MODELLING THE ELECTROPHORETIC MOBILITY OF BASIC DRUGS IN AQUEOUS-METHANOLIC BUFFERS IN CAPILLARY ELECTROPHORESIS

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ABSTRACT

The electrophoretic mobility of three beta-blocker drugs, i.e. nadolol, oxprenolol and pindolol, in sodium acetate buffer containing different concentrations of methanol varying from 0 to 100 percent have been determined by a capillary electrophoresis instrument. The generated experimental data have been employed to evaluate the accuracy of a mathematical model to calculate the electrophoretic mobility at different concentrations of methanol. The proposed model is:

$$\ln \mu_m = f_c \ln \mu_c + f_w \ln \mu_w + K_1 f_c f_w + K_2 f_c^2 f_w$$

where μ is the electrophoretic mobility, f is the volume fraction, subscripts m, c and w are the mixed water-methanol, pure methanol and pure water, respectively, K_1 and K_2 are the model constants. The proposed model produced accurate results and the average percentage deviation between experimental and calculated mobilities was 1.21 % for the data sets studied. This percentage error could be considered as an acceptable error where the relative standard deviation for the repeated experiments is around 2 %.

Key words: Electrophoretic mobility, Mathematical modelling, Beta-blockers, Water-methanol mixture

INTRODUCTION

There is much interest in using capillary electrophoresis in chemical and pharmaceutical analyses. The electrophoretic mobility of analytes is the key factor in the method development stage in capillary electrophoresis. A series of experiments are required to find the best analytical conditions. This will generally be achieved by studying the effects of voltage, pH, ionic strength, buffer additives, organic solvents, sample concentration and temperature. In practice, the analyst holds all parameters constant and the effect of one parameter at a time is studied usually by a trial and error approach. It is obvious that, this method is time consuming and also costly. Therefore, any attempt to predict the optimum analytical conditions is of fundamental importance in capillary electrophoresis. In this work, the possibility of prediction of the electrophoretic mobility of basic drugs in different concentrations of methanol in mixed aqueous-organic solvent electrolyte system has been studied. It is proposed that the model could be employed for predicting unmeasured electrophoretic mobility of analytes to find the optimum organic modifier concentration in order to speed up the method development stage of a new drug. The electrophoretic mobilities of three beta-blockers in mixed water-methanol electrolyte systems have been determined and accuracy of the proposed model has been evaluated against generated experimental data and also collected data sets.

THEORETICAL TREATMENT

Williams and Amidon (1) derived relationships between solute activity coefficient, solute's Henry law constants in pure solvents, and solute free cosolvent and water volume fractions at a constant temperature. These models were used to calculate solute solubility in binary solvents by employing Wohl's method for expressing the excess free energies of mixtures in terms of solute free volume fractions of the components. The three-suffix equation for a binary solvent system is:

$$\ln X_m = f_c \ln X_c + f_w \ln X_w - A_{c-w} f_c f_w (2f_c - 1)\left(\frac{V_2}{V_c}\right) + 2A_{w-c} f_c^2 f_w \left(\frac{V_2}{V_w}\right) + C_2 f_c f_w \qquad (I)$$

where X_c, X_w and X_m represent the mole fraction

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solubility of the solute in the pure cosolvent, water and in the mixed solvent, f_c and f_w are the solute free volume fractions of cosolvent and water, A_{c-w} and A_{w-c} stand for water-cosolvent interaction terms calculated from vapour-liquid equilibrium data, V_2, V_c and V_w are the molar volumes of the solute, cosolvent and water, respectively, and C_2 is the solute-solvent interaction term. Since A_{c-w}, A_{w-c}, V_2, V_c and V_w possess the constant values, Eq. I could be rearrange as II:
or:

$$\ln X_m = f_c \ln X_c + f_w \ln X_w + M_1 f_c f_w + M_2 f_c^2 f_w \qquad (III)$$

where M_1 and M_2 are the model constants and equal to $\left[A_{c-w}\left(\frac{V_2}{V_c}\right)+C_2\right]$ and $\left[2A_{w-c}\left(\frac{V_2}{V_w}\right)-2A_{c-w}\left(\frac{V_2}{V_c}\right)\right]$, respectively. In this work, the electrophoretic mobility of the analytes have been reproduced using a mathematical model derived from the excess free energy model, i.e. Eq. III. The derived model is expressed as:

$$\ln \mu_m = f_c \ln \mu_c + f_w \ln \mu_w + K_1 f_c f_w + K_2 f_c^2 f_w \qquad (IV)$$

where μ is the electrophoretic mobility, subscripts m, c and w are the mixed water-methanol, pure methanol and pure water, respectively, K_1 and K_2 are the model constants. These constants can be computed by regressing $\ln\mu_m - f_c\ln\mu_c - f_w\ln\mu_w$ against $f_c f_w$ and $f_c^2 f_w$ using a no intercept least squares method (3).

$$\ln X_m = f_c \ln X_c + f_w \ln X_w + \left[A_{c-w}\left(\frac{V_2}{V_c}\right)+C_2\right] f_c f_w + \left[2A_{w-c}\left(\frac{V_2}{V_w}\right)-2A_{c-w}\left(\frac{V_2}{V_c}\right)\right] f_c^2 f_w \qquad (II)$$

EXPERIMENTAL

Instrumentation:
All experiments were performed using a P/ACE system 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 detection at 214 nm. The applied voltage was 20 kV. The CE instrument was interfaced with a microcomputer using system Gold version 1.0 software (Beckman, High Wycombe, UK) for data collection and analysis.

Chemicals:
The analytes nadolol, oxprenolol hydrochloride, pindolol and mesityl oxide, were purchased from Aldrich Chemical Company (Dorset, UK). Methanol, sodium acetate and glacial acetic acid were purchased from BDH (Poole, UK). De-ionised water was used for the preparation of the buffer and sample solutions.

Method:
The stock aqueous sodium acetate buffer was prepared by dissolving 3.28 g sodium acetate and 3.8 ml glacial acetic acid in a 100 ml volumetric flask. The running buffers with 0-90 % v/v methanol were prepared by mixing appropriate volumes of the stock aqueous buffer, de-ionized water and methanol. The buffers were unadjusted for pH in this work. It was ensured that all buffers contained the same volume of glacial acetic acid. The pure methanolic buffer was prepared by dissolving 0.328 g sodium acetate and 0.38 ml glacial acetic acid in a 100 ml volumetric flask. The sample solutions were prepared by dissolution 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 with 1.0 M sodium hydroxide solution (30 min), de-ionized water (30 min) and running buffer (30 min). The experiments were performed after pre-washing with sodium hydroxide solution (0.1 M) for 1 min and running buffer for 2 min. All measurements were repeated at least three times. Each sample or sample mixture was injected for 1 second.

Computational analysis:
The electrophoretic mobility of the analytes was calculated by:

$$\mu = 10^9 \times \left[\frac{L_t \cdot L_d}{E}\left(\frac{1}{t_m}-\frac{1}{t_0}\right)\right] \qquad (V)$$

Where L_t and L_d are the total capillary length and length to detector window in meters, E is the applied voltage, t_0 and t_m are migration times for the analytes and the electroosmotic flow in seconds. The accuracy of the calculated mobilities was then examined with respect to the average percentage deviations (APD) which were computed from the expression:

$$APD = \frac{100}{N}\sum_{1}^{N}\left(\frac{|calculated - observed|}{observed}\right) \qquad (VI)$$

where N is the number of experimental data points in each set. The mean of APD is then calculated as an overall criterion. All calculations were carried out using the statistical package for social sciences (SPSS) in a Windows environment.

Table 1. The observed and calculated electrophoretic mobilities alongside the individual percentage deviation (IPD*) of the analytes in different volume fractions of methanol (f_c) and average percentage deviation (APD)

	Nadolol					Oxprenolol					Pindolol				
f_c	Observed	Correlated**		Predicted (Eq. VI)		Observed	Correlated**		Predicted (Eq. VII)		Observed	Correlated**		Predicted (Eq. VIII)	
	μ	μ	IPD	μ	IPD	μ	μ	IPD	μ	IPD	μ	μ	IPD	μ	IPD
0.00	16.19	-	-	-	-	18.51	-	-	-	-	19.21	-	-	-	-
0.10	13.83	13.72	-0.77	13.60	-1.60	15.99	15.86	-0.81	15.73	-1.60	16.31	16.30	-0.07	16.21	-0.58
0.20	11.93	11.95	0.20	11.82	-0.91	13.96	13.98	0.12	13.84	-0.87	14.27	14.25	-0.16	14.16	-0.76
0.30	10.63	10.72	0.87	-	-	12.61	12.70	0.71	-	-	12.80	12.85	0.40	-	-
0.40	9.87	9.93	0.61	9.90	0.30	11.82	11.93	0.92	11.91	0.80	11.91	11.98	0.57	11.98	0.58
0.50	9.54	9.52	-0.25	9.55	0.14	11.63	11.61	-0.16	11.68	0.43	11.55	11.55	0.04	11.61	0.52
0.60	9.56	9.46	-1.05	-	-	11.89	11.74	-1.23	-	-	11.66	11.55	-0.92	-	-
0.70	9.92	9.78	-1.44	9.93	0.08	12.55	12.37	-1.44	12.58	0.25	12.12	11.99	-1.08	12.14	0.13
0.80	10.45	10.53	0.79	10.70	2.43	13.51	13.61	0.71	13.85	2.48	12.84	12.94	0.75	13.10	1.99
0.90	11.59	11.85	2.28	11.99	3.47	15.30	15.67	2.39	15.86	3.67	14.32	14.53	1.47	14.66	2.35
1.00	13.97	-	-	-	-	18.93	-	-	-	-	17.02	-	-	-	-
		APD=	0.92	APD=	1.30		APD=	0.94	APD=	1.50		APD=	0.61	APD=	0.99

* IPD calculated by:

$$IPD = 100\left(\frac{calculated - observed}{observed}\right)$$

** Whole data points fitted to Eq. IV and the back calculated mobilities have been employed to compute the IPD and APD values.

The experiments were carried using a 37 cm (30 cm effective length) × 75 μm I.D. fused silica capillary. The electrolyte was 106 mM acetate buffer (40 mM acetate + 66 mM acetic acid) containing different concentrations of the organic modifier. The applied voltage was 20 kV. Temperature was 25 °C and the wavelength was 214 nm.

RESULTS AND DISCUSSION

Table 1 shows the observed and calculated electrophoretic mobilities, individual percentage deviation (IPD) and APD values for nadolol, oxprenolol and pindolol at different concentration of methanol in running buffer. The electrophoretic mobilities initially decrease with an increase in methanol concentration and reach to a minimum value at f_c=0.50, with further increase in methanol concentration increased to mobilities were observed. This pattern coincides then with viscosity variations in water-methanol mixtures where the maximum viscosity is observed at f_c between 0.40 - 0.60 (4).

The observed mobilities are the mean of at least three experiments and the relative standard deviations are less than 2 %. Each data have been collected in a day, and this is a possible reason for a relatively large RSD value. The experimental mobilities of the analytes were fitted to Eq. (4) and the back-calculated mobilities were compared with observed values by calculating IPD at each methanol concentration. The maximum IPD has been observed at f_c=0.90 for all the analytes studied, which lies in an experimental uncertainty range. The APD values for nadolol, oxprenolol and pindolol are 0.92, 0.94 and 0.61 %, respectively and the overall APD is 0.82 %.

In order to evaluate the prediction capability of the proposed model, 4 data points at f_c=0.00, 0.30, 0.60 and 1.00 have been employed to compute the model constants, i.e. K_1 and K_2. The mobilities at other f_c values were predicted using the trained models, i.e. Eqs. VII-IX:

$$for\ \ nadolol: \quad \ln \mu_m = f_c \ln \mu_c + f_w \ln \mu_w - 1.759 f_c f_w - 0.112 f_c^2 f_w \qquad (VII)$$

$$for\ \ oxprenolol: \quad \ln \mu_m = f_c \ln \mu_c + f_w \ln \mu_w - 1.819 f_c f_w - 0.135 f_c^2 f_w \qquad (VIII)$$

$$for\ \ pindolol: \quad \ln \mu_m = f_c \ln \mu_c + f_w \ln \mu_w - 1.743 f_c f_w - 0.058 f_c^2 f_w \qquad (IX)$$

The predicted mobilities which were compared with the experimental values using the IPD and APD values are shown in Table 1. The trained models are able to predict the electrophoretic mobilities within an acceptable error range. The produced APD for nadolol, oxprenolol and pindolol are 1.30, 1.50 and 0.99 %, respectively and the overall APD is 1.26%. The maximum IPD is 3.97 % for oxprenolol at f_c=0.90 which is still acceptable when it is compared with an experimental uncertainty.

To investigate the accuracy of the proposed model further, a set of electrophoretic data of other analytes have been employed. The detail of data sets, the obtained APD values and their references have been shown in Table 2. The overall APD value is 1.28 % for all 16 data sets of basic and acidic compounds in different running buffers containing various concentrations of methanol. The resulting mobilities are in an acceptable error range and the maximum APD is 4.99 %. For mobility data in phosphate buffer, systems 5 - 9, the electrophoretic mobility at f_c=1 were not determined because of the solubility problems with phosphate ions in the higher concentrations of methanol and one may modify the proposed model as:

$$\ln \mu_m = f_w \ln \mu_w + J f_c + K_1 f_c f_w + K_2 f_c^2 f_w \qquad (X)$$

where J is a curve-fitting parameter. From this equation, it is possible to select 4 mobility data points at different f_c values to train Eq. X for prediction purposes.

As a general conclusion, the proposed model produced reasonably accurate results and it is possible to employ the model to speed up the method development phase in capillary electrophoresis, where an organic modifier is required in the running buffer to improve the solubility or the separation efficiency of the method.

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Table 2. The electrophoretic mobility data of basic and acidic compounds in aqueous-methanolic mixed solvent electrolyte systems, the buffering agent, the range of methanol volume fraction in the mixture, average percentage deviations (APD), number of data points (N) and the reference

No.	Analyte	Running buffer	f_c range	APD	N	Reference
1	Monomethylamine	Acetate+imidazole	0-1	0.49	9	5
2	Dimethylamine	Acetate+imidazole	0-1	0.70	9	5
3	Diethylamine	Acetate+imidazole	0-1	4.99	9	5
4	Triethylamine	Acetate+imidazole	0-1	1.42	9	5
5	Benzoic acid	Phosphate	0-0.45	0.51	9	6
6	Phenylacetic acid	Phosphate	0-0.45	0.59	9	6
7	p-Aminobenzoic acid	Phosphate	0-0.45	0.47	9	6
8	p-Hydroxybenzoic acid	Phosphate	0-0.45	1.08	9	6
9	β-Naphthoxybenzoic acid	Phosphate	0-0.45	0.49	9	6
10	Propranolol	Acetate	0-1	1.36	11	7
11	Timolol	Acetate	0-1	1.42	10	7
12	Atenolol	Acetate	0-1	1.63	11	7
13	Alprenolol	Acetate	0-1	1.25	11	7
14	Acebutalol	Acetate	0-1	1.32	11	7
15	Labetalol	Acetate	0-1	1.46	10	7
16	Metoprolol	Acetate	0-1	1.36	11	7
			Mean=	1.28		

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