

Optimization of the Nonaqueous Capillary Electrophoresis Separation of Metal Ions Using Mixture Design and Response Surface Methods

Cevdet DEMİR*, Yasin YÜCEL

*University of Uludağ, Faculty of Science and Arts, Department of Chemistry,
16059 Bursa-TURKEY
e-mail: cevdet@uludag.edu.tr*

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Mixture experimental design was used to enhance the separation selectivity of metal ions in nonaqueous capillary electrophoresis. The separation of cations (Ag, Fe, Cr, Mn, Cd, Co, Pb, Ni, Zn and Cu) was achieved using imidazole as UV co-ion for indirect detection. Acetic acid was chosen as an electrolyte because its cathodic electroosmotic flow permits faster separation. The composition of organic solvents is important to achieve the best separation of all metal ions. Simplex lattice design and response surface methods have proved to be an effective tool to optimize the composition of organic solvents in nonaqueous capillary electrophoresis. Full and reduced experimental design models were compared to obtain the optimum composition of solvents. Contour plots were presented to visualize the effect of the selected solvents on electrophoretic mobility. The highest electrophoretic mobilities were obtained with higher percentages of acetonitrile for all metal ions. Root mean square error (RMSE) and F-test were used to evaluate the models.

Key Words: Nonaqueous capillary electrophoresis, metal ions, mixture design, response surface methods.

Introduction

Improvement of separation selectivity is of fundamental importance in capillary electrophoresis (CE). Significant improvements were obtained in separation selectivity using different mixtures of 2 or more organic solvents. The majority of CE separations have been accomplished using aqueous buffers as background electrolytes, but a limited number of studies in nonaqueous media have shown the usefulness of this approach¹⁻⁴. The potential of nonaqueous CE is of particular interest for higher mobility and faster separation than with aqueous electrolytes.

One of the most important features of organic solvents is that their physical and chemical properties differ widely (Table 1)⁵. Selectivity manipulation in nonaqueous CE can be achieved simply by changing

*Corresponding author

the organic solvent or varying the proportions of solvents. Methanol and ethanol are amphiprotic solvents that act as proton donors or acceptors if there are other proton donors or acceptors in the separation system⁶. However, there are differences in their proton donor or acceptor capabilities. Aprotic solvents, such as acetonitrile, which have very weak autoprotolysis constants, can only accept protons. The viscosity of acetonitrile is low when compared to that of methanol, ethanol and water. It has an inverse effect on electrophoretic mobility that allows the separations in a reasonable time. Organic solvents may exhibit strong absorbance of ultraviolet (UV) light, so that indirect UV or other detection methods are required. Imidazole can be used for the UV co-ion, which has a similar electrophoretic mobility as a range of metal ions. If different selectivity for the metal ions is required additives such as small organic acids can be added. Because of the difference in affinity to the selector the mobility of metal ions decreases, resulting in different migration times and selectivities.

Table 1. Physical properties of the solvents⁵.

Solvent	Boiling point (°C)	Viscosity (mPa s)	Dielectric constant	Autoprotolysis constant	Surface tension (10 ⁻² N m ⁻¹)
Methanol	64.7	0.545	32.70	17.20	2.212
Ethanol	78.3	1.078	24.55	18.88	2.190
Acetonitrile	81.6	0.341	37.50	≥ 33.30	2.760
Water	100.0	0.890	78.39	14.00	7.181

The mixture design and response surface methods have been used to optimize the organic solvent selectivity in liquid chromatography⁷. Coenegracht et al.⁸ used the mixture design approach for the simultaneous optimization of analysis time and resolution in reversed-phase ion pair liquid chromatography. This approach has been used in the optimization of formulation of food⁹, oil spill dispersant¹⁰, paint¹¹, polymers¹², concrete¹³, glass¹⁴ and ceramic products¹⁵. There is no literature to our knowledge on the optimization of the composition of organic solvents using mixture design in nonaqueous capillary electrophoresis. The separation selectivity can be enhanced with a combination of 2 or more solvents by means of a mixture design approach. In a mixture design, the total amount of organic solvent is held constant and the mobility of the components changes when the proportions of the solvents are changed. The main purpose of this methodology is to verify how the mobilities of metal ions are affected by the variation of the composition of the mixture components. The simplex lattice design method can be used if separation is not achieved using the single solvent system. Ten experiments are performed; 3 are single systems, 6 are binary systems and 1 is a ternary system. The electrophoretic mobility of each metal ion is calculated in each of the 10 experiments. The determination of the best compositions of organic solvents can be accomplished by using response surface diagrams. The simplified model, which defines a so-called response surface, may correlate the mobility to the proportions of organic solvents. This yields the quantitative estimation of mobility of any composition of the solvent system.

The aim of this study was to investigate the influence of nonaqueous capillary electrophoresis on the separation selectivity of metal ions using mixture design. The mixture design approach proves its capability to optimize the composition of organic solvents for the best separation of metal ions in nonaqueous capillary electrophoresis.

Experimental

Chemicals

All metal ion solutions were prepared from nitrate salts (Merck, Darmstadt, Germany) using deionized water. Imidazole was purchased from Merck (Merck, Schuchardt, Hohenbrunn, Germany). Acetic acid, methanol, ethanol and acetonitrile were purchased from Merck (Merck, Darmstadt, Germany).

Apparatus

All measurements were performed using the CRYSTAL 100 capillary electrophoresis system equipped with an UV detector. Platinum wire electrodes were inserted into the buffer vials for connection to the electrical circuit. The fused silica capillary (53 cm \times 50 μ m i.d.) was used in all analysis. The electropherograms were recorded and integrated with PC 1000 software, Version 3.5 (Thermo Separation Products, San Jose, CA, USA) equipped with a Spectra SYSTEM SN 4000 interface.

Electrophoretic conditions

Before measurements the capillary was always flushed for 10 min with 1M NaOH, deionized water and running buffer, consecutively. Metal cations were indirectly detected on-column by UV absorbance at 214 nm. The sample was introduced into the capillary by a 5 s hydrostatic injection. The measurements were performed at room temperature. A positive voltage of 20 kV was applied for separation.

Preparation of stock solutions

Stock standard solutions containing 1000 μ g ml⁻¹ of 10 cations were prepared from their nitrate salts. A mixture of 10 metal ions containing 1 μ g ml⁻¹ each was prepared from stock solution. Imidazole (20 mM) and acetic acid (30 mM) were used as the background carrier electrolyte. Before injection to CE the solutions were filtered through a 0.45 μ m nylon filter (Millipore, Molsheim, France).

Mixture design

Experimental design and response surface methods have been used in a wide range of applications in the literature^{16–20}. Optimization of solvent mixtures in capillary electrophoresis plays an important role in obtaining the best separation of metal ions. The mixture design approach can be adopted if separation is not achieved using single or binary solvent systems. In a mixture design, the constituents of a mixture given in portions of volume are confined to the assumption that the amounts of the N constituents sum up to 100% or normalized to 1. If the proportion of one solvent is changed, the relative ratio of other solvents is changed. When a mixture design is used to optimize the solvent mixture, the electrophoretic mobility (response) is assumed to depend on the relative proportions of the solvents in the mixture. The selection of the optimum solvent composition was performed using a (k,m) simplex lattice design, and is shown in Figure 1. The mixture design table for the 3 factors is given in Table 2. For the k factors (solvents) the lattice describes all experimental points having the factor levels $0, 1/m, 2/m, \dots, (d-1)/m$ or 1. Ten experiments are performed; 3 are single systems, 6 are binary systems and 1 is a ternary system.

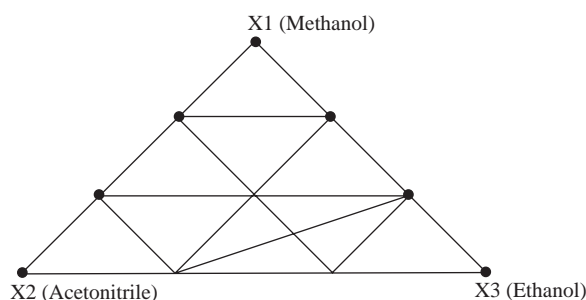


Figure 1. Mixture designs for ternary solvent mixtures (x_1 : methanol; x_2 : acetonitrile; x_3 : ethanol).

Table 2. Simplex lattice design for 3 factors at 5 levels.

Experiment	x_1 (Methanol)	x_2 (Acetonitrile)	x_3 (Ethanol)
1	1	0	0
2	0	1	0
3	0	0	1
4	1/3	2/3	0
5	2/3	1/3	0
6	1/3	0	2/3
7	2/3	0	1/3
8	0	2/3	1/3
9	0	1/3	2/3
10	1/3	1/3	1/3

In mixture designs, the purpose of the experimental design is to model the mixture surface with a form of polynomial equation so that the response for any mixture of solvents can be predicted. The factors (x_1 , x_2 and x_3) are not independent but the sum of all the factors is constant. Scheffé and Cox developed canonical polynomial models with constraints on the coefficients to analyze mixture data¹⁰. Scheffé models are widely used because they are simple and calculation of coefficients is easy. The model can be described as follows.

$$y = b_1x_1 + b_2x_2 + b_3x_3 + b_{12}x_1x_2 + b_{13}x_1x_3 + b_{23}x_2x_3 \quad (1)$$

This quadratic model can be used for obtaining the response surfaces in optimization studies.

Results and Discussion

Experimental design

The simplex lattice design was used in this study to optimize solvent mixtures in nonaqueous capillary electrophoresis. Ten experiments were performed as illustrated in Figure 1 and Table 2. There are 3 single factor experiments, 6 experiments where 1 factor is at 2/3 and the other at 1/3 and 1 experiment where all factors are at 1/3, resulting in 10 experiments in total. There are enough experiments for a full Sheffé model, and so some information about the significance of each parameter could be obtained. The electrophoretic mobilities were calculated for all 10 metal ions (Table 3).

The electrophoretic mobilities determine the velocity of an ion under a set of given capillary electrophoresis conditions. The velocity of the electrophoretic flow (EOF) and the electrophoretic mobilities

provide the apparent mobility in CE. The electrophoretic mobility μ_{eff} of an ion can be determined by

$$\mu_{eff} = \frac{L_t L_d}{V} \left(\frac{1}{t_m} - \frac{1}{t_0} \right)$$

where L_t is the total length of the capillary, and L_d the length of the capillary from the inlet to the detector. V the applied voltage, t_m the migration time of the ion and t_0 the migration time of the neutral marker.

Table 3. Electrophoretic mobilities of Ag, Fe, Cr, Mn, Cd, Co, Pb, Ni, Zn and Cu in organic solvents.

Experiment	Electrophoretic mobilities (μ_a) $\times 1.10^{-4}(\text{cm}^2\text{V}^{-1}\text{s}^{-1})$									
	μ Ag	μ Fe	μ Cr	μ Mn	μ Cd	μ Co	μ Ni	μ Zn	μ Pb	μ Cu
1	4.517	4.247	3.852	2.923	2.686	2.522	2.311	2.259	2.389	2.114
2	4.895	4.517	4.106	2.880	2.636	2.509	2.238	2.189	2.333	1.941
3	4.558	4.283	3.779	2.791	2.529	2.383	2.156	2.070	2.218	1.945
4	4.476	4.265	3.722	2.723	2.466	2.327	2.088	2.012	2.165	1.882
5	5.679	5.372	4.755	4.622	4.265	3.975	2.923	2.807	3.206	2.730
6	3.226	3.096	2.940	2.760	2.664	1.900	1.662	1.590	1.813	1.545
7	3.640	3.206	3.058	2.333	2.119	2.053	1.734	1.696	1.771	1.676
8	4.601	4.359	3.601	2.856	2.615	2.478	2.253	2.179	2.333	2.066
9	5.490	5.096	4.755	4.340	3.627	3.475	2.923	2.529	3.206	2.430
10	4.622	4.417	3.681	2.880	2.636	2.509	2.279	2.185	2.349	2.088

Separation of metal ions in organic solvents

Separation of Ag, Fe, Cr, Mn, Cd, Co, Pb, Ni, Zn and Cu was achieved using a voltage of 20 kV and detected indirectly at 214 nm in methanol, acetonitrile, ethanol and their mixtures. Table 3 presents the electrophoretic mobilities of cations in different solvents. The mobilities in the organic solvents are lowest in pure methanol for Ag, Fe and Cr and increase with acetonitrile and ethanol content.

The metal ions were determined by capillary electrophoresis. The electropherograms in Figures 2-4 show the separation of metal ions in an imidazole/acetic acid electrolyte in methanol, acetonitrile and ethanol, respectively. The effect of the addition of acetonitrile to methanol on the separation selectivity of metal ions was also studied for the imidazole/acetic acid electrolyte. The electropherogram in Figure 5 shows the separation of metal ions (dissolved in deionized water) in an imidazole/acetic acid electrolyte in ternary solvent mixtures.

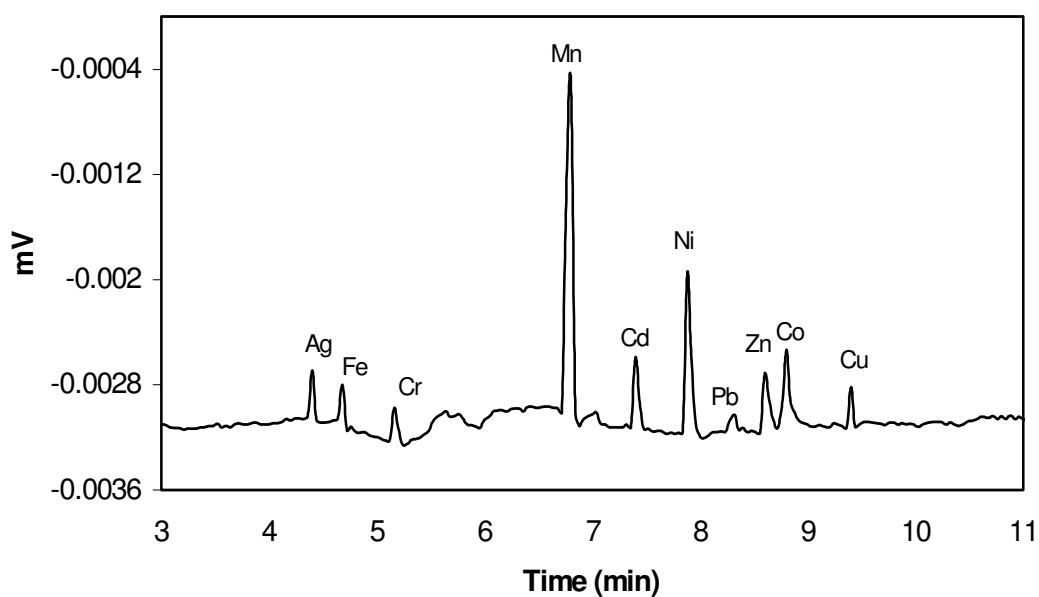


Figure 2. Separation of Ag, Fe, Cr, Mn, Cd, Ni, Pb, Zn, Co, and Cu ($1 \mu\text{g/mL}$) dissolved in deionized water. Experimental conditions: electrolyte, 20 mM imidazole 30 mM acetic acid in methanol; indirect detection at 214 nm; separation voltage, 20 kV; hydrostatic injection from a 10 cm height for 5 s.

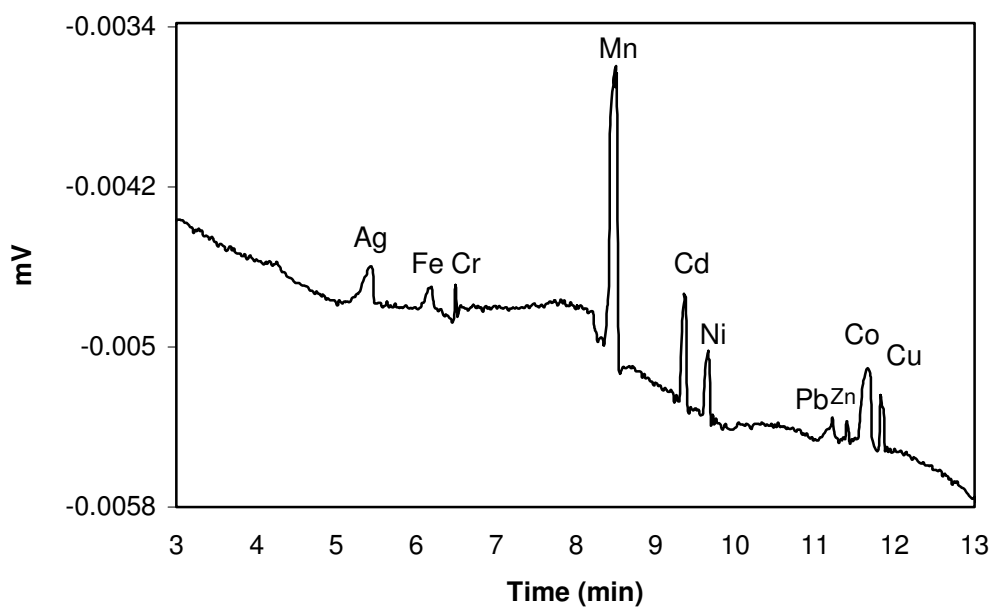


Figure 3. Separation of Ag, Fe, Cr, Mn, Cd, Ni, Pb, Zn, Co, and Cu ($1 \mu\text{g/mL}$) dissolved in deionized water. Experimental conditions: electrolyte, 20 mM imidazole 30 mM acetic acid in acetonitrile; indirect detection at 214 nm; separation voltage, 20 kV; hydrostatic injection from a 10 cm height for 5 s.

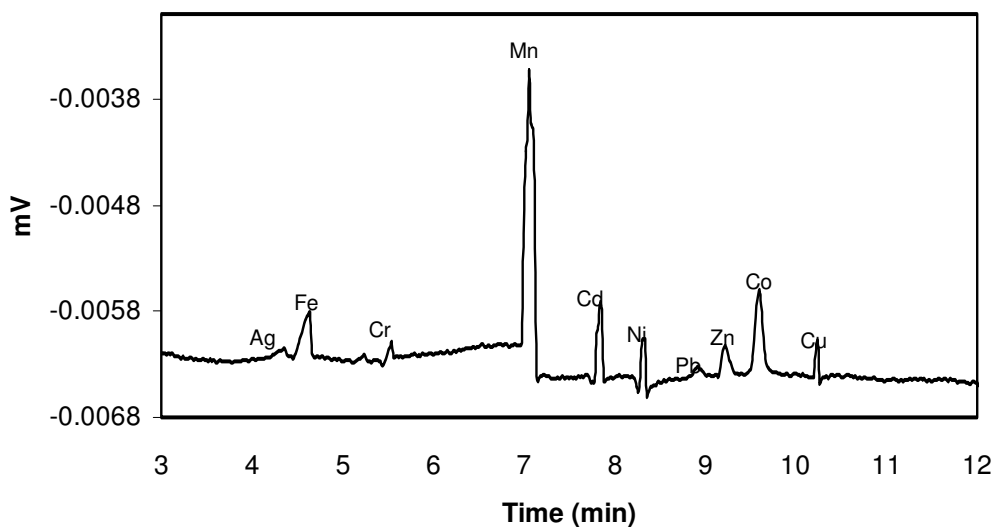


Figure 4. Separation of Ag, Fe, Cr, Mn, Cd, Ni, Pb, Zn, Co, and Cu ($1 \mu\text{g/g}$) dissolved in deionized water. Experimental conditions: electrolyte, 20 mM imidazole 30 mM acetic acid in ethanol; indirect detection at 214 nm; separation voltage, 20 kV; hydrostatic injection from a 10 cm height for 5 s.

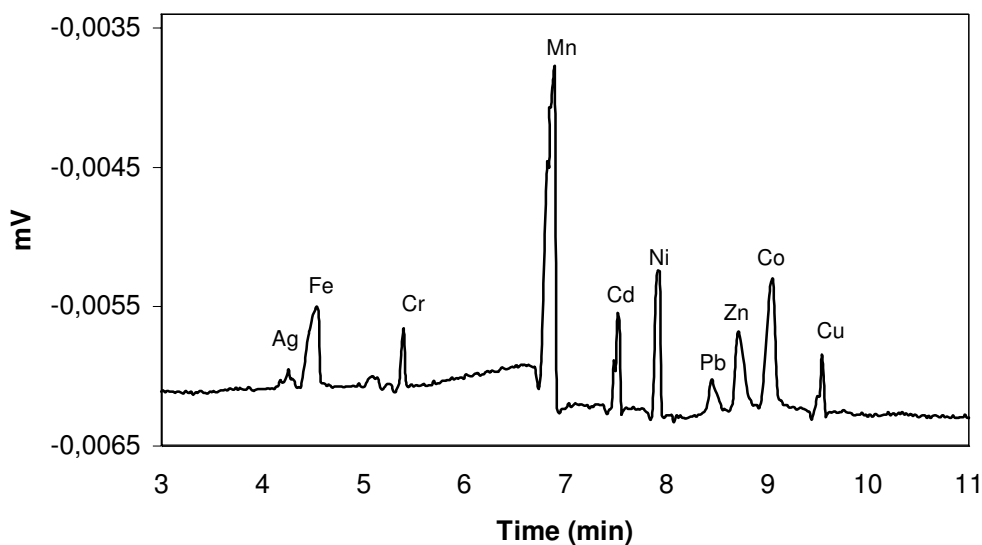


Figure 5. Separation of Ag, Fe, Cr, Mn, Cd, Ni, Pb, Zn, Co, and Cu ($1 \mu\text{g/g}$) dissolved in deionized water. Experimental conditions: electrolyte, 20 mM imidazole 30 mM acetic acid in ternary solvent mixtures (1/3 methanol, 1/3 acetonitrile, 1/3 ethanol); indirect detection at 214 nm; separation voltage, 20 kV; hydrostatic injection from a 10 cm height for 5 s.

Optimization of the CE separation of metal ions

Mixture experimental design and response surface methods are powerful tools in the optimization of the composition of organic solvents for separation of metal ions in capillary electrophoresis. The electrophoretic mobility could be related to the composition of solvents. The mobility is visualized by the height of the response surface for every possible blend of the 3 solvents.

The results are presented as 2-dimensional contour graphs, which show the level of curves of electrophoretic mobility as a function of the composition. The response surfaces for the 10 metal ions are shown in Figures 6-8. The most effective solvent composition can be estimated from the response surface. High electrophoretic mobilities are observed in the high composition of acetonitrile. The highest electrophoretic mobilities are around points 5 (2/3 methanol and 1/3 acetonitrile) and 9 (1/3 acetonitrile and 2/3 ethanol). The results show the importance of utilizing the response surface information to find the regions satisfying the compositions of solvents.

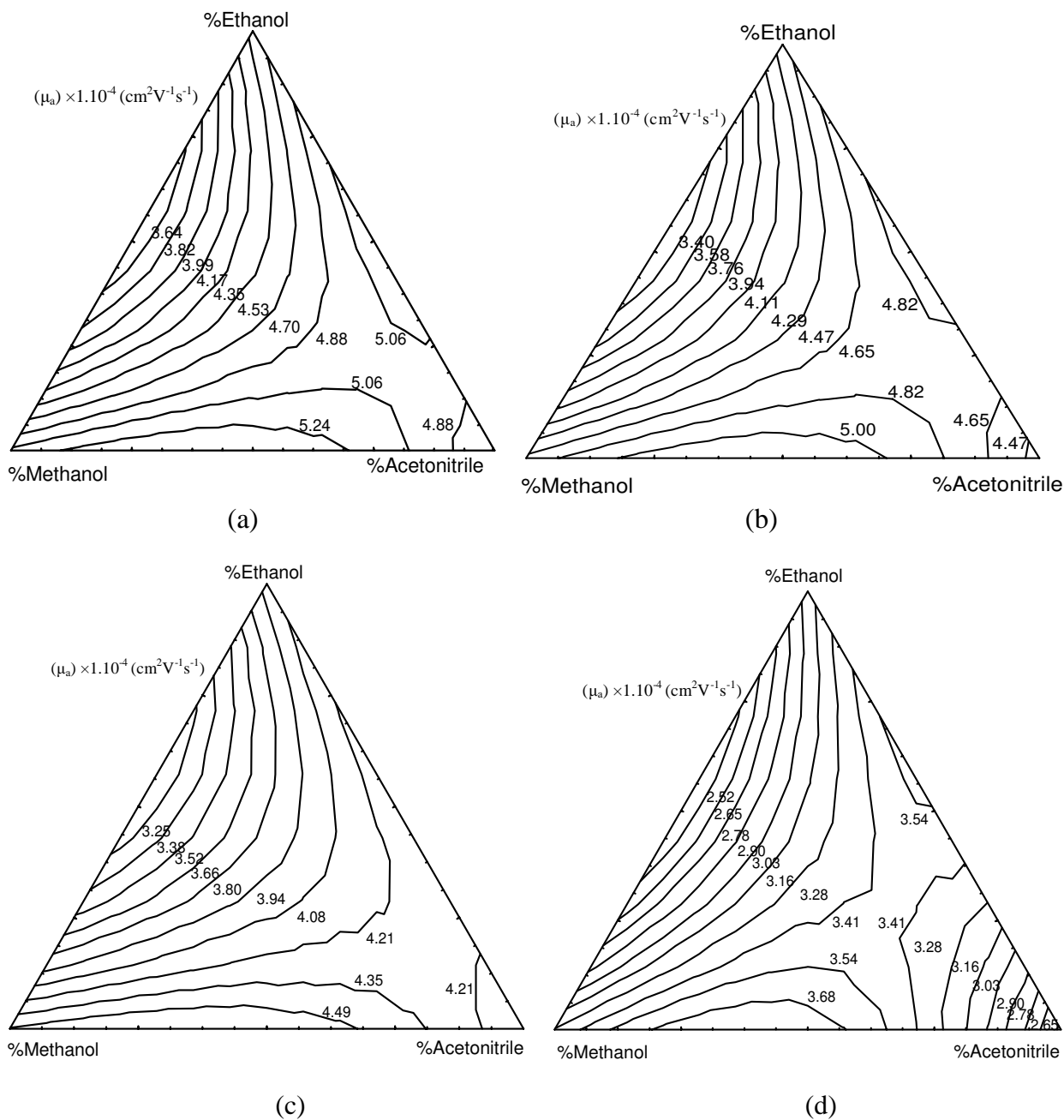


Figure 6. Ternary graphs of elements. (a) Ag, (b) Fe, (c) Cr and (d) Mn.

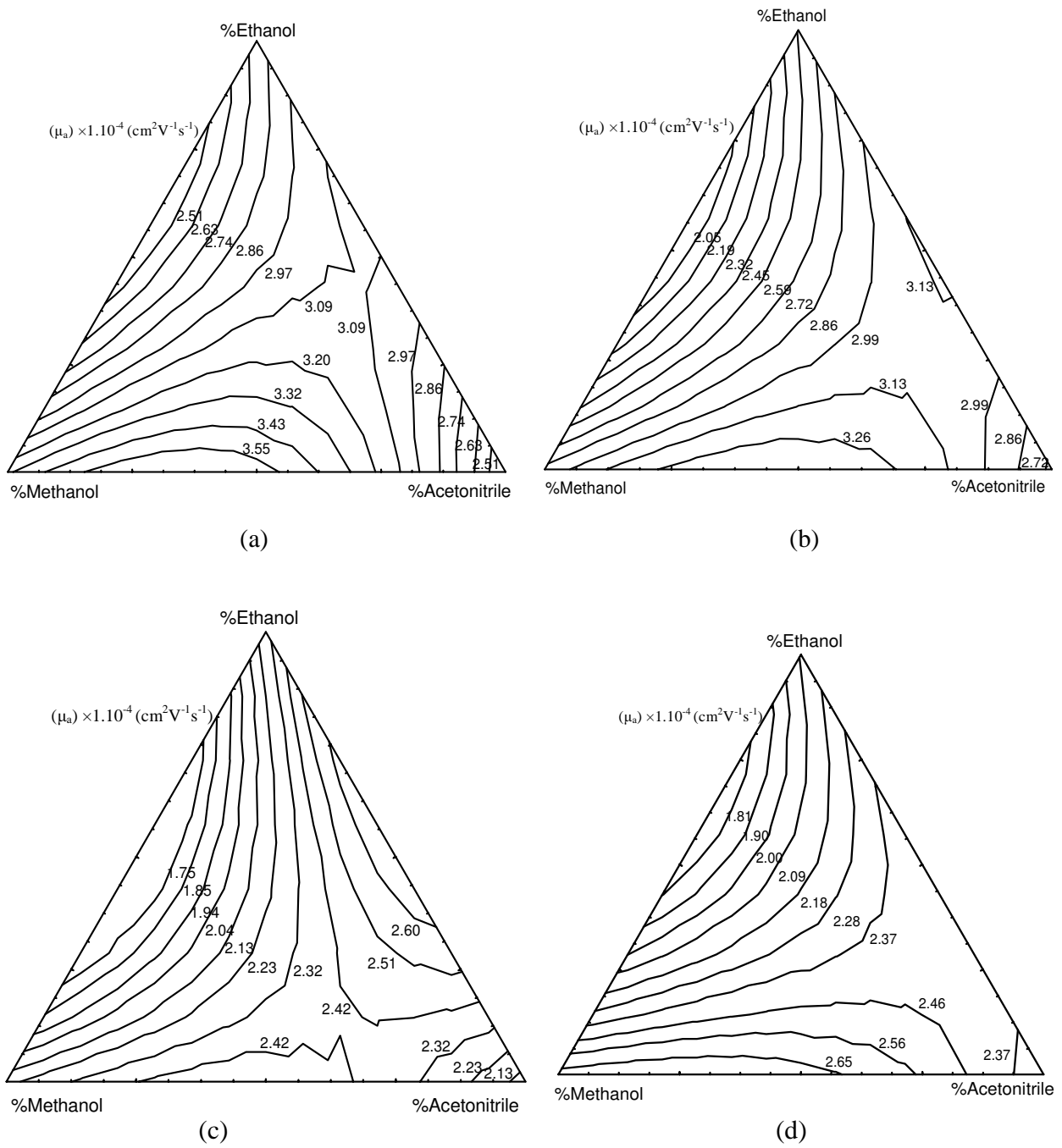


Figure 7. Ternary graphs of elements. (a) Cd, (b) Co, (c) Ni and (d) Zn.

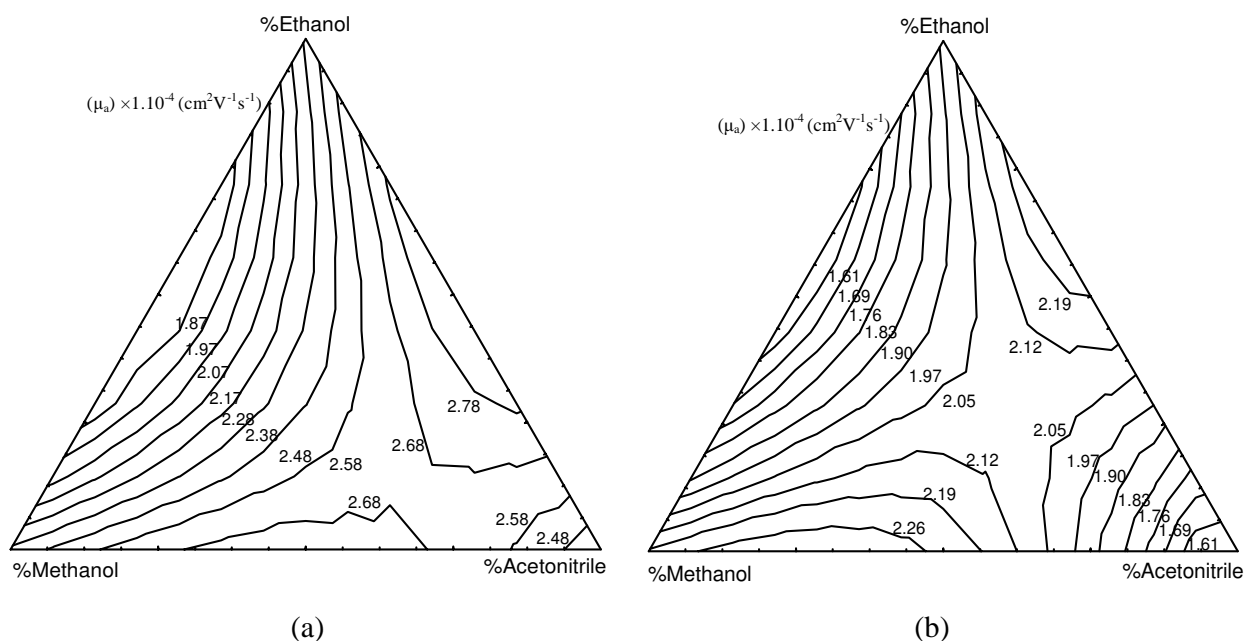


Figure 8. Ternary graphs of elements. (a) Pb and (b) Cu.

The Scheffé model and its reduced model were tested to define the best experimental model. Mathematical models relating electrophoretic mobilities to the factors under study are as follows:

$$y = b_1x_1 + b_2x_2 + b_3x_3 + b_{12}x_1x_2 + b_{13}x_1x_3 + b_{23}x_2x_3 \quad (2)$$

$$y = a_0 + a_1x_1 + a_2x_2 + a_{11}x_1^2 + a_{22}x_2^2 + a_{12}x_1x_2 \quad (3)$$

The relationship between the 2 models can be shown by substituting $x_3 = 1 - x_1 - x_2$ into the equation for model 1. Tables 4 and 5 illustrate the regression coefficients in the models of electrophoretic mobility of Ag, Fe, Cr, Mn, Cd, Co, Pb, Ni, Zn and Cu cations calculated using multiple linear regression (MLR). The positive coefficients corresponding to the interactions between solvents methanol-acetonitrile and acetonitrile-ethanol represent the synergetic effect of the mixture of solvents on the electrophoretic mobility. The negative coefficient of b_{13} indicates that methanol and ethanol are antagonistic towards one another. High electrophoretic mobilities were observed at experimental points 5 and 9 (Table 3) with the binary mixtures. The experimental design model was selected based on root mean square error (RMSE) and F-test criteria. The results of RMSE and F-test are shown in Tables 6 and 7 respectively. As a result, the Scheffé and its reduced experimental design models were compared to obtain the optimum composition of solvents. According to the results the lowest RMSE was obtained using model 1.

Table 4. The regression coefficients of Ag, Fe, Cr, Mn, Cd, Co, Pb, Ni, Zn and Cu for the Scheffé model.

$(b \times 1.10^{-4})$	Ag	Fe	Cr	Mn	Cd	Co	Ni	Zn	Pb	Cu
b_1	4.75	4.41	4.03	3.19	2.91	2.81	2.43	2.37	2.53	2.24
b_2	4.55	4.21	3.78	2.47	2.29	2.17	2.03	2.02	2.07	1.78
b_3	4.62	4.36	3.95	3.14	2.82	2.55	2.25	2.11	2.38	1.99
b_{12}	2.30	2.72	1.63	3.52	3.23	2.92	1.37	1.14	1.76	1.46
b_{13}	-5.42	-5.28	-4.46	-3.18	-2.44	-3.35	-2.85	-2.59	-3.03	-2.21
b_{23}	2.44	2.42	1.53	3.29	2.31	2.69	2.15	1.47	2.50	1.76

Table 5. The regression coefficients of Ag, Fe, Cr, Mn, Cd, Co, Pb, Ni, Zn and Cu for the reduced model.

($\times 1.10^{-4}$)	Ag	Fe	Cr	Mn	Cd	Co	Ni	Zn	Pb	Cu
a_0	4.62	4.36	3.95	3.14	2.82	2.55	2.25	2.11	2.38	1.99
a_1	-5.33	-5.27	-4.42	-3.17	-2.39	-3.10	-2.69	-2.36	-2.89	-1.97
a_2	2.09	2.00	1.12	2.36	1.57	2.11	1.76	1.23	2.02	1.42
a_{11}	5.46	5.33	4.50	3.21	2.48	3.35	2.87	2.61	3.05	2.23
a_{22}	-2.16	-2.15	-1.30	-3.04	-2.10	-2.49	-1.99	-1.33	-2.32	-1.63
a_{12}	5.48	5.78	4.74	3.57	3.50	3.69	2.18	2.36	2.40	1.99

Table 6. Root mean square error (RMSE) of Ag, Fe, Cr, Mn, Cd, Co, Pb, Ni, Zn and Cu for the 2 models.

Model (1.10^{-5})	Ag	Fe	Cr	Mn	Cd	Co	Ni	Zn	Pb	Cu
1	3.77	3.23	3.70	5.66	4.95	4.35	2.34	1.91	3.02	1.92
2	3.84	3.30	3.73	5.69	4.97	4.39	2.37	1.94	3.06	1.95

Table 7. F-test of Ag, Fe, Cr, Mn, Cd, Co, Pb, Ni, Zn and Cu for the 2 models.

Model	DF	F_{Table} (95 %)	Ag	Fe	Cr	Mn	Cd	Co	Ni	Zn	Pb	Cu
F(s1/s2)	9	3.18	1.01	1.01	1.01	1.02	1.02	1.02	1.01	1.01	1.02	1.01

The estimated optimum compositions of the solvents are given in Table 8. The predicted electrophoretic mobilities under these optimum compositions of the solvents are given in Table 9. The optimum compositions correspond approximately to the electrophoretic mobility that indicates the better separation of cations. The optimum compositions of organic solvents for Ag, Fe, Cr, Mn, Cd, Co, Pb, Ni, Zn and Cu were determined according to model 1 (Table 8). The predicted electrophoretic mobilities of these ions were calculated under optimum compositions of methanol, acetonitrile and ethanol (Table 9). The predicted electrophoretic mobilities are in the region of the response surface model for all metal ions.

Table 8. The optimum composition of organic solvents for Ag, Fe, Cr, Mn, Cd, Co, Pb, Ni, Zn and Cu.

Solvent (%)	Ag	Fe	Cr	Mn	Cd	Co	Ni	Zn	Pb	Cu
x_1 (methanol)	11.38	11.06	7.06	18.21	7.44	10.55	28.68	6.93	24.19	24.07
x_2 (acetonitrile)	68.14	65.98	71.58	50.95	46.51	62.25	60.77	68.31	64.51	47.03
x_3 (ethanol)	20.48	22.96	21.36	30.84	46.04	27.20	10.56	24.76	11.29	28.90

Table 9. The predicted electrophoretic mobilities of Ag, Fe, Cr, Mn, Cd, Co, Pb, Ni, Zn and Cu under optimum composition of methanol, acetonitrile and ethanol.

Electrophoretic mobilities ($\text{cm}^2\text{V}^{-1}\text{s}^{-1}$) (μ_a) $\times 1.10^{-4}$	Ag	Fe	Cr	Mn	Cd	Co	Ni	Zn	Pb	Cu
	5.031	4.784	4.252	3.437	3.097	3.095	2.416	2.440	2.682	2.088

Conclusion

Separation of cations (Ag, Fe, Cr, Mn, Cd, Co, Pb, Ni, Zn and Cu) in nonaqueous capillary electrophoresis was achieved using imidazole as the UV co-ion for indirect detection. Simplex lattice design and response

surface methods were employed for the optimization of ternary solvent mixtures (methanol, acetonitrile and ethanol). RMSE and F-test were used to evaluate the models. Two experimental design models were compared to obtain the optimum composition of solvents. The lowest RMSE was obtained using the full model.

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References

1. L.W. Vicki and M.G. Khaledi, **J. Chromatogr. A**, **859**, 203 (1999).
2. K.D. Altria and M.Wallberg, **J. Chromatogr. B**, **714**, 99 (1998).
3. H. Salimi-Mosavi and R.M. Cassidy, **Analytical Chemistry** **68**, 293 (1996).
4. F. Qu, L.M.Lin and Z. Chen, **J. Chromatogr. A**, **1022**, 217 (2004).
5. M.L.Riekkola, M. Jussila, S.P.Porras and I.E. Valko, **J. Chromatogr. A**, **892**, 155 (2000).
6. S.P. Porras and M.L. Riekkola, **J. Chromatogr. A**, **924**, 31 (2001).
7. J.L. Glajch and J.J. Kirkland, **J. Chromatogr. A**, **485**, 51 (1989).
8. P.M.J. Coenegracht, N. Van Tuyen, H.J. Metting and P.J.M. Coenegracht-Lamers, **J. Chromatogr. A**, **389**, 351 (1987).
9. A. Apichartsrangkoon and D.A. Ledward, **Food Chem.** **77**, 317 (2002).
10. P.J. Brandvik and P.S. Daling, **Chemometr. Intell. Lab. Syst.** **42**, 63 (1998).
11. A. Gupta, **J. Appl. Stat.** **28**, 199 (2001).
12. T.C. Wen and W.C. Chen, **J. Appl. Pol. Sci.** **77**, 680 (2000).
13. A.C.B.Ribeiro and I.R. De Almeida, **Mater and Struct.** **33**, 398 (2000).
14. G. Piepel and T. Redgate, **J. Am. Ceram. Society** **80**, 3038 (1997).
15. J.V. Nardi, W. Acchar and D. Hotza, **J. Eur. Ceram. Soc.** **24**, 375 (2004).
16. Y. Yücel and C. Demir, **Talanta** **63**, 451 (2004).
17. T. Naes, F. Bjerke and E.M. Faergestad, **Food Qual. Prefer** **10**, 209 (1999).
18. A. Kamoun M. Chaabouni, M. Sergent and R. Phan-Tan-Luu, **Chemometr. Intell. Lab. Syst.** **61**, 69 (2002).
19. G. Dingstad, B. Egelanddal and T. Naes, **Chemometr. Intell. Lab. Syst.** **66**, 175 (2003).
20. V.C. Morris, C. Hargreaves, K. Overall, P.J. Marriott and J.G. Hughes, **J. Chromatogr. A**, **766**, 245 (1997).