residual	2.8×10^{-1}	29	9.7×10^{-3}		
	Lack of fit	2.3×10^{-1}	22	1.0×10^{-2}	1.4	0.33
	Pure error	5.2×10^{-2}	7	7.4×10^{-3}		
	Corrected total	6.0	49			
Migration time	Model	5.3×10^2	20	27	1.0×10^2	<0.0001
	Residual	7.6	29	2.6×10^{-1}		
	Lack of fit	6.2	22	2.8×10^{-1}	1.5	0.31
	Pure error	1.3	7	1.9×10^{-1}		
	Corrected total	5.3×10^2	49			

Table 5

Peak areas and S/N ratios from the CCD experiment for the optimization of sheath liquid parameters and spray chamber parameters

Sheath liquid parameters					
Exp. #	Experimental parameters			Experimental response	
	%MeOH (v/v)	pH	[NH ₄ OAc] (mM)	Avg. Peak area	Avg. S/N
1	20	8.50	40.0	19110	25
2	50	5.15	22.5	40192	54
3	50	7.25	22.5	32941	46
4	50	7.25	51.9	17091	16
5	50	7.25	22.5	19571	26
6	20	6.00	40.0	9384	14
7	50	7.25	22.5	39556	54
8	100	7.25	22.5	29854	41
9	0	7.25	22.5	N/A	N/A *
10	20	6.00	5.0	N/A	N/A *
11	50	7.25	22.5	45218	54
12	50	7.25	22.5	45938	75
13	50	9.35	22.5	40716	30
14	50	7.25	0.0	N/A	N/A *
15	80	8.50	40.0	25253	28
16	20	8.50	5.0	N/A	N/A *
17	80	6.00	5.0	89457	53
18	50	7.25	22.5	44909	50
19	80	6.00	40.0	35907	67
20	80	8.50	5.0	67074	23

Spray chamber parameters				
Exp. #	Experimental parameters		Experimental response	
	Drying gas flow rate	Drying gas temperature	Avg. Peak area	Avg. S/N

(°C)

(L/min)

1	6.4	200	61319	48
2	5.0	200	70044	47
3	3.6	200	73744	54
4	5.0	200	46001	43
5	5.0	200	77827	48
6	5.0	200	96153	54
7	5.0	129	101409	57
8	6.0	150	77195	45
9	4.0	150	53219	29
10	5.0	200	57994	49
11	5.0	271	39219	21
12	6.0	250	41356	23
13	4.0	250	29377	15

* Data unavailable due to current break down.

Table 6

Regression coefficient of the coded factors and analysis of variance for the response surface models of average peak area and S/N for the optimization of sheath liquid and spray chamber parameters.

Term	Peak area		S/N	
	Coefficient	Prob> F	Coefficient	Prob> F
Sheath liquid parameters				
Intercept	4.1×10^4		49	
F_1 : %MeOH	5.4×10^3	0.26	-5.9	0.33
F_2 : pH	-1.9×10^3	0.29	-6.2	0.16
F_3 : $[\text{NH}_4\text{OAc}]$	-2.0×10^4	0.0010	-17	0.017
$F_1 F_2$			-12	0.071
$F_1 F_3$			20	0.015
$F_2 F_3$			-1.3	0.83
R^2	0.71		0.72	
Adjusted R^2	0.63		0.53	
Predicted R^2	0.43		0.53	
Spray chamber parameters				
Intercept	6.3×10^4		41	
F_1 : DGF	2.3×10^3	0.71	2.0	0.65
F_2 : DGT	-1.8×10^4	0.013	-11	0.027
R^2	0.48		0.41	
Adjusted R^2	0.38		0.29	
Predicted R^2	0.16		-0.16	