MATHEMATICAL REPRESENTATION OF ELECTROPHORETIC MOBILITY IN TERNARY SOLVENT ELECTROLYTE SYSTEMS

ABOLGHASEM JOUYBAN*, HAK-KIM CHAN**, MARYAM KHOUBNASABJAFARI*** and BRIAN JOHN CLARK****

*School of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran, **Faculty of Pharmacy, The University of Sydney, Sydney, Australia, ***Drug Applied Research Centre, Tabriz University of Medical Sciences, Tabriz, Iran and ****School of Pharmacy, University of Bradford, Bradford, UK

ABSTRACT

Electrophoretic mobilities of salmeterol and phenylpropanolamine in capillary zone electrophoresis were determined using acetate buffer in mixed solvents containing different concentrations of water, methanol and acetonitrile. Maximum electrophoretic mobilities for salmeterol and phenylpropanolamine were observed with water-methanol-acetonitrile ratios of 5:50:45 v/v and 3:60:37 v/v, respectively, and minimum mobilities of both compounds occurred in methanol-acetonitrile ratio of 30:70 v/v. The generated experimental data have been used to evaluate a mathematical model to compute the electrophoretic mobility of the analytes in a ternary solvent electrolyte system. The proposed model is:

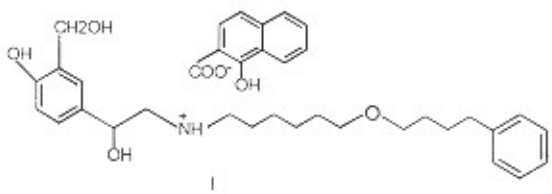
$$\ln \mu_m = f_1 \ln \mu_1 + f_2 \ln \mu_2 + Kf_3 + M_1 f_1 f_2 + M_2 f_1 f_3 + M_3 f_2 f_3 + M_4 f_1^2 f_2 + M_5 f_2^2 f_3 + M_6 f_2^2 f_3 + M_7 f_1 f_2 f_3$$

where μ is the electrophoretic mobility, subscripts m, 1, 2 and 3 refer to mixed solvent and solvents 1-3, respectively, f is the volume fraction of the solvent in the mixed solvent system and M_1-M_7 and K are the model constants calculated by a least squares analysis. The generated experimental data fitted to the model and the back-calculated mobilities were employed to compute the average percentage deviation (APD) as an accuracy criterion. The obtained APD for salmeterol and phenylpropanolamine are 3.10 and 2.21 %, respectively and the low APD values indicate that the model is able to calculate the mobilities within an acceptable error range.

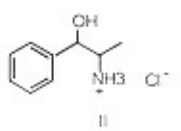
Keywords: Electrophoretic mobility, Mathematical modelling, Ternary solvent, Salmeterol, Phenylpropanolamine

INTRODUCTION

Salmeterol (I) is a long acting beta (β) adrenergic drug which is used to manage nocturnal asthmatic crisis. It has a basic functional group and is formulated as xinafoate salt for lung delivery. Phenylpropanolamine (II) is an alpha (α) adrenergic drug, which is regularly used in nasal decongestant formulations. Because of the low solubility of salmeterol in water (1), for their assay by capillary electrophoresis (CE) the use of mixed aqueous organic modifiers is desirable and in this study these drugs were used as models to calculate electrophoretic mobility of the analytes in an electrolyte system. Aqueous and non-aqueous mixed solvent buffer systems have been used in many validated CE methods. The main advantage of using these mixed solvent buffer systems are to



I



II

improve the selectivity, efficiency and resolution of the separation in developing a CE method (2-4). The binary mixed solvent buffer systems have been used in many separations, however, they are not able to solve every problem and sometimes

Correspondence: Dr. A. Jouyban, Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz 51664, Iran. Email: jouyban@tbzmed.ac.ir, Fax: +98 411 3344798.

addition of a third solvent is required. Several ternary mixed solvent buffers have been employed in validated capillary electrophoretic methods (5-6). These mixtures possess different characteristics in comparison with aqueous or non-aqueous buffers. The most important reasons for the changes in the electrophoretic behaviors of ions in mixed solvent running buffers are differences in; pKa values, viscosities, dielectric constants, electroosmotic behavior and conductivity of the electrolytes and analytes. Generally a combination of these effects is responsible for any changes in the electrophoretic mobility and also separation efficiency of the system. In establishment the condition for a separation a trial and error approach is often used by the analyst to optimize the concentration of the organic modifiers in running buffer. However, this procedure may take a long time and is therefore costly. In a recent paper (7), a mathematical model was proposed to calculate the electrophoretic mobility in binary solvent electrolyte systems. By developing such an equation, the trial and error approach can be replaced by a more rational method and also the generated experimental data can be evaluated to determine possible outliers when redetermination is required. In this article an extended form of the model for computation of the electrophoretic mobility in ternary solvent systems is described. The applicability of the proposed model to real data has been investigated by the use of the generated experimental mobility data of salmeterol and phenylpropanolamine in water-methanol-acetonitrile mixtures.

THEORETICAL TREATMENT

In the earlier work (7), a solution model was proposed to compute the electrophoretic mobility of analytes in binary solvent electrolyte systems. It showed accurate results for correlation of the mobility of 16 analytes in water-organic modifier mixtures (7). The model was:

$$\ln \mu_m = f_1 \ln \mu_1 + f_2 \ln \mu_2 + J_1 f_1 f_2 + J_2 f_1^2 f_2 \quad (I)$$

where μ is the electrophoretic mobility, subscripts m, 1 and 2 refer to the mixed solvent, solvents 1 and 2, respectively, f is the volume fraction of the solvent in the mixed solvent system and J_1-J_2 are the model constants calculated by a least squares analysis. When electrophoretic mobility in pure solvent 2 electrolyte system (μ_2) is not available or not applicable (for example insolubility of buffering agents in pure solvent 2), a modified version of the model may be employed, where C and J_1-J_2 are the model constants.

$$\ln \mu_m = f_1 \ln \mu_1 + C f_2 + J_1 f_1 f_2 + J_2 f_1^2 f_2 \quad (II)$$

In the case of a ternary solvent system, it is possible to include similar binary interaction terms for the solvents (i.e. f_1f_3, $f_1^2f_3$, f_2f_3, $f_2^2f_3$) and a ternary interaction term ($f_1f_2f_3$), and as a result an extended form of the model could be expressed as:

$$\begin{aligned} \ln \mu_m = {} & f_1 \ln \mu_1 + f_2 \ln \mu_2 + K f_3 + M_1 f_1 f_2 \\ & + M_2 f_1 f_3 + M_3 f_2 f_3 + M_4 f_1^2 f_2 \\ & + M_5 f_2^2 f_3 + M_6 f_2^2 f_3 + M_7 f_1 f_2 f_3 \end{aligned} \quad (III)$$

in which subscript 3 refers to solvent 3, K, M_1-M_7 are the model constants calculated by fitting ($\ln\mu_m$-$f_1\times\ln\mu_1$-$f_2\times\ln\mu_2$) against f_3, f_1f_2, f_1f_3, f_2f_3, $f_1^2f_2$, $f_1^2f_3$, $f_2^2f_3$ and $f_1f_2f_3$ using a no intercept least squares analysis. The model could be derived from an original solution model to calculate the solubility in ternary solvent mixtures proposed by Williams and Amidon (8).

EXPERIMENTAL

Instrumentation

All experiments were performed using a programmable capillary electrophoresis instrument (model P/ACE 5510, Beckman Instruments, High Wycombe, UK) and a 75 μm i.d.×37 cm length (30 cm to detector) fused silica capillary at 25°C. Samples were injected by pressure mode for 1 sec and analytes were detected by UV at 214 nm. The applied voltage was 24 kV. The CE instrument was interfaced with a microcomputer using Gold software (version 1.0) for the data collection and analysis.

Chemicals

Phenylpropanolamine hydrochloride and mesityl oxide were purchased from Aldrich Chemical Company (Dorset, UK). Salmeterol xinafoate was a gift from Glaxo Wellcome (Ware, UK). Methanol, sodium acetate and glacial acetic acid were purchased from BDH (Poole, UK) and acetonitrile from Riedel-de-Haen (Germany). Deionized water was used in the preparation of the buffer and sample solutions.

Method

A stock aqueous acetate buffer was prepared in a 100ml volumetric flask by dissolving 3.28 g of sodium acetate and 3.8 ml of glacial acetic acid in

pure water and/or methanol. The running buffers with binary and/or ternary solvents were prepared by mixing appropriate volumes of the stock buffer, deionized water, methanol and acetonitrile. The buffers were unadjusted for pH in this work. It was ensured that all buffers that we used contained the same volume of glacial acetic acid (0.38 ml/100ml) and sodium acetate (0.328 g/100ml). The expected pH for aqueous buffer was 4.7 based on Henderson-Haslbalch equation. The sample solutions were prepared in diluted running buffer solutions. Mesityl oxide was added to the sample solutions as a neutral marker.

Electrophoretic procedure

When a new capillary was used, the capillary was washed consecutively with 1M sodium hydroxide solution (1.0M), de-ionized water and running buffer for 30 minutes. The experiments were performed after pre-washing the capillary with sodium hydroxide solution (0.1M) for 1 min and running buffer for 2 min. All measurements were repeated at least three times. Each sample was injected for 2 seconds.

Computational analysis

The electrophoretic mobility of analytes was calculated by:

$$\mu = \frac{L_t \cdot L_d}{E}\left(\frac{1}{t_m} - \frac{1}{t_0}\right) \qquad (IV)$$

where L_t and L_d are the total capillary length and capillary length to the detector window in meters, E is the applied voltage, t_0 and t_m are migration times (in second) for the analytes and the electroosmotic flow.

The accuracy of the calculated mobilities was then examined with respect to the average percentage deviations (*APD*) which were computed from the expression:

$$APD = \frac{100}{N}\sum^{N}\left(\frac{|calculated - observed|}{observed}\right) \qquad (V)$$

where N is the number of experimental data points in each set. The mean value of APD was then calculated as an overall accuracy criterion. All calculations were carried out by the use of the statistical package for social sciences (SPSS).

RESULTS AND DISCUSSION

Table 1 shows the average electrophoretic mobilities and standard deviations of salmeterol and phenylpropanolamine in the solvent systems containing different concentrations of water, methanol and acetonitrile. Minimum and maximum mobilities of salmeterol were observed with water-methanol-acetonitrile ratios of 0:30:70 v/v and 5:50:45 v/v, respectively. The corresponding ratios for phenylpropanolamine were 0:30:70 v/v and 3:60:37 v/v, respectively. A non-linear relationship exists between ln μ_m and the solvent composition. The electrophoretic mobilities in pure aqueous and methanolic running buffers have been reported here. Because of solubility problem with sodium acetate, pure acetonitrile running buffer was not used in this work.

The experimental mobility data (Table 1) were fitted to Eq. III and the resulting trained model for salmeterol I is:

$$\ln\mu_m = 2.57f_1 + 2.66f_2 - 0.55f_3 - 2.93f_1f_2 + 14.35f_1f_3 + 9.59f_2f_3 + 1.97f_1^2f_2 - 13.22f_2^2f_3 - 6.28f_2^2f_3 - 18.51f_1f_2f_3 \qquad (VI)$$

R>0.999, F=1226, N=29, APD=3.10 %, p<0.0005

and for phenylpropanolamine II is:

$$\ln\mu_m = 2.57f_1 + 2.66f_2 + 0.38f_3 - 2.38f_1f_2 + 1288f_1f_3 + 8.05f_2f_3 + 1.42f_1^2f_2 - 11.66f_2^2f_3 - 4.97f_2^2f_3 - 16.64f_1f_2f_3 \qquad (VII)$$

R>0.999, F=3566, N=29, APD=2.21 %, p<0.0005

In these calculations, the robustness of the relationship between ln μ_m and the volume fractions of the solvents was indicated by higher F and lower p values. The resulting average percentage deviations lie in the experimental uncertainty. Therefore; by using the proposed model, it is possible to correlate the electrophoretic mobility data in ternary solvent mixtures. The mean APD for two sets was 2.66 %, which is an acceptable error range where the relative standard deviation between repeated experiments is ~ 4 %. Figures 1 and 2 show the computer generated three-dimensional plots of the electrophoretic mobility at different solvent compositions employing equations VI and VII. As it is seen from these graphs one cannot easily recognize the concentration at which a mixture of the analytes could be resolved. The graphical representations and recognition of the possibility of resolution is even impossible in the case of the use of a quaternary solvent system. However, by using mathematical models it is possible to calculate the mobility values. A small computer

Table 1. The experimental electrophoretic mobility (10^{-9} $m^2V^{-1}s^{-1}$) data for salmeterol and phenylpropanolamine in different solvent compositions

	Volume fraction			Salmeterol		Phenylpropanolamine	
No	Water	Methanol	Acetonitrile	Mean	SD	Mean	SD
1	0.00	0.30	0.70	7.43	0.18	13.12	0.08
2	0.00	0.50	0.50	13.31	0.06	22.59	0.24
3	0.00	0.70	0.30	15.26	0.24	25.74	0.22
4	0.00	0.90	0.10	15.12	0.05	25.19	0.07
5	0.00	1.00	0.00	14.23	0.21	23.66	0.34
6	0.03	0.60	0.37	15.93	0.11	26.35	0.03
7	0.05	0.50	0.45	16.17	0.07	25.92	0.25
8	0.07	0.53	0.40	14.27	0.11	24.00	0.28
9	0.10	0.45	0.45	15.02	0.00	24.75	0.00
10	0.10	0.47	0.43	14.57	0.13	24.39	0.18
11	0.10	0.60	0.30	14.19	0.06	23.82	0.11
12	0.17	0.33	0.50	14.90	0.00	24.87	0.00
13	0.20	0.40	0.40	13.28	0.06	23.14	0.04
14	0.20	0.47	0.33	12.95	0.22	22.60	0.27
15	0.23	0.20	0.57	14.48	0.00	24.82	0.00
16	0.30	0.35	0.35	12.82	0.08	22.30	0.04
17	0.30	0.70	0.00	8.58	0.09	15.82	0.09
18	0.33	0.33	0.33	12.67	0.05	22.35	0.12
19	0.47	0.20	0.33	12.61	0.12	22.82	0.27
20	0.50	0.25	0.25	11.51	0.05	20.88	0.10
21	0.50	0.33	0.17	10.06	0.04	18.50	0.07
22	0.50	0.50	0.00	8.29	0.03	15.53	0.07
23	0.70	0.15	0.15	11.25	0.06	20.62	0.06
24	0.70	0.20	0.10	10.46	0.03	19.14	0.07
25	0.70	0.30	0.00	9.52	0.10	17.56	0.13
26	0.90	0.05	0.05	12.25	0.07	22.36	0.15
27	0.90	0.07	0.03	12.00	0.13	21.87	0.18
28	0.90	0.10	0.00	11.78	0.01	21.35	0.12
29	1.00	0.00	0.00	13.04	0.02	23.81	0.20

The experiments were carried out at least in triplicate with a 37 cm (30 cm effective length) × 75 μm I.D. fused silica capillary. The electrolyte was 106 mM acetate buffer containing different concentrations of the organic modifiers. The applied voltage was 24 kV. Temperature was 25 °C and the wavelength was 214 nm.

program is able to predict the possibility of such a resolution. In CE operation the electrophoretic mobility in mixed solvent running buffers may be influenced by changes in the size of the solvated ions, variations in the dielectric constant of the electrophoresis medium and changes in pKa values of analytes in mixed solvents (9).

The solvation of the solutes in the electrophoresis medium and hetero conjugation of buffer electrolytes with the analytes can affect the mobility in mixed solvent running buffers (10). Differences in the viscosity and altered electro osmotic behaviour are the other effects of the mixed solvent. The curve-fitting parameters of the models represent the overall effects in the solution. To estimate these overall effects, one may determine the mobility in a limited number of different compositions of the solvent mixture to compute the model constants and then to predict the mobility at all other possible combinations of the solvent concentrations. It should be noted that electrophoretic mobility is one aspect of the method optimization process and other aspects such as pH, ionic strength, voltage, concentration of analytes in the sample solution and temperature should be fine tuned to give the best separation efficiency.

As a general conclusion, the proposed model

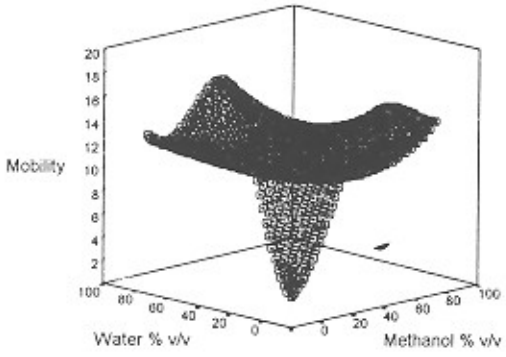


Fig. 1. Electrophoretic mobility of salmeterol in different percents of water and methanol in the ternary mixtures (The experiments were carried out at least in triplicate with a 37 cm (30 cm effective length) × 75 µm I.D. fused silica capillary. The electrolyte was 106 mM acetate buffer containing different concentrations of the organic modifiers. The applied voltage was 24 kV. Temperature was 25°C and the wavelength was 214 nm).

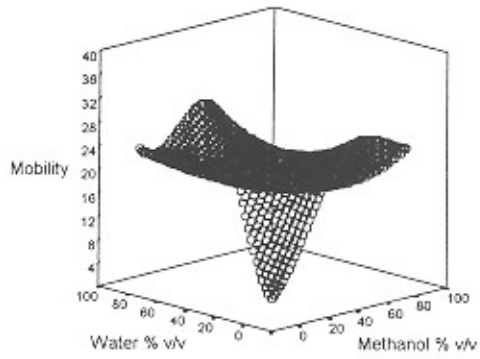


Fig. 2. Electrophoretic mobility of phenylpropanolamine in different % of water and methanol in the ternary mixtures. (The experiments were carried out at least in triplicate with a 37 cm (30 cm effective length) × 75 µm I.D. fused silica capillary. The electrolyte was 106 mM acetate buffer containing different concentrations of the organic modifiers. The applied voltage was 24 kV. Temperature was 25°C and the wavelength was 214 nm).

showed accurate results to calculate electrophoretic mobility in ternary mixed solvent running buffers. Concerning the general use of the model, it is proposed that in developing CE methods the model could be employed to optimize the solvent composition when a ternary mixed solvent system is required.

By using this mathematical procedure, it would be possible to avoid the lengthy trial and error approach and thus save the time and reduce the cost of development of an analytical method in CE.

ACKNOWLEDGMENTS

The authors would like to thank the Australian Department of Education, Training and Youth Affairs and the University of Sydney, Australia for providing the IPRS and IPA scholarships. The authors acknowledge Glaxo-Wellcome, UK for the salmeterol xinafoate powder and Drug Applied Research Center, Tabriz University of Medical Sciences, Iran for providing facilities.

REFERENCES

1. Jouyban-Gh., A., York, P., Hanna, M., Clark, B.J. (2001) Solubility prediction of salmeterol xinafoate in water-dioxane mixtures. Int. J. Pharm. 216: 33-41.
2. Bjornsdottir, I., Tjornelund, J., Hansen, S.H. (1998) Nonaqueous capillary electrophoresis, Its applicability in the analysis of food, pharmaceuticals and biological fluids. Electrophoresis 19: 2179-2186.
3. Sarmini, K., Kenndler, E. (1997) Influence of organic solvents on the separation selectivity in capillary electrophoresis. J. Chromatogr. A 792: 3-11.
4. Kuhn, R., Hoffstetter-Kuhn, S. (Eds.) (1993) Capillary Electrophoresis Principles and Practice, Springer-Verlag, Berlin, 12-48.
5. Tjornelund, J., Hansen, S.H. (1996) Determination of impurities in tetracycline hydrochloride by non-aqueous capillary electrophoresis. J. Chromatogr. A 737: 291-300.
6. Leung, G.N.W., Tang, H.P.O., Tso, T.S.C., Wan, T.S.M. (1996) Separation of basic drugs with non-aqueous capillary electrophoresis. J. Chromatogr. A 738: 141-154.

7. Jouyban, A., Chan, H.K., Khoubnasabjafari, M., Jouyban, N., Clark, B.J. (2001) Modeling the electrophoretic mobility of basic drugs in aqueous-methanolic buffers in capillary electrophoresis. DARU 9: 1-5.
8. Williams, N.A., Amidon, G.L. (1984) Excess free energy approach to the estimation of solubility in mixed solvent systems III: Ethanol-propylene glycol-water mixtures. J. Pharm. Sci. 73: 19-23.
9. Tjornelund, J., Hansen, S.H. (1999) Non-aqueous capillary electrophoresis of drugs: Properties and application of selected solvents. J. Biochem. Biophys. Methods 38: 139-153.
10. Okada, T. (1997) Non-aqueous capillary electrophoresis of bronsted acids as heteroconjugated anions. J. Chromatogr. A 771: 275-284.