

Migration Characteristics of Hydrophobic Organophosphorus Pesticides in Micellar Electrokinetic Chromatography with Buffer and Organic Modifiers

Wan Aini Wan Ibrahim, S. M. Monjurul Alam and A. B. Sulaiman

Chemistry Department, Faculty of Science, Universiti Teknologi Malaysia,
81310 Skudai, Johor.

Abstract : In this work, the effect of buffer and the addition of organic solvents on the electrophoretic mobility and electroosmotic flow (*EOF*) mobility were investigated. It was found that, electrophoretic mobility of pesticides has strong linear correlation with the buffer concentrations that attributed to the influence of *EOF*. *EOF* in phosphate and borate buffer was only significantly different when acetonitrile was used. In the same buffer medium, *EOF* was also significantly different in different modifier, but their differential influences on the linear decrease of *EOF* mobility with respect to buffer concentration were absent. On the other hand, increase of buffer concentration and addition of different modifier did not cause any significant changes in the retention. And finally, correlation between octanol-water partition coefficient ($\log P$) and log value of capacity factors was investigated.

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Introduction

The initial pioneering work of micellar electrokinetic chromatography (MEKC) was performed by Terabe *et. al.* [1, 2]. The separation mechanism in MEKC results from the combination of electrophoretic movement of free ions in the applied potential field, and the differential partitioning of neutral analytes between the charged migrating micelles and aqueous buffer phase. All neutral analytes elute between the retentions of *EOF* marker (t_{eo}) and micellar marker (t_m). t_{eo} is the time it takes the *EOF* to sweep an unretained solute past the detector, and t_m is the time it takes a solute that is irreversibly solubilized in the micelle to pass the detector. The buffer solution in MEKC contains a surfactant at a concentration in excess of its critical micelle concentration (cmc).

Although MEKC is a very versatile separation technique offering a simpler and faster approach to the analysis of a large number of neutral and ionic compounds [3-5], and separation of various pesticides have been also reported [6, 7], however, to our knowledge, very little research has dealt with the application of the MEKC technique to the analysis of organophosphorus pesticides (OPPs) [8, 9]. This is probably due to the experimental difficulties encountered, resulting from their very high hydrophobic characteristics and strong interactions with the micelles as evidenced from the relatively long separation times. In an earlier work we reported the separation of a batch of five OPPs by MEKC using SDS surfactant [10].

In this article, we examined the effect of the range of buffer concentration and the type of modifiers on the electrophoretic mobility of the pesticides. It is assumed that the mobilities are

important characteristics and are used to describe the migration of compounds, and their precise values enable further optimization of separation conditions. Trends and variations in electrophoretic mobility with respect to the above-mentioned factors were explored. As the retention properties provide fundamental information concerning the distribution of the analytes between the aqueous phase and the micellar phase, therefore, effect of buffer and modifiers on capacity factor were also investigated. Finally, the relationship between the log value of capacity factor ($\log k$) and their hydrophobicities ($\log P$ values) were also evaluated for these OPPs.

Experimental

1. Standards and chemicals

5 OPPs [Methidathion (Mt), Diazinon (Dz), Quinalphos (Qu), Profenofos (Pr), and Chlorpyrifos (Ch)] were obtained from Dr. Ehrenstorfers GmbH laboratory (Augsburg, Germany). The structures and their selected properties are shown elsewhere [11]. All other chemicals and solvents were of analytical-reagent grade or HPLC grade, and were used as received.

Stock standards (< 2500 ppm) of the individual pesticides were prepared by dissolving the standards in methanol. Running standards concentration were from 50 to 200 ppm, and were made by diluting the stock standards with methanol (*EOF* marker) containing 25 ppm Sudan III (micelle marker). 100 mM stock phosphate or borate buffers (pH 9.25) were prepared in distilled deionized (DD) water. In this study 5, 10, and 20 mM phosphate (ph) and borate (bor) were used as background electrolyte (BGE) by

making appropriate dilutions from stock solution. As the OPPs are neutral compounds, a charged surfactant is needed to carry these OPPs during the MEKC run. An anionic surfactant, SDS was added to the BGE to give a final solution of 10 mM SDS as this is well above its critical micelle concentration (cmc) value (8.1 mM in water at 25 °C) [12]. 5 % v/v acetonitrile (AcN) and methanol (MeOH) were also added to the BGE as modifier, because higher concentration of organic modifiers would increase the cmc value of SDS [13, 14]. A 5 % v/v mixture of methanol and acetonitrile (1:1 v/v) was also used as a third (mixed) modifier in the BGE. 5, 10, and 20 mM mixed buffer of phosphate and borate (1:1) was also used with the mixed modifier as the BGE. All running buffers were filtered through 0.45 μm nylon filter disc from Whatman (Clifton, New Jersey, USA).

2. Instrumentation

All experiments were conducted on a CE-L1 capillary electrophoresis system, from CE Resources, Singapore. CE-L1 is a modular system consisting of an auto injector with a 50-position sample carousel, a dual polarity high-voltage power supply, and a variable-wavelength UV-Vis detector (SPD – 10A VP, Shimadzu) with an on-capillary detection cell. The system is computer-controlled, with an integrated software package allowing for comprehensive hardware management and data analysis (*Chromatography Station*). Throughout the experiments, a positive voltage of 25 kV was employed. The fused silica capillary (50 μm I.D. \times 360 μm O.D) was supplied by Polymicro Technologies (Australia). A length of 82 cm was cut, and a UV detection window was created at 42 cm downstream of the capillary. Samples were introduced to the capillary for 10s at 2.8 kPa injection and detected at 200 nm. Detailed run methods are presented in earlier reports [10].

3. Calculation of electrophoretic mobility, EOF mobility and capacity factors

Electrophoretic mobility (μ) of analytes was calculated from the observed migration times with the following equations [15]:

$$\mu = \frac{L_t L_d}{V t_r} \quad (1)$$

where L_t and L_d are the total capillary length and length to the detector window in m, respectively. V is the applied voltage; t_r is the migration times for the analytes in seconds. The mobility of the electroosmotic flow marker can be calculated according to Eq. (1) by substituting the migration

time of the electroosmotic flow marker (t_{eo}) for the migration time of the analyte (t_r).

Capacity factors, k of each OPPs was determined at least in duplicate using the following equation [1]:

$$k = \frac{(t_r - t_{eo})}{t_{eo} \left(1 - \frac{t_r}{t_m}\right)} \quad (2)$$

where, t_r is the solute retention time, and t_{eo} and t_m is the EOF elution time and micelle elution time, respectively.

Results and Discussions

1. Effect of buffer and modifiers on electrophoretic mobility

The electrophoretic mobility of five OPPs was plotted against the concentration of phosphate and borate buffers with each modifier. Mobility of each pesticide decreased sharply with an increase of buffer concentration and showed strong linear relationship ($r^2 \sim 0.99$) in both buffer systems. The mobility of methidathion (least hydrophobic one) and chlorpyrifos (most hydrophobic one) in both buffer systems is presented in Figure 1 and 2 respectively. A representative electropherogram is presented in Figure 3. There are a few unidentified peaks in the electropherogram which could be from impurities that were associated with the standards before it was mixed.

Reciprocal dependence of solute electrophoretic mobility (μ) on the square root of buffer concentration has been demonstrated and the following expression was given [15]:

$$\mu \cong \frac{e}{3 \times 10^7 |Z| \eta \sqrt{C}} \quad (3)$$

where, e , Z , η , and C are defined as excess charge in solution per unit area, charge of the ion, viscosity of the solution and concentration, respectively. However, electrophoretic mobilities are also dependent on the properties of the silica capillary surfaces due to the contribution from the EOF. Therefore, the linear relationship of the mobilities with the increase in buffer concentration is due to the fact that the migration times of the compounds are highly correlated to the migration time of the EOF marker. In this context, effects of buffer and modifiers on EOF were evaluated. Figure 4 shows the effect of phosphate and borate buffer

concentrations on *EOF* mobility with three kinds of modifiers. As the buffer concentrations were increased, zeta (ζ) potential of the capillary wall decreased, therefore, sharp linear decreases in *EOF* with strong correlation ($r^2 = 0.99$) were observed. In MEKC, indications on the comparative effects of phosphate and borate buffer in any separation in relation with either solute mobility or *EOF* mobility is hard to find from the literature. In one study, selective features of borate buffer were described as poly anionic species of borate buffer has the ability to

form complexes with particular solutes [16]. However, from the comparison it is found here that with this particular concentration range, influences of phosphate and borate buffer on *EOF* and as well as on the electrophoretic mobility of pesticides is almost insignificant except in one occasion where *EOF* is significantly different in phosphate and borate buffer when acetonitrile is used as modifier ($t = 4.8 \gg t_{0.05(2)4} = 2.78$).

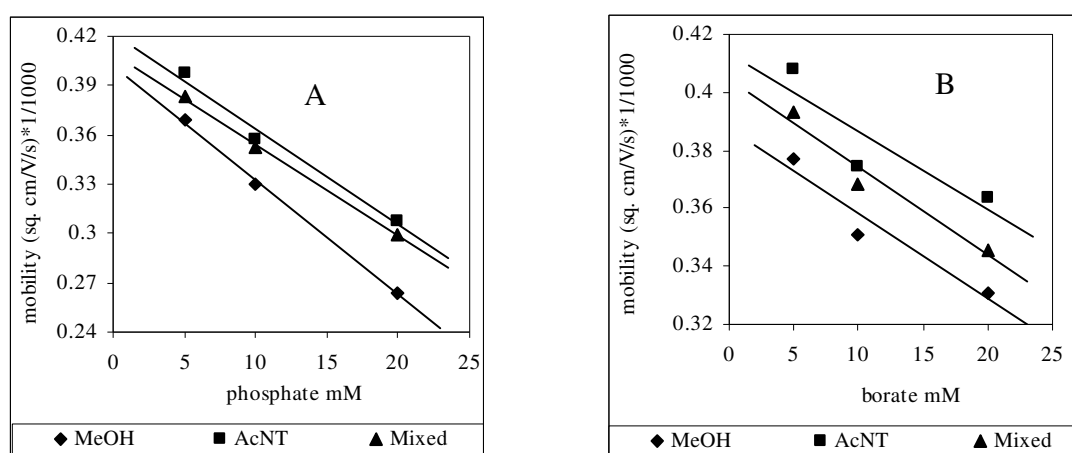


Figure 1 : Electrophoretic mobility of methidathion in (A) phosphate and (B) borate buffer with 5 % v/v of three different modifiers.

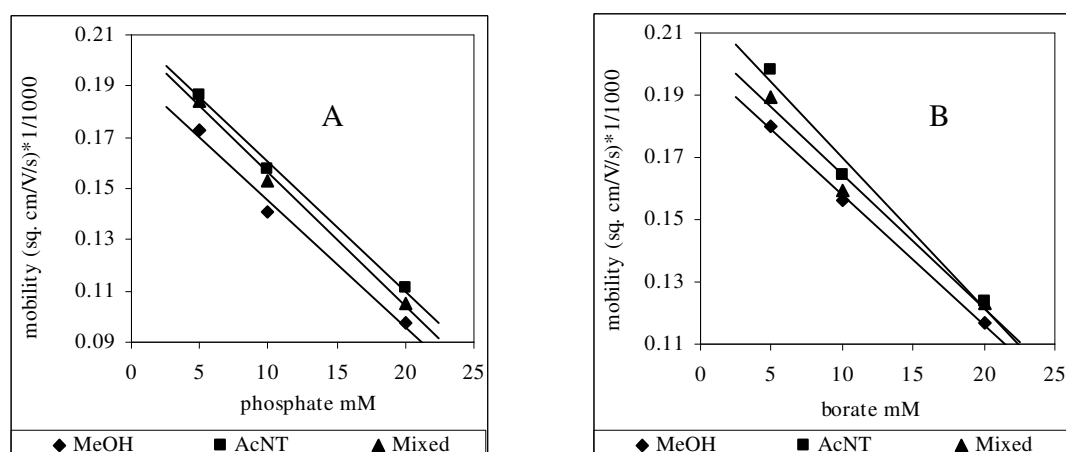


Figure 2 : Electrophoretic mobility of chlorpyrifos in (A) phosphate and (B) borate buffer with 5 % v/v of three different modifiers.

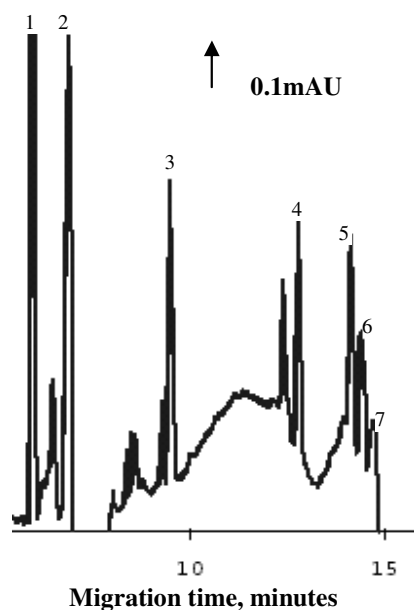


Figure 3 : A representative electropherogram of the separation of 5 OPPs in MEKC. Applied potential 25 kV, 10 mM SDS-2.5 mM Na_2HPO_4 -2.5 mM $\text{Na}_2\text{B}_4\text{O}_7$, pH 9.25, 5 % v/v 1:1 mixture of methanol and acetonitrile; sample injection 10s by low pressure (2.8 kPa); Peaks: 1 = methanol; 2 = methidathion (200 ppm); 3 = diazinon (200 ppm); 4 = quinalphos (50 ppm); 5 = profenofos (50 ppm); 6 = chlorpyrifos (50 ppm); 7 = Sudan III (25 ppm). Unidentified peaks were probably impurities from standard or solution.

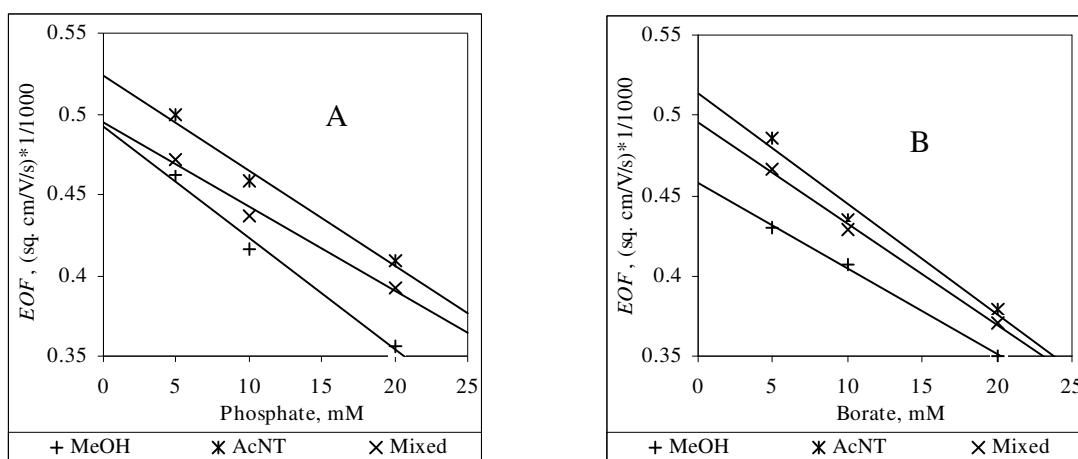


Figure 4 : EOF mobility in (A) phosphate and (B) borate buffer with 5 % v/v of three different modifiers.

Separation of analytes in MEKC is often accomplished by adding organic modifiers to the background electrolyte in order to vary the extent of chemical interactions so that the electrophoretic mobility of each individual solute can be effectively differentiated from each others [13,14]. Therefore, effective separation of solutes may be achieved in the presence of specific level of organic modifiers.

However, organic modifier affects electrophoretic mobility of the analytes [17] and EOF [18, 19].

Electrophoretic mobility is very sensitive to small changes in the viscosity of the solution. According to the Debye-Huckel-Henry theory, the viscosity of the solution shows a reciprocal relationship with electrophoretic mobility (μ) of an ion by the following equation [17]

$$\mu = \frac{q}{6\pi\eta r} \quad (4)$$

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. By ignoring any changes in solvation of the ions, above equation was rewritten [17] as a function of η

$$\mu = \frac{A}{\eta} \quad \text{or} \quad \frac{1}{\mu} = \frac{\eta}{A} \quad (5)$$

where A is a constant value equal to $(q/6\pi r)$. Therefore, knowledge of the viscosity of water-organic modifier mixtures over the entire concentration range is important with relation to the solute mobility. On the other hand, EOF usually is suppressed in the presence of organic modifiers and decreases with increasing fraction of organic solvent and trend can be explained by changes in the dielectric properties of the electric double layer and of the charge generation on the inner surface of bare-silica capillary [18]. Here, the effect of three modifiers on both the electrophoretic mobility and EOF mobility is found always significant ($t = 2.9 > t_{0.05(2) 4} = 2.78$). From Figure 1 & 2, it can be seen that mobility is always highest in acetonitrile and lowest in methanol, while the mixed modifier produces the moderate values, and features are clearer in electroosmotic mobility (Figure 4). This may show their relative contribution for the increase of viscosity in running buffer. However, to test whether these three modifiers have significantly different effect on the EOF with relation to the increase of buffer concentration, slopes of three regression lines were compared, and F value was calculated [20]. Since both calculated F values for phosphate and borate buffer (0.008 and 0.004 respectively) are far less than the value from the statistical table ($F_{0.05(1), 2, 3} = 9.55$) [20], therefore, even there are some variation in regression coefficient (slope) among regression lines, effect of three different modifiers on EOF mobility with relation to the increase of buffer concentration is virtually insignificant. It is assumed that for the small volume fraction (5 % v/v), the identity of these modifiers (methanol, acetonitrile, or their 1:1 mixture) seems to be less important in modifying the EOF [21].

2. Influence of buffer and modifiers on capacity factors

Capacity factor k provides fundamental information concerning the distribution of the

analytes between the aqueous phase and the micellar phase and is linearly related to the phase ratio V_{MC}/V_{AQ} , according to the following equation [2]:

$$k = K (V_{MC} / V_{AQ}) \quad (6)$$

where, K is the thermodynamic distribution coefficient for a solute between the micellar phase and the aqueous phase, and V_{MC} and V_{AQ} are the volume of the micellar and the aqueous phase, respectively. In MEKC, phase ratio may change during the course of the separation, unlike liquid chromatography, since it depends on micelle concentration [22], which can be affected by the higher buffer concentration, addition of organic solvent or by the increase of temperature for Joule heating. Therefore, changes in capacity factor with the increase of buffer concentration and organic modifier through modifying the distribution coefficient and phase ratio is worth to investigate.

k of five pesticides in phosphate and borate buffer with each modifier are presented in Table 1, and in mixed buffer with mixed modifier in Table 2. Variations in k values for methidathion, diazinon and quinalphos are little with respect to the increase of either buffer concentrations or to the kind of modifiers. In both buffer systems, profenofos and chlorpyrifos have significantly very high values and variations are quite drastic. For instance, k value of chlorpyrifos in 5 mM phosphate buffer with mixed modifier is only 27, but in 10 mM phosphate buffer with methanol modifier k value is as high as 321. Capacity factors in mixed buffer also followed similar trends for each pesticide (Table 2). High values of capacity factors are not uncommon especially when separation of similar pesticides was tried in SDS surfactant system [8]. The wide variation of k values of profenofos and chlorpyrifos would produce a poor resolution between them, and between chlorpyrifos and micelle marker respectively.

Statistical paired t -test among each pair of all sets of buffer and modifiers revealed that variation of capacity factors are quite insignificant with an increase of both buffer concentrations from 5 to 20 mM and with same concentration of each buffer, influence of modifiers are also insignificant (t value < 2 , where $t_{0.05(2) 4}$ is 2.78). The increase in the migration times of EOF marker and micellar marker by the increase of buffer concentrations might have occurred concomitantly; as a result, changes in elution windows were insignificant. Therefore, neither any consistent increase nor decrease were found in k values with the increase of buffer concentrations with any kind of modifiers added to it.

Table 1 : Capacity factor for 5 OPPs at three different concentrations of phosphate and borate buffer with 5 % v/v of three different modifiers

Buffer	modifier	Mt		Dz		Qu		Pr		Ch	
		ph	bor	ph	bor	ph	bor	ph	bor	ph	bor
5 mM	MeOH	0.46	0.26	1.7	1.6	7.6	6.3	25	25	36	41
	AcN	0.47	0.36	1.6	1.6	7.9	7.1	33	47	50	59
	mixed	0.42	0.35	1.5	1.7	7.2	6.9	26	31	27	70
10 mM	MeOH	0.46	0.28	1.7	1.9	11	8.3	75	34	321	78
	AcN	0.49	0.28	1.5	1.7	7.8	8.0	30	57	64	74
	mixed	0.41	0.28	1.6	1.8	9.4	7.1	33	25	51	45
20 mM	N	0.54	0.09	1.7	1.8	11	8.4	38	34	62	37
	Acnt	0.50	0.06	1.6	1.7	9.7	7.5	64	37	88	41
	mixed	0.47	0.11	1.6	1.7	10	7.5	56	27	71	31

Table 2 : Capacity factor for 5 OPPs in mixed buffer (phosphate and borate 1:1) with 5 % v/v mixed modifiers

Buffer	modifier	Mt	Dz	Qu	Pr	Ch
5 mM	mixed	0.33	1.5	5.7	13	85
10 mM	mixed	0.41	1.8	11	113	143
20 mM	mixed	0.29	1.7	11	86	175

3. Correlation between log value of capacity factor and log *P*

In recent years the octanol/water partition coefficient has become a key parameter in studies of the environmental fate of organic chemicals and it has been found to be related to water solubility [23], soil/sediment adsorption coefficients [24], and bioconcentration factors for aquatic life [25]. Because of its increasing use in the estimation of the other properties, log *P* is considered a required property in studies of new or problematic chemicals. It has been reported that in MEKC investigation, log value of capacity factors of several aromatic compounds and corticosteroids were found to be linearly correlated with their log *P* values [26]. Therefore, MEKC-based method of nonpolar hydrophobic organophosphorus

pesticides can logically be extended to correlate their retention behavior with their hydrophobicities.

The values of log *P* for five pesticides (Table 3) were obtained from references [27-29]. Referred log *P* values in cited articles are either obtained by traditional shake-flask method or by indirect calculations from correlation studies. Therefore, values of log *P* of any particular pesticides varied significantly. And, it is assumed that the mean of all referred log *P* values of each pesticide might be closer to its authentic value, for this reason their mean log *P* values were used in correlation studies with capacity factors. Similarly, as the capacity factor of each pesticide did not change significantly over the range of both buffer concentrations with each modifier, therefore, their mean values were also used in this correlation studies.

Table 3 : log *P* values of pesticides [27-29]

Name	Range of log <i>P</i>
Chlorpyrifos	4.7 – 5.3
Diazinon	3.02-3.86
Methidathion	1.58-2.42
Profenofos	4.68-4.82
Quinalphos	3.04-4.44

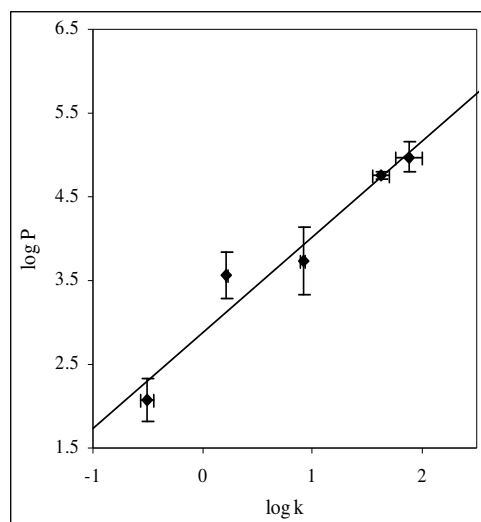


Figure 5 : Relationship between mean $\log k$ and mean $\log P$; data point (from left to right): 1st-methidathion, 2nd- diazinon, 3rd - quinalphos, 4th - profenofos, and 5th – chlorpyrifos.

While plotting was done between mean \log value of capacity factors and the mean of all available referred $\log P$ value, a strong linear relationship ($y = 1.138x + 2.87$) was obtained (Figure 5), and the correlation coefficient ($r = 0.975$) is significantly higher than if the regression was done against any single value of $\log P$ from one reference (data not shown here).

In Figure 5, x axis error bar shows the standard error of the mean of the capacity factor and the y-axis error bar shows the standard error of the mean of all referred $\log P$ values. It is to be noted that variation in terms of standard error are quite high in the referred $\log P$ values of all pesticide except for profenofos (4th data point), while only the standard error of the capacity factor of chlorpyrifos is significantly higher than the other four.

Conclusion

Results of the present study shows that the strong linear correlation between electrophoretic mobility of the five organophosphorus pesticides and the buffer concentrations was the result of the direct influence of *EOF* mobility. A consistent linear decrease of *EOF* mobility with an increase of both phosphate and borate buffer concentration can easily be explained through the change of electrical double layer potential of the capillary wall and with respect to the increase of viscosity of the electrophoretic medium.

Addition of a small amount (5 % v/v) of methanol, acetonitrile, or their 1:1v/v mixture to the buffer, clearly demonstrate their specific influences on the migration, and therefore, in phosphate and borate buffer, *EOF* was found to be significantly different for each of those three modifiers. However, only in the presence of acetonitrile, *EOF* mobility

was found to be significantly different in the two buffer systems. And, the covariance statistics showed that their differential influences on the linear decrease of *EOF* mobility with respect to buffer concentration were insignificant. With any kind of organic modifier, used in this study, the k values of each pesticide were found to be independent in phosphate, borate and mixed buffer in the range of 5 – 20 mM, indicating that the variation in elution window was negligibly small due to the proportional decrease of *EOF* mobility. Relationship between $\log k$ and $\log P$ was found to be linear. Therefore, when the direct measurement of $\log P$ of a particular pesticide in a laboratory is found to be difficult and impractical, then the use of this simple linear model can justifiably be used if the test compounds possess similar chemical properties.

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