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Calculation of electrophoretic mobilities in water–organic modifier mixtures in capillary electrophoresis

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Abstract

In order to correlate/predict electrophoretic mobility data in the mixture of water+organic modifier four equations have been presented and examined. The experimental mobilities of five analytes were determined in a water–methanol mixture. These data have been used to assess the accuracy and predictability of the models. Also, some previously published mobility data in water–organic modifier mixtures has been employed for further evaluation of the models. The models produced accurate results and the means of percentage deviations were in the range of 0.66–1.30.

Keywords: Electrophoretic mobility; Background electrolyte composition; Mathematical modelling; Organic acids

1. Introduction

In separation techniques, such as capillary electrophoresis (CE), the organic modifier affects dissociation constant, separation efficiency and effective electrophoretic mobility of the analytes, pH, viscosity, dielectric constant, electroosmotic flow and conductivity of the background electrolyte. During method development in CE to develop an optimised separation the analysts generally have to employ a large number of experiments, which is often costly and time-consuming. Quantitative structure–retention relationships (QSRRs) have extensively been used to explain separation mechanisms and predict retention behaviour in analytical chemistry. However, only a few reports have investigated the quantitative correlation between the analytical parameters and the obtained responses in CE. These reports however, have shown the possibility of systematic optimisation techniques for method development to replace the trial and error approaches often used by industry. An example by Fu and Lucy developed empirical expressions for the prediction of electrophoretic mobilities of mono amines [1] and aliphatic carboxylic acids [2]. They correlated the mobility of the analytes with the molecular weight, hydration number, molar volume and dissociation constant by using non-linear equations. Also Liang et al. [3] correlated the electrophoretic mobility of flavonoids to topological indices but with relatively high prediction error (≈10%).

According to the Debye–Huckel–Henry theory [4], the viscosity of the solution shows a reciprocal relationship with mobility. Electrophoretic mobility

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is very sensitive to small changes in the viscosity of the solution. Therefore, knowledge of the viscosity of water–organic modifier mixtures over the entire composition range is beneficial to allow rapid method development. In addition the electrophoretic mobility is affected by any variations in pK_a, buffer conductivity and ζ potential. As a result, it is useful to establish a practical method for quantitatively predicting the electrophoretic mobility of the analytes as a function of the background electrolyte composition. Such predictive methods can be used for rationale method development. With higher organic modifier concentration a longer run time will be needed. After carrying out a minimum number of experiments and by using the models, one can optimise the best concentration of the organic modifiers to achieve the best separation efficiency.

The aim of this paper is to show the applicability of generated mathematical models for correlating and predicting the electrophoretic mobility of the analytes in mixed aqueous–organic modifier background electrolytes with respect to the concentration of the organic modifier. The models also provide a means of screening experimental data to check for possible outliers in data and, therefore, indications where re-determination is necessary.

2. Theoretical treatment

Electrophoretic mobility (μ) of an ion is expressed by the Debye–Huckel–Henry theory [4]

$$\mu = \frac{q}{6\pi\eta r} \tag{1}$$

where q is the charge on the particle, η denotes the viscosity of the background electrolyte and r is the Stokes' radius of the analyte.

When an organic modifier is added to an aqueous solution, it affects the viscosity of the solution. By ignoring any changes in solvation of the ions, we can rewrite Eq. (1) as a function of η

$$\mu = \frac{A}{\eta} \tag{2}$$

or

$$\frac{1}{\mu} = \frac{\eta}{A} \tag{3}$$

where A is a constant value equal to $(q/6\pi r)$. It has been shown that η can be represented as a power series of the organic modifier concentration in binary mixtures [5]. Thus,

$$\eta = B_0 + B_1 f_c + B_2 f_c^2 + B_3 f_c^3 \tag{4}$$

where B_0–B_3 are the curve-fit parameters and f_c is the volume fraction of the organic modifier in the mixture. Combination of Eqs. (3) and (4) yields

$$\frac{1}{\mu} = C_0 + C_1 f_c + C_2 f_c^2 + C_3 f_c^3 \tag{5}$$

where C_0–C_3 are the curve-fit parameters and equal to B_0/A, B_1/A, B_2/A and B_3/A, respectively. Logarithmic transformation in the left-hand side improves the accuracy of the model

$$\ln \mu = D_0 + D_1 f_c + D_2 f_c^2 + D_3 f_c^3 \tag{6}$$

in which D_0–D_3 are the new curve-fit parameters. The least-squares analysis which is available on commercial software, allows us to calculate these constants. Previously it has been shown that there are other variable arrangements for Eq. (6) which produce more accurate results [6]. This form of the equation is the combined nearly ideal binary solvent/Redlich–Kister (CNIBS/R-K) equation [7]. These models (Eqs. (6)–(9)) have basically been presented for calculating solute solubilities in mixed solvents. In addition, the modified Wilson model has also produced acceptable results for solubility data [8]. This model was introduced to calculate the infinitely diluted activity coefficient of solutes in mixed stationary phases in gas chromatography [9].

In the work here on capillary electrophoresis the applicability of the models to correlate the mobility data with respect to the concentration of the organic modifier is tested. The CNIBS/R-K model is expressed by

$$\ln \mu = f_c \ln \mu_c + f_w \ln \mu_w + L_0 f_c f_w + L_1 f_c f_w (f_c - f_w) \tag{7}$$

where μ_c and μ_w denote the electrophoretic mobilities in organic modifier and water, respectively, f_w denotes the volume fraction of water and L_0–L_1

are the curve-fit parameters. These constants are calculated by fitting $\ln \mu - f_c \ln \mu_c - f_w \ln \mu_w$ against $f_c f_w$ and $f_c f_w(f_c - f_w)$. In the case where μ_c is unknown, it is possible to use

$$\ln \mu = f_w \ln \mu_w + J f_c + L_0 f_c f_w + L_1 f_c f_w (f_c - f_w) \quad (8)$$

where J is the model constant which can be considered as an extrapolated value of $\ln \mu_c$. The model constants are computed by fitting $\ln \mu - f_w \ln \mu_w$ against f_c, $f_c f_w$ and $f_c f_w(f_c - f_w)$ by using a no intercept least-squares analysis.

It has been shown that the accuracy of the modified Wilson model for calculating solute solubilities in mixed solvent systems was comparable with that of the CNIBS/R-K model, and it can be employed for correlating the mobility data in mixed solvents as an alternative equation. The modified Wilson model is

$$\ln \mu = 1 - \frac{f_c(1 + \ln \mu_c)}{f_c + \lambda_1 f_w} - \frac{f_w(1 + \ln \mu_w)}{\lambda_2 f_c + f_w} \quad (9)$$

Since $(1 + \ln \mu_c)$ and $(1 + \ln \mu_w)$ are constant values for each analyte, one can assume that these terms are the model constants and there is no need to determine the mobility values in pure aqueous and pure organic modifier buffers. The simplified form of Eq. (9) is

$$\ln \mu = 1 - \frac{J_1 f_c}{f_c + \Lambda_1 f_w} - \frac{J_2 f_w}{\Lambda_2 f_c + f_w} \quad (10)$$

where λ_1, λ_2, J_1, J_2, Λ_1 and Λ_2 are the model constants. These constants are computed by using a non-linear least-squares analysis which is available on commercial statistical packages.

3. Experimental

3.1. Instrumentation

All experiments were performed using a P/ACE system 5510 (Beckman Instruments, High Wycombe, UK) and a 37-cm (30 cm to detector)×75-μm I.D. fused-silica capillary at 25°C. Samples were injected by pressure mode for 2 s and analytes were detected by direct UV detection at 254 nm. The applied voltage was 20 kV. The CE instrument was interfaced with a microcomputer using system Gold version 1.0 software for data collection and analysis.

3.2. Chemicals

The analytes used, 4-hydroxybenzoic acid (HBA), phenylacetic acid (PA), β-naphthoxyacetic acid (NA) and mesityl oxide, were purchased from Aldrich (Dorset, UK). Methanol, disodium hydrogenphosphate anhydrous, sodium dihydrogen phosphate monohydrate, 4-aminobenzoic acid (ABA) and benzoic acid (BA) were purchased from BDH (Poole, UK). Deionised water was used for preparing the buffer and sample solutions.

3.3. Method

The stock aqueous phosphate buffer was prepared by dissolving 7.1 g (Na_2HPO_4) and 6.9 g ($NaH_2PO_4 \cdot H_2O$) in a 100-ml volumetric flask. The running buffers with 0–45% (v/v) methanol were prepared by mixing appropriate volumes of the stock buffer, deionised water and methanol. We used buffers which were unadjusted for pH in this work at 10 m*M* concentration. The samples were prepared at a concentration of 2 m*M* in aqueous solution. Mesityl oxide was added to the sample solutions as a neutral marker.

3.4. Electrophoretic procedure

When a new capillary was used, the capillary was washed with sodium hydroxide solution (1.0 *M*) for 30 min, deionised 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 in triplicate. Each sample or sample mixture was injected for 2 s.

3.5. Computational analysis

The electrophoretic mobility of analytes was calculated by

$$\mu = \frac{L_t L_d}{E} \cdot \left(\frac{1}{t_m} - \frac{1}{t_0} \right) \quad (11)$$

where L_t and L_d are the total capillary length and length to detector window in m, respectively, E is the applied voltage, t_m and t_0 are migration times for the analytes and the electroosmotic flow in seconds, respectively. The electroosmotic mobility (μ_{eo}) was calculated by

$$\mu_{eo} = \frac{L_t L_d}{E t_0} \quad (12)$$

The accuracy of the theoretically calculated mobilities was examined with respect to the average percentage deviations (APDs) which were computed from the expression

$$\text{APD} = \frac{100}{n} \sum_{1}^{N} \left(\frac{|\text{theoretical} - \text{observed}|}{\text{observed}} \right)$$

where n is the number of experimental data points in each set. The mean of APD is then calculated as an overall criterion for the comparison of the models. The one-way analysis of variance and Duncan's multiple range test were used for statistical evaluating of the APD values from the different equations.

All calculations were carried out by using the statistical package for social sciences (SPSS) in a Windows environment.

4. Results and discussion

Table 1 shows the electroosmotic mobility and electrophoretic mobility of the analytes studied here (see Fig. 1). The mobility differences between analytes are varied with the concentration of the organic modifier in the running buffer. The parallel behaviours are observed for set 1 (PA, BA and ABA) and set 2 (HBA and NA). This means that one can efficiently separate these sets in any methanol concentration. There are also non-parallel behaviours for set 3 (HBA and NA), set 4 (HBA and PA), set 5 (ABA and NA) and set 6 (PA and NA). This means that the best separation efficiency of the analytes will be achieved in a given concentration of the organic modifier which can be calculated by the proposed equations. It is obvious that the mixed aqueous–organic modifier buffers affect other factors including peak shape, the theoretical plate numbers and Joule heating. However, in this work, we focused on the effect of mixed buffer systems on the mobility of the analytes. The general decreasing pattern was observed for electroosmotic flow with increasing the concentration of organic modifier.

Table 2 shows APD values for Eqs. (5), (6), (8) and (10). The accuracy order of the correlative models (the model constants computed by using whole data points) is Eq. (8)>Eq. (6)>Eq. (5)>Eq. (10). The corresponding order for the predictive equations (the model constants computed by employing a minimum number of experiments, i.e., four data points) is Eq. (10)>Eq. (8)>Eq. (6)>Eq. (5). These results indicate that Eq. (10) is a more accurate model where the analyst wishes to optimise the concentration of the organic modifier by carrying out a minimum number of experiments.

In order to test the applicability of the expressions further, the experimental mobility values for benzoate derivatives were collected from the literature [10,11]. The list of analytes with their experimental mobilities at $f_c = 0$ and $f_c = 0.75$ and APD values for the different models are shown in Table 3. This time Eq. (8) is the most accurate, followed by Eqs. (6), (5) and (10). The result of the analysis of variance shows that the difference between the mean APD values for equations studied are statistically significant ($P < 0.05$). In order to identify which means differ from others, Duncan's multiple range test is employed. The results of this test indicate that mean differences between Eqs. (6) and (8) with Eqs. (5) and (10) are significant. From a mathematical standpoint Eqs. (6) and (8) are essentially the same and this is illustrated by Eq. (8) being easily converted to Eq. (6) by a simple algebraic manipulation [6]. As a result the accuracy differences for these models (Eqs. (6) and (8)) are insignificant.

As was mentioned in Section 1, the mathematical models can be employed for screening experimental data for possible outliers. By further careful examination of the results in Table 3, all four correlative equations produced relatively high APD values for the model compound number 29. It means that in this data set (3-hydroxybenzoate mobility in 1-propanol + water) there is at least one outlier data point that affects the developed correlative equations.

As indicated in the introduction, during method development in CE, an organic modifier is sometimes added to the aqueous running buffer but this

Table 1
The experimental (Exp.) and the calculated electrophoretic mobility (10^{-9} m^2 V^{-1} s^{-1}) of the analytes in different concentrations of methanol (f_c) for Eqs. (5), (6), (8) and (10)[a]

Analyte	f_c	Exp.	Eq. (5)	Eq. (6)	Eq. (8)	Eq. (10)
BA	0.00	30.43	30.27	30.30	30.43	30.44
	0.05	27.76	28.11	28.08	28.13	28.04
	0.10	26.23	26.07	26.05	26.06	25.96
	0.15	24.30	24.23	24.23	24.22	24.15
	0.20	22.65	22.61	22.63	22.62	22.60
	0.25	21.26	21.25	21.26	21.25	21.28
	0.30	19.94	20.12	20.12	20.12	20.18
	0.35	19.34	19.23	19.21	19.23	19.28
	0.45	18.12	18.13	18.13	18.13	18.09
PA	0.00	28.51	28.21	28.29	28.51	28.38
	0.05	25.60	26.12	26.05	26.13	26.09
	0.10	24.02	23.99	23.94	23.94	23.78
	0.15	22.26	22.02	22.02	22.00	21.83
	0.20	20.28	20.30	20.33	20.31	20.26
	0.25	18.91	18.87	18.90	18.89	18.98
	0.30	17.63	17.73	17.74	17.75	17.94
	0.35	16.94	16.88	16.87	16.88	17.08
	0.40	16.25	16.31	16.30	16.31	16.36
	0.45	16.07	16.03	16.04	16.03	15.75
ABA	0.00	27.41	27.17	27.24	27.41	27.31
	0.05	24.73	25.10	25.04	25.09	25.10
	0.10	22.95	23.01	22.96	22.97	22.83
	0.15	21.32	21.09	21.09	21.07	20.91
	0.20	19.42	19.41	19.44	19.42	19.36
	0.25	18.03	18.02	18.04	18.03	18.11
	0.30	16.81	16.90	16.91	16.91	17.09
	0.35	16.13	16.06	16.05	16.06	16.24
	0.40	15.44	15.49	15.48	15.49	15.54
	0.45	15.22	15.20	15.20	15.20	14.95
HBA	0.00	28.73	28.29	28.39	28.73	28.56
	0.05	26.01	26.77	26.69	26.81	26.70
	0.10	24.96	24.94	24.88	24.88	24.64
	0.15	23.60	23.10	23.10	23.06	22.85
	0.20	21.28	21.42	21.46	21.42	21.37
	0.25	19.98	20.00	20.03	20.02	20.17
	0.30	18.82	18.87	18.88	18.89	19.17
	0.35	18.22	18.06	18.04	18.07	18.34
	0.40	17.34	17.58	17.56	17.57	17.64
	0.45	17.57	17.46	17.48	17.46	17.05
NA	0.00	25.39	25.17	25.22	25.39	25.31
	0.05	22.72	23.07	23.04	23.10	23.05
	0.10	21.18	21.16	21.13	21.14	20.99
	0.15	19.64	19.50	19.49	19.48	19.36
	0.20	18.11	18.10	18.11	18.09	18.07
	0.25	16.99	16.96	16.97	16.97	17.05
	0.30	15.99	16.07	16.07	16.08	16.24
	0.35	15.46	15.41	15.41	15.42	15.57
	0.40	14.92	14.98	14.97	14.98	15.01
	0.45	14.81	14.78	14.78	14.77	14.54

[a] The experiments were carried out with a 37-cm (30 cm effective length)×75-μm I.D. fused-silica capillary. The electrolyte was 10 m*M* phosphate buffer containing different concentrations of methanol (f_c). The applied voltage was 20 kV. The temperature was 25°C and the wavelength 254 nm. The key to the identity of the analytes is found in Section 3.

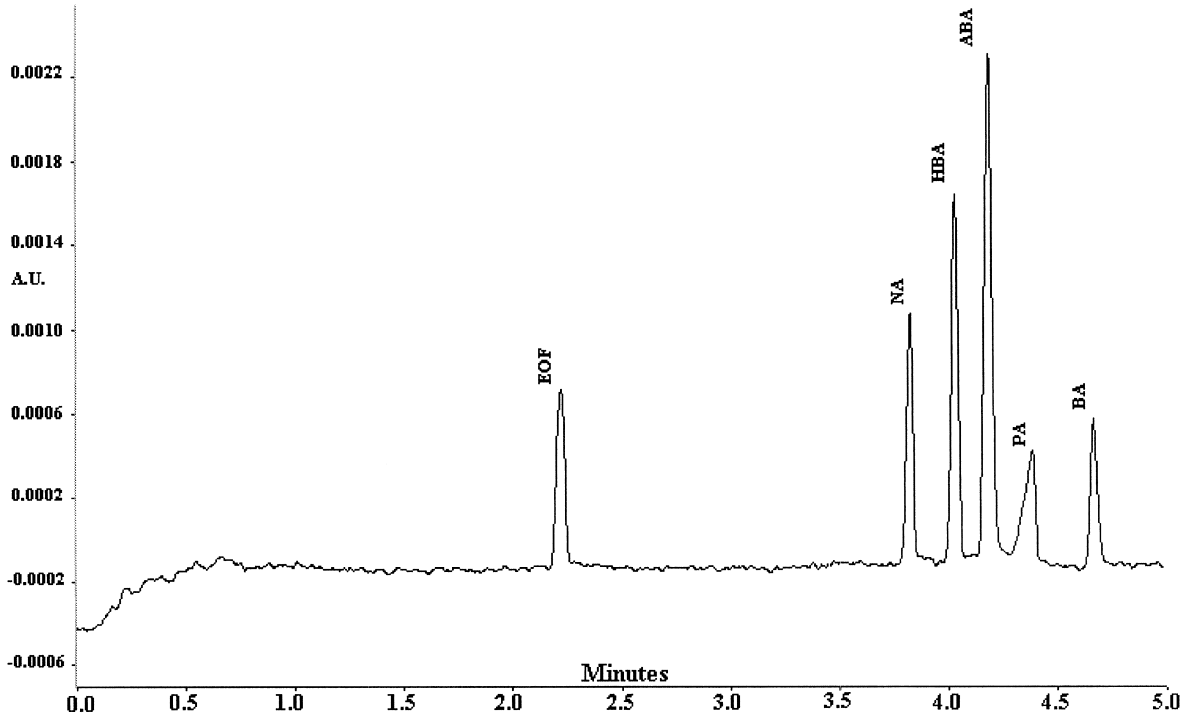

Fig. 1. A sample electropherogram of the anlaytes studied. Fused silica capillary 75-μm I.D.×37 cm (30 cm), running buffer 10 m*M* phosphate buffer containing 20% (v/v) methanol (f_c=0.20), applied voltage 20 kV, temperature 25°C, wavelength 254 nm. The EOF is measured from the peak for the electroosmotic flow marker (mesityl oxide) and the key to the analytes is found in Section 3.

results in changes in some fundamental factors, e.g., dissociation constants of analytes, silanol groups and buffering agents, viscosity of the running buffer, electroosmotic flow, etc. The simplest way to represent these parameters on the mobility of the analytes is to measure the mobility values of a limited number of concentrations of organic modifier and then to use this data to predict the mobility at other possible compositions of the organic modifier. In order to evaluate the applicability of this assumption, the experimental mobility values of five determined analytes at 0, 15, 30 and 45% (v/v) methanol in

Table 2
The average percentage deviation (APD) values calculated from the four equations[a]

Analytes	APD for correlative Eqs.[b]				APD for predictve Eqs.[c]			
	(5)	(6)	(8)	(10)	(5)	(6)	(8)	(10)
BA	0.5012	0.4777	0.4561	0.5177	1.0320	0.9044	0.8733	0.7612
PA	0.6084	0.5717	0.5348	1.1004	1.1876	1.1337	1.1309	0.7016
ABA	0.5364	0.4569	0.4242	0.9846	1.1406	1.0728	1.0689	0.5393
HBA	1.0463	1.0383	0.9692	1.6197	2.1946	2.1057	2.0997	1.5985
NA	0.4816	0.4572	0.4439	0.9350	0.8836	0.8274	0.8334	0.4555
Mean	0.6348	0.6004	0.5656	1.0315	1.2878	1.2088	1.2012	0.8112

[a] The key to the identity of the analytes is found in Section 3 and the experimental conditions are as Table 1.
[b] The model constants are computed by using whole data points and the mobilities are back-calculated based on these model constants.
[c] The experimental mobility values at 0, 15, 30 and 45% (v/v) are employed to compute the model constants and then the mobility values at other data points are predicted based on the computed model constants.

Table 3
The mobility range of analytes and average percentage deviations (APDs) calculated from tabulated data using the four equations

No.[a]	Analytes	Mobility range[b]		Equations			
		$\mu_{f_c=0.75}$	μ_w	(5)	(6)	(8)	(10)
1	Benzoate	22.01	33.27	0.1651	0.3704	0.3541	0.2941
2	2-Hydroxybenzoate	23.25	36.29	0.4925	0.2670	0.2553	0.4489
3	3-Hydroxybenzoate	19.01	31.17	0.1805	0.0118	0.0112	0.3018
4	4-Hydroxybenzoate	18.33	31.08	0.1685	0.0271	0.0259	0.2988
5	2,3-Dihydroxybenzoate	20.62	32.62	0.7490	0.4980	0.4761	0.7483
6	2,4-Dihydroxybenzoate	19.41	32.53	0.9203	0.6966	0.6659	1.0044
7	3,4-Dihydroxybenzoate	17.16	29.68	0.0042	0.2399	0.2294	0.0020
8	3,5-Dihydroxybenzoate	15.87	28.97	1.1395	0.9384	0.8971	1.0931
9	2,4,6-Trihydroxybenzoate	19.76	34.00	0.7194	0.4980	0.4761	0.7495
10	3,4,5-Trihydroxybenzoate	15.29	27.69	0.2498	0.0540	0.0516	0.2124
11	2-Methylbenzoate	21.04	31.59	0.5474	0.3503	0.3349	0.4651
12	3-Methylbenzoate	20.09	31.53	0.7655	0.5740	0.5487	0.6444
13	4-Methylbenzoate	19.98	31.51	0.6577	0.4127	0.3945	0.4814
14	2,4-Dimethylbenzoate	20.14	29.21	1.5549	1.2285	1.1744	1.3990
15	2,5-Dimethylbenzoate	20.68	29.36	1.2553	0.9633	0.9209	1.1152
16	3,4-Dimethylbenzoate	20.08	29.38	0.9718	0.7076	0.6765	0.8317
17	3,5-Dimethylbenzoate	20.24	28.94	1.1686	0.9065	0.8666	1.0402
18	2-Nitrobenzoate	21.10	32.78	0.5317	0.2770	0.2648	0.3422
19	3-Nitrobenzoate	21.02	32.27	0.8362	0.5978	0.5714	0.6817
20	4-Nitrobenzoate	20.98	32.65	0.3501	0.1129	0.1079	0.2269
21	3,4-Dinitrobenzoate	20.17	31.03	0.8483	0.5552	0.5307	0.6199
22	3,5-Dinitrobenzoate	20.98	30.63	0.8637	0.5351	0.5116	0.6008
23	2,4,6-Trinitrobenzoate	20.32	28.42	0.8471	0.4555	0.4355	0.5747
24	2-Chlorobenzoate	20.92	32.34	0.4569	0.1977	0.1890	0.2455
25	3-Chlorobenzoate	21.03	32.63	0.0867	0.2177	0.2081	0.1320
26	4-Chlorobenzoate	20.49	32.25	0.3394	0.1036	0.0990	0.1915
27	Benzoate	9.34	33.27	0.5853	0.7887	0.7541	0.5923
28	2-Hydroxybenzoate	10.95	36.29	0.6402	0.2239	0.2141	0.5715
29	3-Hydroxybenzoate	6.84	31.17	2.9746	1.4238	1.3611	4.4681
30	4-Hydroxybenzoate	7.06	31.08	0.1586	1.0117	0.9672	2.8874
31	2,3-Dihydroxybenzoate	9.49	32.62	0.6177	0.2630	0.2514	0.7995
32	2,4-Dihydroxybenzoate	7.46	32.53	0.3056	0.6834	0.6534	2.2915
33	3,4-Dihydroxybenzoate	7.20	29.68	0.5902	1.2835	1.2271	1.8774
34	3,5-Dihydroxybenzoate	7.19	28.97	0.5271	0.3611	0.3452	2.1825
35	2,4,6-Trihydroxybenzoate	8.12	34.00	2.2435	0.5424	0.5185	2.6846
36	3,4,5-Trihydroxybenzoate	7.57	27.69	0.8582	1.3458	1.2867	0.7594
37	2-Methylbenzoate	9.37	31.59	1.0419	0.3104	0.2968	1.9246
38	3-Methylbenzoate	9.17	31.53	1.5023	0.7419	0.7092	1.3229
39	4-Methylbenzoate	9.31	31.51	1.0238	0.5573	0.5327	0.2932
30	2,4-Dimethylbenzoate	8.61	29.21	2.1209	1.2409	1.1862	1.7448
41	2,5-Dimethylbenzoate	9.09	29.36	1.7109	0.9920	0.9483	1.4018
42	3,4-Dimethylbenzoate	9.63	29.38	2.8604	1.9305	1.8454	2.2765
43	3,5-Dimethylbenzoate	9.12	28.94	1.8461	1.1636	1.1124	1.4268
44	2-Nitrobenzoate	9.50	32.78	0.2006	0.5315	0.5082	2.6601
45	3-Nitrobenzoate	10.09	32.27	1.3180	0.5522	0.5279	1.0618
46	4-Nitrobenzoate	9.78	32.65	0.8629	0.2096	0.2003	0.5467
47	3,4-Dinitrobenzoate	9.23	31.03	2.9774	1.9235	1.8387	2.3560
48	3,5-Dinitrobenzoate	9.80	30.63	1.9552	1.1678	1.1164	1.6032
49	2,4,6-Trinitrobenzoate	8.41	28.42	0.8540	0.0838	0.0802	2.5345
40	2-Chlorobenzoate	9.64	32.34	1.7301	0.9654	0.9229	1.9871
51	3-Chlorobenzoate	9.77	32.63	2.7819	1.8630	1.7808	2.1890
52	4-Chlorobenzoate	9.66	32.25	2.6593	1.8364	1.7554	2.0575
Mean				1.0334	0.6883	0.6580	1.1778
SD				0.8039	0.5126	0.4900	0.9298

[a] The binary mixture for numbers 1–26 is methanol + water [10] and for numbers 27–52 is 1-propanol + water [11]. The number of data points is 5.

[b] The electrophoretic mobility (10^{-9} m^2 V^{-1} s^{-1}) in pure aqueous buffer (μ_w) and at maximum concentration of the organic modifier ($\mu_{f_c=0.75}$).

binary mixtures were examined in order to compute the models' constants. Then, the mobility values at other methanol concentrations were predicted by the proposed models. The resulting APDs for Eqs. (5), (6), (8) and (10) are 1.3, 1.2, 1.2 and 0.8%. These low prediction errors illustrate that one can use the

models to predict the mobility of the analytes at other methanol concentrations. It should be added that reported experimental mobility uncertainty is generally about 2.4–3.8% [1] and the prediction errors found are, therefore, within an acceptable error range. However, Eq. (8) is the preferred equation, because (1) the model constants can be calculated by a simple least-squares analysis which can be provided by scientific calculators and commercial software and (2) one can extend the number of model constants to provide more accurate correlations/predictions by employing more curve-fitting parameters, i.e., $L_2 f_c f_w (f_c - f_w)^2$ and $L_3 f_c f_w (f_c - f_w)^3$, for a highly curve-linear ln μ–f_c relationship.

In order to extend the applicability of the proposed models to positively charged analytes which possess a wider mobility range, the electrophoretic mobility data for monomethyl ammonium (ranging from 32 to 53×10^{-9} m^2 V^{-1} s^{-1}) were fitted to the proposed equations. The mobility of monomethyl ammonium was determined in acetate buffer containing 0–100% (v/v) methanol, where imidazole is the background absorber [12]. The APD values for Eqs. (5), (6), (7) and (10) are 0.4612, 0.4355, 0.4178 and 0.4149, respectively. As another application for the proposed models, the electrophoretic mobility data generated for two β-blocker drugs, timolol and propranolol, in mixed water–methanol acetate buffers at 20, 30 and 40°C have been employed. The models produced similar results to the above discussed analytes and mean APD values were 0.8425, 0.8023, 0.7923 and 0.8067 for Eqs. (5), (6), (7) and (10), respectively. These low APD values for different acidic and basic analytes show that the proposed equations can be applied for other analytes over a complete composition range of the organic modifier ($f_c = 0$–1) in mixed aqueous–organic modifier buffers.

In conclusion, all derived equations showed accurate results and these models can be used for screening experimental data or predicting electrophoretic mobility at other solvent compositions by interpolation. Since the expected mobility–f_c relationships are linear or curve-linear, thus the models are applicable to any analyte in mixed solvent running buffers. As an example, for a linear relationship, Eq. (8) includes two first terms, i.e., f_c and f_w, and for curve-linear relationships the third and the fourth terms will be included in the process of least-squares analysis. The higher the curvature the more significant the model constants which are included in the calculation. A similar discussion is the case for Eqs. (5), (6) and (10). However, it is recommended that Eq. (8) produced more accurate results where a large number of experimental data is employed for computing the model constants, and Eq. (10) is the best model where a minimum number of experiments is considered for building the model. As it has been shown above, by just four experimental mobility determinations for each analyte, one can predict mobility at other possible solvent compositions with percentage error about 1%. These predicted mobilities can be used by the analyst to speed up the method development process.

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