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SEPARATION OF ORGANOPHOSPHORUS PESTICIDES USING MICELLAR ELECTROKINETIC CHROMATOGRAPHY

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Abstract. Separation of six organophosphorus pesticides (mevinphos, methidathion, diazinon, profenofos, quinalphos and chlorpyrifos) has been investigated using micellar electrokinetic chromatography (MEKC) with on-column UV-Vis detection. Separations involved using anionic sodium dodecyl sulphate (SDS) surfactant and phosphate or borate buffer as carrier electrolytes. Separations have been found incomplete regardless of changing all sort of possible factors such as buffer types, buffer and surfactant concentrations, addition of organic modifier, methanol and pH of the running buffer. Irrespective of the combinations of levels of factors, mevinphos alone appeared always as a distinguishable strong peak followed by diazinon and methidathion. Even in the absence of any surfactant in buffer matrices, mevinphos and diazinon were detected but methidathion, profenofos, quinalphos and chlorpyrifos were undetected. The use of 6 mM β–cyclodextrin (β–CD), 20 mM borate buffer with 40 mM SDS and buffer pH of 9.5 resulted in the best separation of mevinphos, diazinon and methidathion. The results found in this study might reflect the solubility and hydrophobicity properties of the pesticides.

Key words: Organophosphorus Pesticides (OPPs), Micellar electrokinetic chromatography, Sodium dodecyl sulphate, β–cyclodextrin, UV-Vis

Abstrak. Pemisahan enam pestisid organofosforus (mevinfos, metidation, diazinon, prefenofos, kuinalfos dan klorpirifos) telah dikaji menggunakan teknik kromatografi elektrokinetik misel (MEKC) dengan pengesan ultralembayung-nampak. Proses pemisahan dilakukan dengan menggunakan surfaktan anionik natrium dodesil sulfat (SDS) dan larutan penimbal borat atau fosfat sebagai elektrolit pembawa. Pemisahan yang dilakukan didapati tidak lengkap walaupun telah dilakukan perubahan terhadap pelbagai faktor seperti jenis larutan penimbal, kepekatan surfaktan dan larutan penimbal, penambahan pengubahsuai metanol dan juga pengubahsuaian pH larutan penimbal yang digunakan. Tanpa penggunaan dan pengubahsuaian faktor di atas, pestisid mevinfos sahaja selalu muncul sebagai puncak yang tajam diikuti oleh diazinon dan metidation. Walaupun tanpa kehadiran surfaktan SDS dalam matrik larutan penimbal, mevinfos dan diazinon dapat dikesan manakala pestisid OPPs yang lainnya tidak dapat dikesan. Penggunaan β–siklodekstrin (β–CD) 6 mM, penimbal borat 20 mM dengan SDS 40 mM dan pH penimbal 9.5 memberikan keputusan terbaik bagi pemisahan pestisid mevinfos, diazinon dan metidation. Keputusan dalam kajian ini mencerminkan kaitan antara keterlarutan dan sifat hidrofobik pestisid.

Kata kunci: Pestisid organofosforus, kromatografi elektrokinetik misel, natrium dodesil sulfat, β–siklodekstrin, Ultralembayung-Nampak

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1.0 INTRODUCTION

At present, organophosphorus pesticides (OPPs) probably represent the largest single class of pesticides worth more than several billions of US dollar for yearly application in the world [1] and its use is in the direction of an increasing trend. So the mass-balance and distributions of applied pesticides into various environmental compartments need to be understood carefully. Therefore, importance of extraction, isolation and separation of persisted portion of applied pesticides and their transformation products are the pressing needs of the time. In order to ensure that these substances are not present at hazardous levels in environment samples, analytical methods have been developed and implemented for many years. These primarily involve gas or liquid chromatography with various means of detection [2-5]. The determination of low concentrations of OPPs pesticides in various environmental matrices requires, in addition to highly selective and sensitive detection techniques, the application of efficient extraction and thorough cleanup procedures to enable a quantitative as well as qualitative determination and separation to be implemented.

Capillary Electrophoresis (CE) has been successfully used to achieve efficient separation of a vast range of analytes, ranging from small inorganic cations [6,7] to the larger biological molecules [8] and even in pharmaceutical industries [9]. The application range of capillary electrophoresis has been extended by MEKC approach that allows the separation of uncharged molecules with the satisfactory level of resolution. By using only buffers, nonionic substances like most of the pesticides may not be separated as long as any kind of surfactants are not used. The micellar solution is prepared by dissolving a surfactant into a buffer solution. The micelle migrates electrophoretically under the influence of the electrical field. Once a solute is introduced into a micellar system, it will partition between the hydrophobic micellar phase and the aqueous phase with a particular partition coefficient, depending on the polarity of the analyte [10]. MEKC has been successfully applied in numerous analyses. It has been used in the analysis of compounds of environmental interest, pharmaceuticals [11-13], proteins-peptides [14] and so on. MEKC offers a promising alternative for OPPs analysis in that it is inherently fast and offers the potential for high separation efficiency. However, the lack of charge and hydrophobic nature of most OPPs results in difficulties for electrophoretic separation and detection. So the key aspect for an efficient separation of the pesticides is the amount of surfactant added to the buffer. So far, only a few articles can be found where MEKC has been used as the separation technique for determining some herbicides [15] and other pesticides [16-21].

In this study we explore the use of MEKC for the separation of six neutral OPPs by using on-column UV detection. This is because the use of MEKC for the separation of OPPs is rarely reported in the literature [16, 17]. In this study the effect of buffer matrices with sodium dodecyl sulphate (SDS) as surfactant were studied in the separation of six widely used OPPs in Malaysia. The OPPs used in this study are mevinphos, methidathion, chlorpyrifos, quinalphos, profenofos and diazinon.

2.0 EXPERIMENTAL

2.1 Apparatus

Capillary Electrophoresis System (CE – L1) was procured from CE Resources Pte Ltd. Singapore. A UV-Vis detector (SPD – 10A VP Shimadzu) was used for detection of OPPs. Software for data acquisition was *Chromatography Station* for Windows.

2.2 Chemicals

All pesticides were analytical standards purchased from Dr. Ehrenstorfer GmbH laboratory (Augsburg, Germany). Structures of pesticides used in the studies are presented in Figure 1 and their selected properties in Table 1. Stock solutions of the pesticides were prepared by dissolving appropriate amounts of the corresponding standards in *n*-hexane. Working standard solutions were prepared by serial dilution of the corresponding stock solutions also in *n*-hexane. A mixed solution containing 50 ppm of each pesticide in *n*-hexane (unless specified) was prepared from each stock standard and was used for separation run.

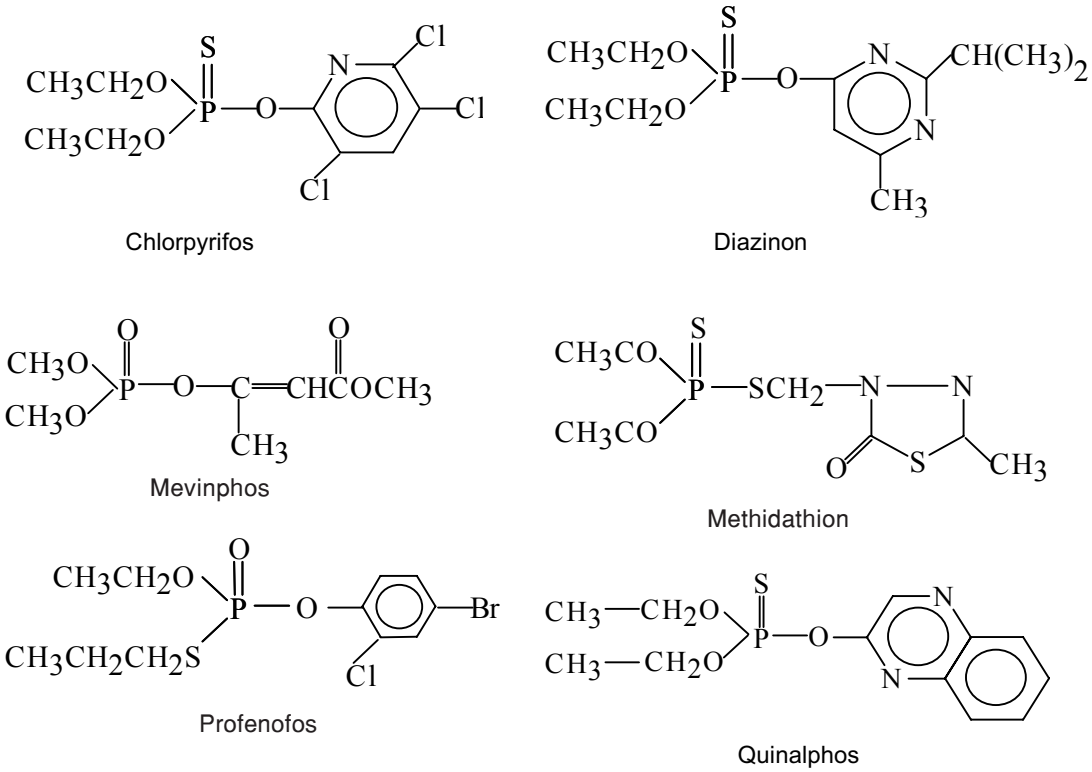

Figure 1 Structure of the pesticides used in this work

Table 1 Selected properties of investigated pesticides [22].

Name (State at RT)	Mol. Formula (m.p./b.p./°C)	Mol. Wt.	S_w ($g\ L^{-1}$)	Log K_{ow}
Mevinphos (liquid)	$C_7H_{13}O_6P$ (bp 106-107.5)	224.16	Miscible	–
Methidathion (solid)	$C_6H_{11}N_2O_4PS_3$ (mp 39-40)	302.31	0.25(20°C)	4.73
Diazinon (liquid)	$C_{12}H_{21}N_2O_3PS$ (bp 83-84)	304.35	5.35×10^{-2} (20°C)	3.30 (25°C)
Quinalphos (Liquid)	$C_{12}H_{15}N_2O_3PS$ (bp 142)	298.3	2.4×10^{-2} (20°C)	NF
Profenofos (liquid)	$C_{11}H_{15}BrClO_3PS$ (bp 110)	373.64	2.0×10^{-2} (20°C)	1.9
Chlorpyrifos (solid)	$C_9H_{11}Cl_3NO_3PS$ (bp decom. 160)	350.59	0.73×10^{-3} (20°C)	4.7 – 5.3

S_w = Solubility in water 20°C; NF = Not Found in available references.
K_{ow} = Octanol/Water Partition Coefficient;

SDS was purchased from Fisher Scientific, Loughborough, U.K.; β–CD from Fluka Chemika, Switzerland; Sodium hydroxide pellets was from Merck; *n*-hexane from Fisher Scientific (Fair Lawn, New Jersey, USA); methanol from BDH (Poole, England) and hydrochloric acid (37.5%) from Sigma. Di-sodium hydrogen-12-hydrate buffer was obtained from Riedel-de Haen (Ridel-de-Haen AG. D-3016 Seelze 1, Germany)) and di-sodium tetraborate decahydrate from Merck (Darmstadt, Germany). β–CD were obtained from Sigma and distilled deionised water was prepared with a Milli-Q system from Millipore, USA.

2.3 Preparation of background electrolyte

SDS, β–CD and methanol where necessary, were added to the buffer and the volume was adjusted with deionised water. Buffer, SDS, β–CD and methanol concentrations are reported based on the final volume of the mixture. pH values refer to the pH of the pure buffer solutions. Concentrations of SDS and buffers were selected in such a way that excessively high current were avoided.

2.4 Operating conditions

The MEKC separation was performed in an uncoated fused-silica capillary (45 cm × 75 mm i.d., total length 80 cm) with a positive power supply of 25 kV. Electropherograms

were recorded at 215 nm. Sample injections in all separation runs were performed hydrodynamically for 10 seconds. All experiments were conducted in an air-conditioned laboratory at an ambient temperature of 25°C.

2.5 Run Methods

At the beginning of each day, the capillary was rinsed for 10 min with 1.0 M NaOH solution followed by distilled deionised (DD) water for 10 min and then conditioned with running buffer for 10 min. Between each run, capillary was also conditioned by running buffer for 3 to 5 min and before shutting down at the end of each working day, capillary was rinsed with DD water for 10 minutes followed by passing air. Identification of peaks was performed by injection of a solution of a single standard and comparison of the migration times.

3.0 RESULTS AND DISCUSSION

3.1 Effect of Buffer Systems

An increase of buffer concentrations, irrespective of their types resulted in a decrease of the electroosmotic flow while an increase in pH makes it faster. The buffer concentration, studied here in the range from 10 to 25 mM and pH were maintained always in the basic range (pH 9.5), as electroosmotic flow is determined by the surface charge on the capillary wall and, therefore, the mobility is low in the acidic range and increases strongly at higher pH values of the buffer. Similar to the buffer concentration, an increase of surfactant concentrations also leads to higher migration times of the analytes. The number of micelles, the micelle shape, size and conformation may change significantly as the surfactant concentration in the aqueous phase gets higher. Therefore, the concentration of SDS should not exceed 100 mM unless otherwise stated.

By using only phosphate buffer with various levels of SDS surfactants (10 mM – 100 mM), only three out of six pesticides can be detected (Figure 2). Under these set of buffer mixture, quinalphos, profenofos and chlorpyrifos might have been strongly adsorbed by the micelles and coeluted with them. Higher concentrations of SDS did not improve the separation and resulted in higher migration time. Addition of organic modifier methanol also did not improve the separation of the OPPs. However, when borate buffer was used with SDS surfactant, only mevinphos and diazinon were separated. In this case, diazinon was detected before mevinphos and the sensitivity was significantly improved by using higher surfactant concentration with the addition of 20 % methanol (Figure 3). Profenofos, quinalphos, chlorpyrifos and methidathion were also undetected when borate buffer was used.

These results would resemble the water solubilities of mevinphos, methidathion and diazinon. To confirm these results, samples were run with buffer medium without any surfactant added. In theory, this is no longer MEKC, as long as any kind of surfactant

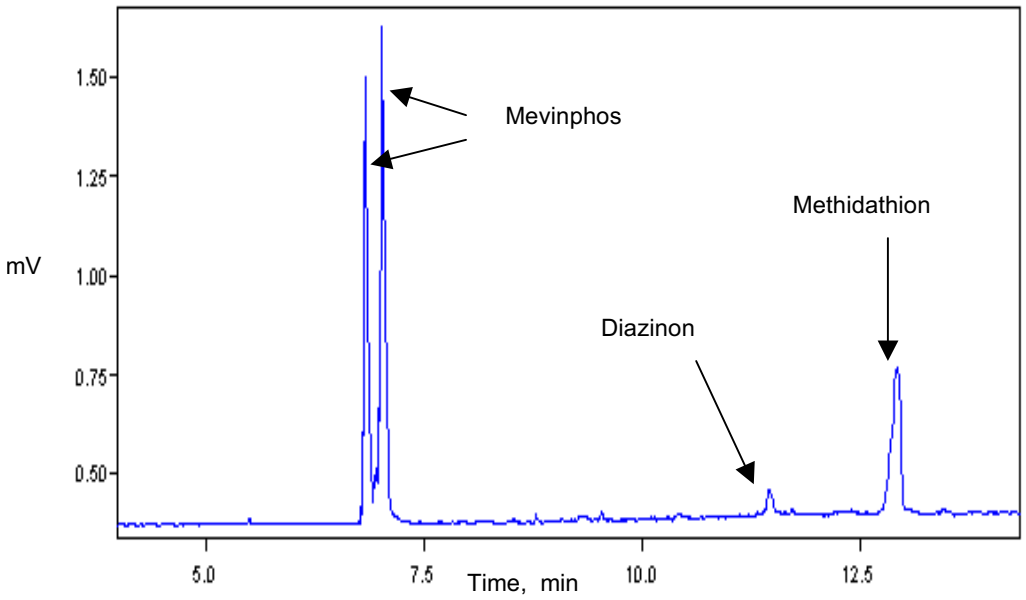

Figure 2 Electropherogram of 50 ppm each of mevinphos, methidathion and diazinon; conditions: 25 mM Phosphate, 10 mM SDS, pH 9.5, UV detection at 215 nm, 25 kV separation voltage. Hydrodyamic injection for 10 s

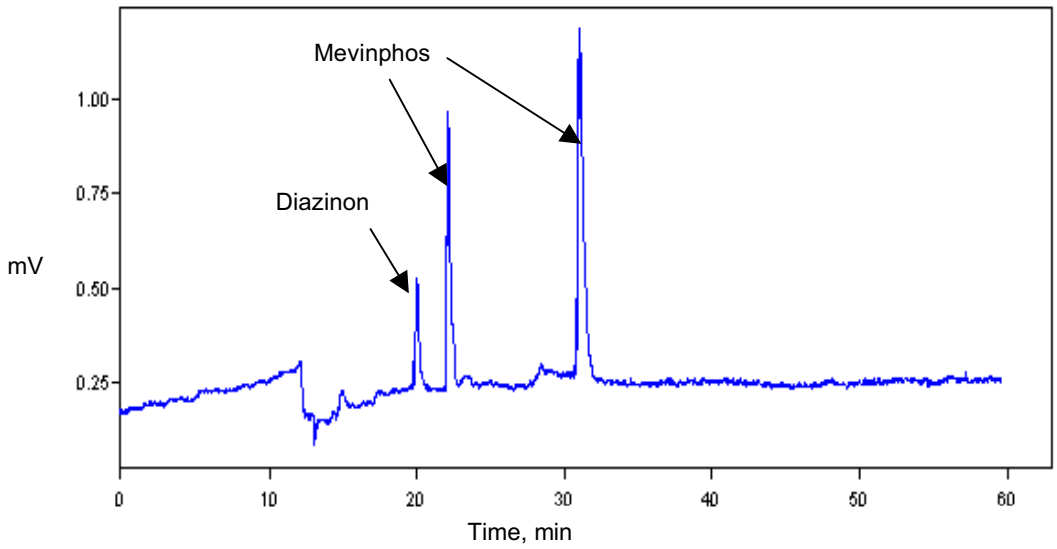

Figure 3 Electropherogram of Mevinphos (18 ppm) and Diazinon (90 ppm); Conditions: 20 mM Borate, 80 mM SDS, pH 9.5, 20 % MeOH, UV detection at 215 nm, 25 kV separation voltage. Hydrodynamic injection for 10 s

is not added to the system. In this case, mevinphos and diazinon both gave a pair of peaks (electropherogram are not shown). Interestingly methidathion having a higher solubility compared to diazinon (Table 1) did not give a peak.

3.2 EFFECT OF β–CD IN SEPARATION

Cyclodextrins are oligosaccharides composed of several glucopyronase units. They are very popular especially as chiral selectors but their unique capability to form inclusion complexes is also very useful in an achiral environment. The beneficial use of CD for the enantiomeric separation in MEKC had been reported [18,19]. Owing to the hydrophobic character of the internal cavity of CDs, non-ionic aromatic and alkyl groups form inclusion type complexes and thus selective separation can be performed effectively.

Good separation was obtained in 25 minutes for the three (mevinphos, diazinon and methidathion) pesticides by addition of β–CD to the borate buffer (Figure 4). It was noticed that β–CD also enhanced the peak-height of both mevinphos and diazinon possibly by increasing their solubilities in aqueous medium. Due to the peaks identification on the basis of single standard migration time, it was found that mevinphos and diazinon both generated a pair of peaks. The addition of β–CD to the buffer displaces the distribution pattern of the pesticides among the micellar, CD cavity and the water phase. Profenofos, quinalphos and chlorpyrifos were still not detected under this condition. The reason why mevinphos and sometimes diazinon produced pair of peaks could be the high pH level and significant temperature rises by Joule heating might cause a substantial hydrolysis of several pesticides [22] within

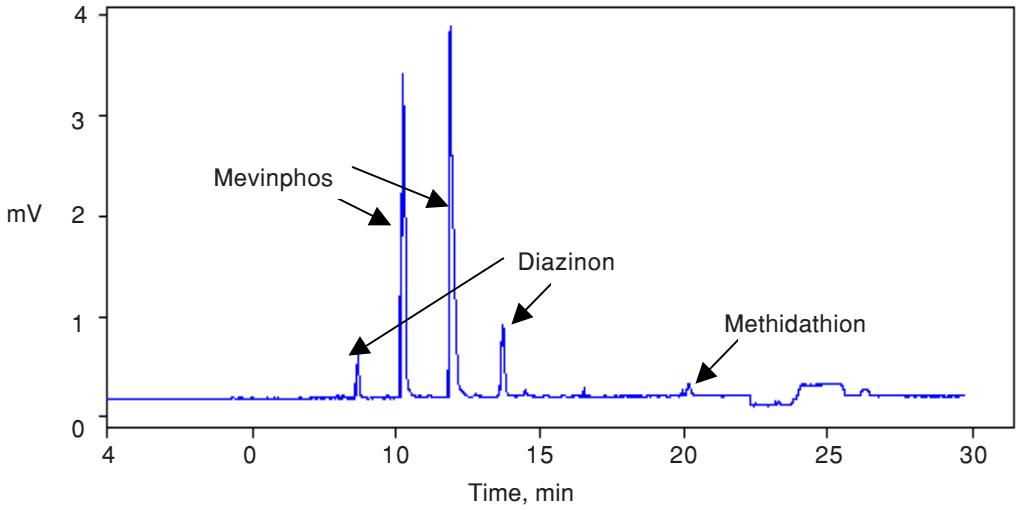

Figure 4 Electropherogram of 50 ppm each of mevinphos, methidathion and diazinon; Conditions: 20 mM Borate, 40 mM SDS, 6 mM β-CD, pH 9.5, UV detection at 215 nm, 25 kV separation voltage, hydrodynamic injection:10 s

the range of migration time. In the pH range of 3.1-10.4, diazinon can hydrolyse to give several mobalitia. To date, no study has been known to be conducted on diazinon and mevinphos using MEKC.

4.0 CONCLUSIONS

This exploratory studies found that separation of OPPs was incomplete regardless of changing all sort of possible factors such as buffer types, buffer and surfactant concentrations, addition of organic modifier, methanol and pH of the running buffer. Highly hydrophobic compounds such as quinalphos, profenofos and chlorpyrifos absorb so strongly to the micelles as even addition of any organic modifier such as methanol and by forming inclusion complexes with added β-CD were not sufficient to separate them. Effect of solvent of sample mixture on separation also needs to be evaluated. Consideration of the effect of minute changes in temperature on separation selectivity would be worth trying, but not considered here due to the limited capability of the instrument. Generally, temperature is positively correlated with critical micellar concentration of the surfactant, moreover, it influences the partition of the analytes between the phases. Further optimisation is needed, as there may be interaction between the factors considered in the studies.

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