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ELECTROPHORETIC MIGRATION OF ORGANOPHOSPHORUS PESTICIDES AS A FUNCTION OF SEPARATION POTENTIAL IN MICELLAR ELECTROKINETIC CHROMATOGRAPHY: A BASIS OF PREDICTING THE RETENTION FACTORS

W. AINI W. IBRAHIM[1], S. M. MONJURUL ALAM[2] & A. B. SULAIMAN[3]

Abstract. Retention factors (k') in Micellar Electrokinetic Chromatography (MEKC) are very often calculated against the mobility of fully retained hydrophobic micelle markers, which are not abundant for universal use. An alternative approach was proposed to predict the k' values on the basis of a functional relationship between the solutes retention times and separation potentials using organophosphorus pesticides (OPPs) as reference hydrophobic compounds. The use of the proposed simple linear model was evaluated for another set of hydrophilic OPPs and its practicality was defined.

Keywords: Micellar Electrokinetic Chromatography (MEKC), hydrophobic OPPs, hydrophilic OPPs

Abstrak. Dalam kromatografi elektrokinetik misel, faktor penahanan (k') biasanya dikira menggunakan mobiliti misel penanda hidrofobik yang tertahan sepenuhnya dan penanda ini tidak banyak didapati untuk kegunaan universal. Satu pendekatan alternatif dicadangkan di sini untuk meramalkan nilai k' berdasarkan fungsi hubungan antara masa penahanan zat terlarut dan keupayaan pemisahan menggunakan pestisid organofosforus sebagai sebatian rujukan hidrofobik. Penggunaan model linear yang ringkas ini dinilai untuk satu set pestisid organofosforus hidrofilik lain.

Kata kunci: Kromatografi elektrokinetik misel (MEKC), OPPs hidrofobik, OPPs hidrofilik

1.0 INTRODUCTION

Micellar Electrokinetic Chromatography (MEKC) [1, 2] is a relatively new mode of capillary electrophoresis (CE) that has expanded considerably in the last two decades. The separation mechanism of neutral analytes in MEKC is based on their differential distribution between the mobile aqueous phase and the pseudo-stationary micellar phase. The two phases move with different electrophoretic mobilities and the analytes migrate at the velocities between the electroosmotic flow (EOF) velocity and the velocity of the micelle. The capacity (retention) factor, k' provides fundamental information concerning the distribution of the analytes between the aqueous and micellar phases [2] and is expressed as:

[1,2&3]Chemistry Department, Faculty of Science, Universiti Teknologi Malaysia, 81310 Skudai, Johor.

$$k' = \frac{t_R - t_0}{t_0\left(1 - \frac{t_R}{t_m}\right)} \quad (1)$$

In this equation, t_R is the retention time of an individual neutral analyte, t_0 is the time for an unretained neutral compound to reach the detector (the *EOF*), and t_m is the travel time to the detector of a compound (micelle marker) that is fully retained in the micelle and dragged by the *EOF* against its own migration, when the medium is not highly acidic and an uncoated fused silica capillary is used. Therefore, k' is dependent on t_0 and t_m, which is called the elution window, within which neutral analyte must migrate to the detector. The narrower the elution window, the less the peak capacity, that is the maximum number of peaks separated within the time window. The existence of this elution window is a major limitation because resolution in MEKC is only optimal for k' values in the range of 1 to 5 [1].

For hydrophobic compounds, determination of capacity factors in MEKC is limited [3], as its resolution with the micelle marker is often poor. The characteristic of a micelle marker is that it should be highly hydrophobic and its solubility in the desired sample matrices (sometimes aqueous rich) is also an important criterion for its selection. Sudan III is a common micelle marker in MEKC [2, 4], but its sensitivity in absorbance detection mode is diminished with the variation of the detection wavelengths. If the detection of a method is based on UV-VIS absorbance and the selected wavelength for the particular mixture of analytes is higher for instance, in which Sudan III cannot be detected, then finding an alternative marker is inevitable.

In MEKC, higher separation potential is always favored for the short analysis time and higher separation efficiency if the generated current permits [5-7]. In the earliest work, Terabe *et al.* reported that nonlinear relationship existed between the analyte velocities and the applied potentials [2]. Nonlinear relationships between applied potentials and analytes migration were also reported thereafter [5, 8]. The increase of temperature by high-applied potential actually reduces the viscosity of the medium and to this effect, current and solute velocities are increased concomitantly. Therefore, a linear relationship between the current generated and the solute velocity is attained [2] but not between the applied potentials and the solute velocities. However, if the Joule heating is adequately controlled through an efficient heat dissipation system, only then linear relationship between the solute velocity and applied potential can be attainable [6].

The recurrent features of nonlinear relationships that existed between the applied potentials and the migration times has prompted us to verify the nature of curvatures and the possibility of correlating the equation entities with the retention properties of analytes to be tested. In this work, five selected organophosphorus pesticides (OPPs, "batch 1"), having different hydrophobicities, were separated using MEKC, and their

log k' values were calculated. Thereupon, by consecutive runs in a range of applied potentials, the retention time of each pesticide was plotted against the potentials and coefficients of these lines were further regressed with their respective k' values and was used as a linear model to predict the capacity factors of a new set of three OPPs ("batch 2"). Batch 2 OPPs was separated in the same MEKC method where Sudan III cannot be used as a micelle marker due to the higher detection wavelength that was unsuited for its detection.

2.0 EXPERIMENTAL

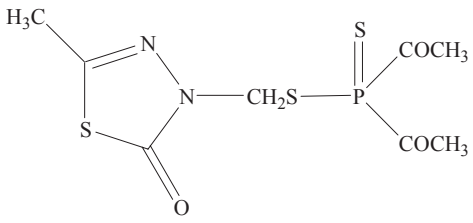
All eight OPPs were obtained from Dr. Ehrenstorfer GmbH laboratory (Augsburg, Germany). The structures of batch 1 (methidathion, diazinon, quinalphos, chlorpyrifos and profenofos) are shown in Figure 1(a). Batch 2 is a set of three water soluble OPPs (phosphamidon, dicrotophos and monocrotophos) and their structures are presented in Figure 1(b). All other chemicals (e.g. SDS surfactant, buffers) and solvents are of analytical-reagent grade or HPLC grade, purchased from various manufacturers and were used as received. The apparatus and experimental conditions were as described previously [9, 10]. The uncoated fused silica capillary (50 mm ID) was obtained from SGE, Victoria, Australia. A total length of 82 cm and effective length of 42 cm was used. Considering the very poor water solubility of chlorpyrifos and profenofos, working standards were prepared in pure methanol (*EOF* marker) with 25 ppm Sudan III. To maintain a comparable sample matrix, methanol was also used as the solvent for the batch 2 OPPs even though they are quite water soluble. Buffers were filtered with a nylon filter disc (Whatman, Clifton, New Jersey, USA). Three injections were made for each CE conditions.

3.0 RESULTS AND DISCUSSION

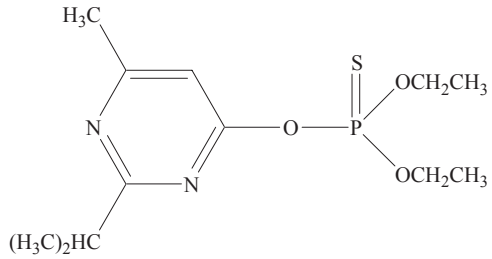
3.1 Separation of Batch 1 OPPs Using MEKC Method

MEKC separation of batch 1 OPPs were obtained at little above the critical micelle concentration of SDS with mixed buffer and mixed organic solvents. A representative electropherogram is shown in Figure 2(a). Retention order in MEKC is generally described by the solute hydrophobicity [2]. Here, the retention order is found to be consistent with the respective water solubilities [9] except for profenofos which is eluted after chlorpyrifos. Theoretically, relationship between hydrophobicity (in the scale of octanol-water partition coefficient, K_{ow}) and the water solubility of the compounds would be linear [11]. However, the reported log K_{ow} values for the OPPs of batch 1 are inconsistent with their level of water solubilities, presumably those reported log K_{ow} values are not authentic by any means. The capacity factors are calculated on the basis of *EOF* velocity and the velocity of Sudan III and the log k' values are given in Table 1.

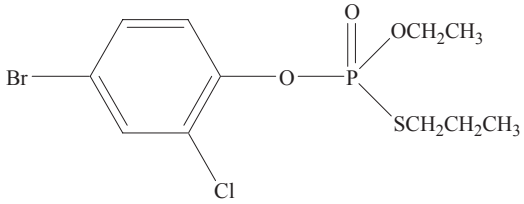
(a)



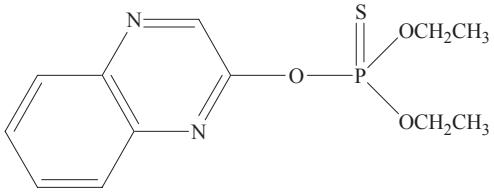
Methidathion



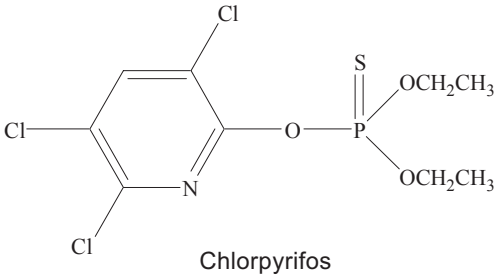
Diazinon



Profenofos

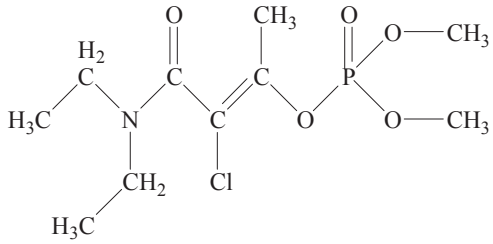


Quinalphos

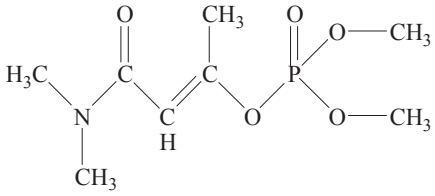


Chlorpyrifos

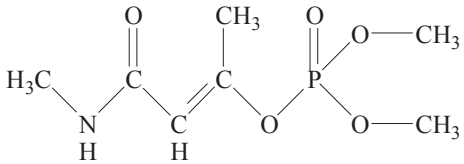
(b)



Phosphamidon



Dicrotophos



Monocrotophos

Figure 1 Structure of the 2 batches of OPPs studied. (a) Batch 1 of hydrophobic OPPs; (b) Batch 2 of hydrophilic OPPs

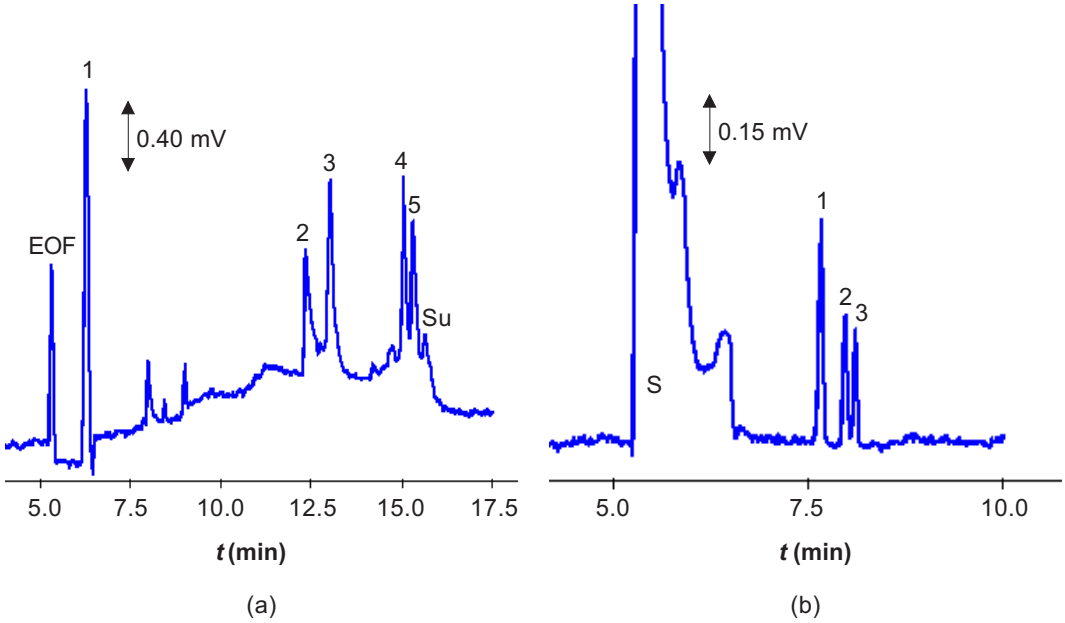


Figure 2 Separation of batch 1 (a) and batch 2 (b) OPPs in MEKC. Running buffer: SDS 10 mM, 10 mM 1:1 phosphate & borate buffer and 5 % 1:1 methanol & acetonitrile (pH 9.25); applied potential 25 kV, and sample injection 10s at 2.8 kPa. In (a) detection at 202 nm; peaks: *EOF* (methanol); 1, methidathion (200 ppm); 2, diazinon (200 ppm); 3, quinalphos (50 ppm); 4, chlorpyrifos (50 ppm); 5, profenofos (50 ppm). In (b) detection at 230 nm; peaks: S, system peak (responsive to basic buffer where *EOF* marker peak is also merged); 1, phosphamidon (200 ppm); 2, dicrotophos (200 ppm); 3, monocrotophos (200 ppm).

Consecutive runs of the same standard mixture of batch 1 were carried out in 20, 15 and 10 kV of applied potentials. As attributed in other earlier works [2, 5, 8], the increase of the migration time of each pesticide followed a concave curvature path with a decrease in applied potentials (Figure 3(a)). This curvilinear relationship is reproducibly fitted by a simple power regression equation ($y = ax^{-1}$) with distinctive coefficients for each OPP. The coefficient, *a,* was found to have a linear relationship with the log value of their k' (see Figure 3(b)). The following equation of the straight line (Figure 3(b)) is obtained with $r^2 = 0.982$

$$\log k' = 0.0131a - 2.549 \quad (2)$$

All regression equations and the goodness of fit (r^2) are presented in Table 2.

3.2 Separation of Batch 2 OPPs Using the Same MEKC Method

An electropherogram of the separation of batch 2 OPPs is shown in Figure 2(b). All parameters remained the same as in earlier separation except a 230 nm detection wavelength was used as its detection at 202 nm gave very poor sensitivity. The baseline

Table 1 Calculated log k' of batch 1 of hydrophobic OPPs

OPPs	Calculated log k'
Methidathion	–0.53
Diazinon	0.76
Quinalphos	0.90
Chlorpyrifos	1.50
Profenofos	1.66

of this separation is better compared to batch 1, but the detection sensitivities are considerably low. Note that the response scale of Figure 2B(b) is different from that of Figure 2(a). Possibly, non-aqueous sample matrix (methanol) is not suited for these aqueous soluble OPPs and if the sample is prepared in a similar matrix to the separation buffer, then this would enhance the sensitivity. Effects of separation potentials of 25 to 10 kV on migration times are shown in Figure 4. With an increase of applied potentials, migration time of pesticides also followed a concave curvature path and again reproducibly fitted by the same power regression equation ($y = ax^{-1}$). All regression equations and r^2 values are presented in Table 3. Their log k' values were calculated

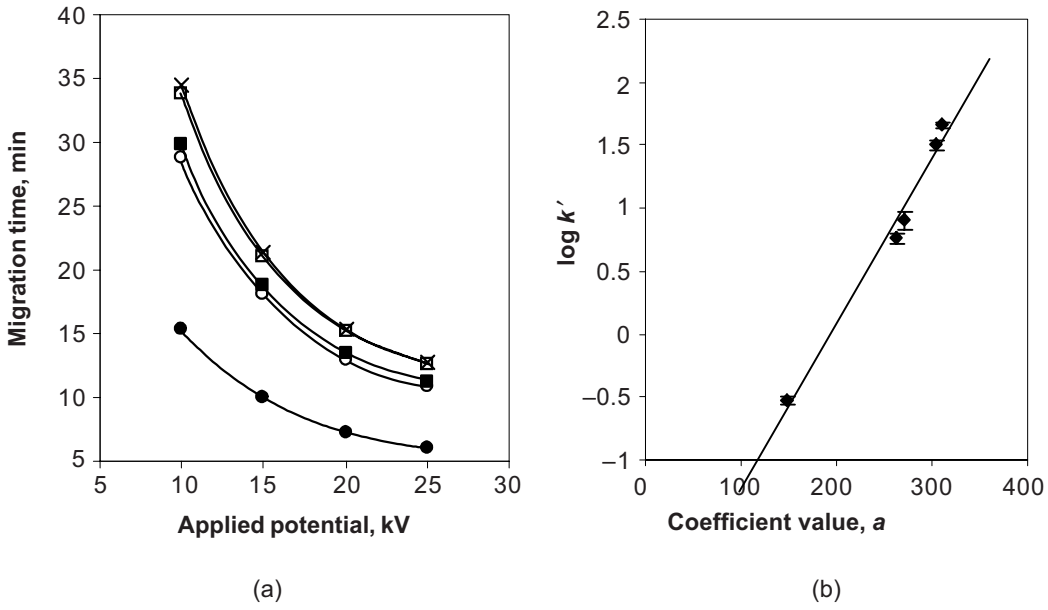


Figure 3 (a) Effects of applied potentials on migration time of the batch 1 OPPs. Conditions as in Figure 2(a). Analyte identity: ● methidathion, ○ diazinon, ■ quinalphos, □ chlorpyrifos, ✕ profenofos. (b) Relationship between the coefficient values of the regression lines against the log value of the capacity factors for the set of hydrophobic OPPs; error bars represent standard error

Table 2 Equations and r^2 for the relation between potentials and migration time of batch 1 OPPs

Pesticides	Methidathion	Diazinon	Quinalphos	Chlorpyrifos	Profenofos
Equation	$y = 148.83x^{-1}$	$y = 262.56x^{-1}$	$y = 271.33x^{-1}$	$y = 304.88x^{-1}$	$y = 310.50x^{-1}$
r2	0.997	0.997	0.996	0.996	0.996

from the linear equation (2) of Figure 3(b) and presented in Table 3 (column 4).

On the basis of those calculated log k' values, it was predicted that these three OPPs would appear between methidathion and diazinon, if all eight OPPs (both batches) were tried to be separated in a single run with a suitable detection tools (e.g. diode array). This assumption is easy to attribute if both electropherograms (2 (a) and (b)) of separate runs are overlapped. However, the elution of three OPPs after methidathion was inconsistent to their higher water soluble nature. Moreover, their elution pattern was also reciprocal to what is described on the basis of hydrophobic interaction with micelles [2]. Phosphamidon will be more hydrophobic, as two ethyl groups were attached to the terminal N atom and one chlorine atom was also present. Similarly, dicrotophos would be slightly more hydrophobic than monocrotophos, as it has an extra methyl group. In the highly basic buffer, these three polar OPPs may

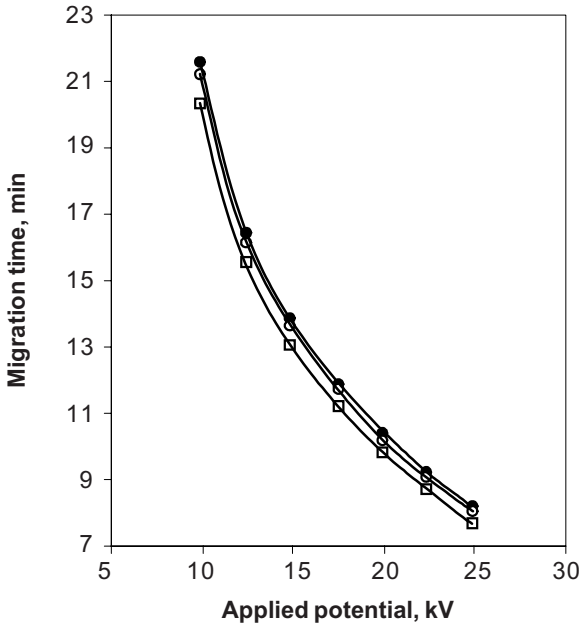


Figure 4 Effects of applied potential on migration time of the batch 2 OPPs. Conditions: see Figure 2(b). □ phosphamidon, ○ dicrotophos, ● monocrotohos

Table 3 Equations and r^2 of the relation between potentials and migration time of batch 2 OPPs

OPPs	Equation	r^2	log k'*
Phosphamidon	$y = 192.14x^{-1}$	0.999	-0.032
Dicrotophos	$y = 200.03x^{-1}$	0.999	0.071
Monocrotophos	$y = 203.24x^{-1}$	0.999	0.113

* calculated values, from Figure 3(b)

temporarily gain anionic characters. The migration of anionic analytes had little affinity for the hydrophobic interior of SDS micelles. The theory of charged analytes becomes more complex owing to the fact that the unbound analytes have an electrophoretic mobility of their own and migrate toward the electrode of opposite charge. A rigorous treatment of this situation accounted for the effects of both separation mechanisms independently and required knowledge of how the analytes migrated in the absence of micelles. Another set of experiment is necessary to define the observed peak order with plausible causes, but this is not within the scope of the study.

Nowadays, hydrophobicity, expressed as log K_{ow}, is considered as one of the most important physico-chemical properties in relation to bioaccumulation and toxicity of numerous organic compounds [3, 11, 12]. Considering a similar partition mechanism in micelle-water and octanol-water system for the vast range of organic compounds, linear relationship between the log k' of neutral analytes (in MEKC) and their log K_{ow} values is usually approximated, and the model is used reliably as an alternative way to measure the log K_{ow} of congeneric hydrophobic compounds [3, 12]. The alternative approach proposed in this is study to estimate the capacity factors using the coefficient of regression lines based on the relationship between retention time and applied potentials. This would result in a rapid estimation of log K_{ow} values using both linear models. It is to be noted that the capacity factors of separating analytes in MEKC is sometimes reported as a function of micelle concentration, therefore, the approach of predicting log k' values, where high surfactant is used, may find limitation.

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