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# Optimization of separation and migration behavior of chloropyridines in micellar electrokinetic chromatography

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**Abstract**

The separation and migration behavior of pyridine and eight chloropyridines, including three monochloropyridines, four dichloropyridines, and 2,3,5-trichloropyridine were investigated by micellar electrokinetic chromatography using either sodium dodecyl sulfate (SDS) as an anionic surfactant or SDS–Brij 35 mixed micelles. Various parameters such as buffer pH, SDS concentration, Brij 35 concentration and methanol content that affect the separation were optimized. Complete separation of these chloropyridines was optimally achieved with a phosphate buffer containing SDS (30 m*M*) and methanol (10%, v/v) at pH 7.0. The resolution and selectivity of analytes could be considerably affected by the addition of methanol and/or Brij 35 to the background electrolyte. The migration order of these chloropyridines depends primarily on their hydrophobicity. However, electrostatic interactions may also play a significant role in the determination of the migration order of the positional isomers of chloropyridines. 

*Keywords:* Buffer composition; Micelles, mixed; Chloropyridines; Pyridines

## 1. Introduction

Pyridine and chloropyridines are widely used as intermediates or insecticides in some chemical and agricultural industries [1–5]. For example, chlorpyrifos used as an insecticide is derived from chloropyridines [1]. In the production process of chloropyrifos, pyridine is first chlorinated to pentachloropyridine. During the chlorination reaction of pyridine, other lower chlorinated pyridine isomers in addition to pentachloropyridine are also present as by-products. Moreover, chloropyridines are of environmental concern. Thus, the development of an efficient method for separating these pyridine compounds is desirable.

Although various chromatographic methods and hyphenated techniques, including GC [6–8], GC–MS [9] and HPLC [7,10] have been applied to separate some mixtures of chlorinated pyridine, complete separation of chloropyridine isomers is still not achieved by aforementioned methods.

Capillary electrophoresis (CE) has become a popular and powerful technique to separate diverse analytical samples [11–14]. This technique provides high resolution, great efficiency, rapid analysis and small consumption of solvent in comparison with HPLC. In recent years, the application of this

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technique to the analysis of environmental pollutants has gained considerable attention. However, to our knowledge, a systematic study on the separation of chloropyridines is still lacking. In this study, micellar electrokinetic chromatography (MEKC) using sodium dodecyl sulfate (SDS) as an anionic surfactant was employed to obtain baseline separation of a mixture of pyridine and eight chloropyridines, including three monochloropyridines, four dichloropyridines and 2,3,5-trichloropyridine. The optimization of various parameters that may affect the separation is taken into consideration. Moreover, factors that influence the migration order of these chloropyridines are examined.

## 2. Experimental

### 2.1. Chemicals

Pyridine, three monochloropyridines and Brij 35 were obtained from Tokyo Kasei Kogyo (TCI, Tokyo, Japan); four dichloropyrines were purchased from Sigma-Aldrich (St. Louis, MO, USA); 2,3,5-trichloropyridine and sodium dodecyl sulfate were supplied from Merck (Darmstadt, Germany). All other chemicals were of analytical grade obtained from various suppliers. Deionized water was prepared with a Milli-Q system (Millipore, Bedford, MA, USA).

A stock solution containing 1000 μg/ml of each analyte dissolved in methanol was prepared. Standard sample solutions were prepared by mixing the stock solution with methanolic solutions to obtain a desired concentration.

### 2.2. Apparatus

Capillary electrophoretic experiments were carried out on a Beckman Coulter P/ACE System Model 5500 (Fullerton, CA, USA) equipped with a photodiode array detector for absorbance measurements. Uncoated fused-silica capillaries purchased from Polymicro Technologies (Phoenix, AZ, USA) were used. The dimensions of the capillary were 67 cm× 50 μm I.D. The effective length of the capillary is 60 cm from the injection end of the capillary. Analytes were detected by on-column measurements of UV absorption at 214 nm or 200 nm as specified. The CE systems were interfaced with a microcomputer and a laser printer using MDQ software for data acquisition. All CE experiments were performed at 25°C, unless otherwise specified. For pH measurements, a pH meter (Suntex model SP-701, Taipei, Taiwan) with an accuracy of 0.01 pH unit was used.

### 2.3. Procedures

When using a new capillary column, the capillary was washed for 60 min with NaOH solution (1.0 *M*) at 60°C, followed by washing for 10 min with water at the same temperature and for 10 min with water at 25°C. Before each injection, the capillary was flushed with the buffer solution for 5 min. The capillary was washed with NaOH solution (0.1 *M*) and water to keep the electroosmotic flow (EOF) to normal when needed.

The buffer solutions were prepared by mixing stock solutions of $NaH_2PO_4$ (100 m*M*), SDS and methanol at varied ratios and then adjusted to the desired pH value with KOH solution (0.1 *M*).

All solutions were degassed by sonication and passed through a membrane filter (0.22 μm) before use. The electroosmotic mobility ($\mu_{eo}$) was determined with methanol as a neutral marker and the electrophoretic mobility of the micelles was determined with Sudan III as a micelle marker. Samples were injected in a hydrodynamic mode during 2 s. Analytes were identified by the spiking technique.

### 2.4. Calculations

The electrophoretic mobility of analytes were calculated from the observed migration times with the equation:

$$\mu_{ep} = \mu - \mu_{eo} = \frac{L_d L_t}{V} \cdot \left( \frac{1}{t_m} - \frac{1}{t_{eo}} \right) \quad (1)$$

in which $\mu_{ep}$ is the electrophoretic mobility of the analyte tested, $\mu$ is the apparent mobility, $\mu_{eo}$ is the electroosmotic mobility, $t_m$ is the migration time of a solute peak measured directly from the electropherogram, $t_{eo}$ is the migration time for an uncharged solute, $L_t$ is the total length of capillary, $L_d$ is the length of capillary between injection and detection, and $V$ is the applied voltage.

## 3. Results and discussion

### 3.1. Effect of buffer pH

The effects of buffer pH on the migration behavior and separation of pyridine and eight chloropyridines in the absence and presence of SDS micelles was investigated. Fig. 1 shows the variations of the electrophoretic mobility of pyridine and chloropyridines as a function of buffer pH in the range 1.5–8.0 without the addition of SDS surfactant to the buffer solution. The p$K_a$ values of pyridine and chloropyridines can be determined from the variation of the effective electrophoretic mobility of each analyte as a function of buffer pH, according to the following equation [15–17]:

$$\mu_{\text{eff}} = \alpha_{\text{BH}^+}\mu_{\text{BH}^+} = \frac{[\text{H}_3\text{O}^+]}{[\text{H}_3\text{O}^+] + K_a} \cdot \mu_{\text{BH}^+} \qquad (2)$$

where $\mu_{\text{eff}}$ is the effective electrophoretic mobility of a protonated basic analyte ($BH^+$), $\mu_{\text{BH}^+}$ and $\alpha_{\text{BH}^+}$ are the limiting electrophoretic mobility and mole fraction of the protonated form of a basic analyte, and $K_a$ is the acid dissociation constant of $BH^+$. The p$K_a$ values determined for pyridine, 4-chloropyridine, and 3-chloropyridine are 4.95, 3.65, and 2.70 respectively, and the p$K_a$ values of 2-chloropyridine and dichloropyridines are estimated to be less than 1.0. Therefore, all analytes are practically in the neutral form at pH 7.0 and complete separation of these analytes is experimentally impossible without the addition of surfactant molecules or electrolyte modifiers to the background electrolyte at pH 7.0.

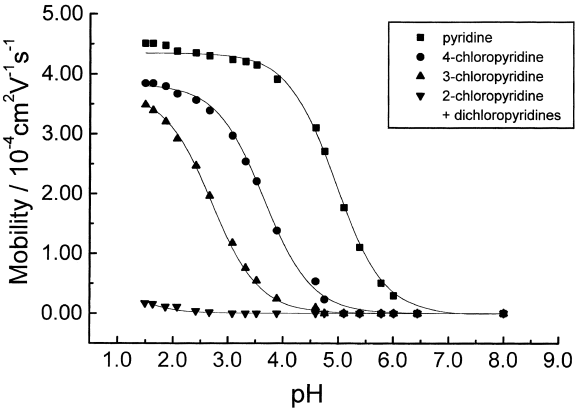


Fig. 1. Variations of the electrophoretic mobility of pyridine and chloropyridines as a function of buffer pH without the addition of SDS micelles to the background electrolyte. Experimental results are represented by data points; predicted mobility curves are shown by solid lines. Background electrolyte: 10 m*M* phosphate buffer containing 10% (v/v) methanol at varied buffer pH. Capillary: 67 cm×50 μm, I.D. Other operating conditions: 30 kV, 25°C. Sample concentration, 50 μg/ml. Detection wavelength, 214 nm.

The effect of buffer pH in MEKC was then examined in the range 5.6–10.0 for these analytes in a phosphate buffer containing 30 m*M* SDS. In this pH range, the EOF increases almost linearly with increasing pH at pH<7, but in a lesser extent at pH>7, whereas the electrophoretic mobility of analytes, except pyridine and 4-chloropyridine, does not show significant change with increasing buffer pH, especially at pH>7. The electrophoretic mobility of 4-chloropyridine decreases slightly from $-6.6\cdot10^{-5}$ to $-4.0\cdot10^{-5}$ $\text{cm}^2\,\text{V}^{-1}\,\text{s}^{-1}$, whereas that of pyridine decreases appreciably with increasing buffer pH in the range 5.6–7.0. This is probably resulted from the micelle-shifted p$K_a$ values of pyridine and 4-chloropyridine. It is found that pyridine and 2-chloropyridine are barely separated at pH 5.6, but they are well resolved at pH in range 6.0–7.0. Hence, effective separation of these analytes is optimally achieved in MEKC at pH 7.0.

### 3.2. Effect of SDS concentration

Fig. 2 shows the variations of the electrophoretic mobility of pyridine and chloropyridines obtained in a phosphate buffer containing varied concentration of SDS and 10% (v/v) methanol at pH 7.0. The SDS concentration was varied in the range 0–75 m*M*. As expected, the partition of a solute into SDS micelles is favored with increasing SDS concentration as the electrophoretic mobility of each analyte increases with increasing SDS concentration. On the other hand, the EOF decreases comparatively to a lesser extent with increasing SDS concentration. Consequently, the separation with an increase in SDS concentration results in a longer analysis time. As shown in Fig. 3, a baseline separation of a mixture of pyridine and eight chloropyridines was optimally achieved in 10 min using a phosphate buffer (10

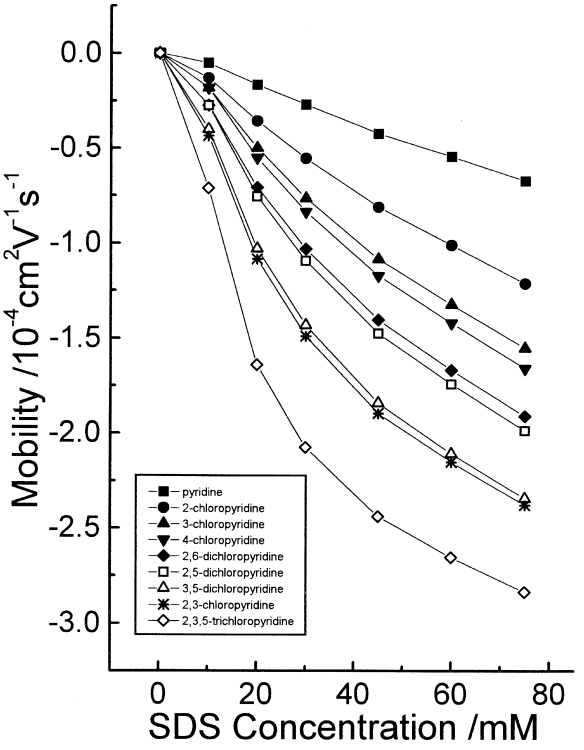


Fig. 2. Variations of the electrophoretic mobility of pyridine and chloropyridines as a function of SDS concentration in MEKC. Background electrolyte: 10 m*M* phosphate buffer containing 10% (v/v) methanol at pH 7.0. Other operating conditions as for Fig. 1.

m*M*) containing 30 m*M* SDS and 10% (v/v) methanol at pH 7.0 with an applied voltage of 30 kV. With this buffer electrolyte, the electrophoretic mobility of SDS micelles using Sudan III as a micelle marker was determined to be $-3.46 \cdot 10^{-4}$ cm$^2$ V$^{-1}$ s$^{-1}$.

### *3.3. Effect of methanol content*

A background electrolyte (BGE) solution composed of $NaH_2PO_4$ (10 m*M*) and SDS (30 m*M*) containing various proportions of methanol (0–20%, v/v) at pH 7.0 was used to examine the effect of methanol content on the EOF and electrophoretic mobility of each solute. The EOF declines almost linearly and the electrophoretic mobility of each analyte also decreases, but to a lesser extent, with increasing methanol content. The decrease in the EOF is attributed to the interaction between methanol and the silanol groups on the capillary wall,

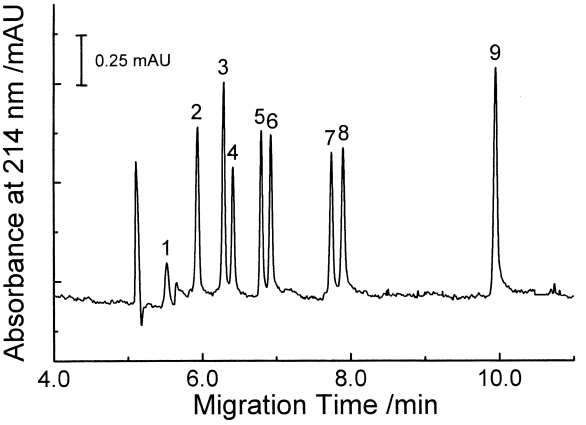


Fig. 3. A typical electropherogram of pyridine and eight chloropyridines obtained with a phosphate buffer (10 m*M*) containing 30 m*M* SDS and 10% (v/v) methanol at pH 7.0. Other operating conditions as for Fig. 2. Peak identification, 1, pyridine; 2, 2-chloropyridine; 3, 3-chloropyridine; 4, 4-chloropyridine; 5, 2,6-dichloropyridine; 6, 2,5-dichloropyridine; 7, 3,5-dichloropyridine; 8, 2,3-dichloropyridine; 9, 2,3,5-trichloropyridine.

resulting in the lowering of the zeta potential of the silica surface. Furthermore, the dielectric constant of the BGE decreases linearly and the viscosity of the BGE also increases in the presence of methanol, thus causing the decrease in the EOF [18]. The migration time of analytes separated in a BGE without the addition of methanol is shorter than that separated in a BGE containing methanol. Unfortunately, 2,3-dichloropyridine and 3,5-dichloropyridine elute together in a BGE without the addition of methanol.

As shown in Fig. 4, the resolution of the peaks of 2,3- and 3,5-dichloropyridine can be improved with addition of methanol. In fact, the peaks of these two analytes are effectively separated with addition of 10% (v/v) methanol, and the peaks are well separated with addition of 20% (v/v) methanol. On the contrary, the separations for the peaks between 2,5- and 2,6-dichloropyridine and for the peaks between 3- and 4-dichloropyridine become worse as methanol content in the buffer electrolyte increases from 10 to 20% (v/v).

### *3.4. Effect of Brij 35 concentration*

A mixed micelle composed of SDS and Brij 35 has been reported to improve separation selectivity [19–23] and to increase the elution range in MEKC

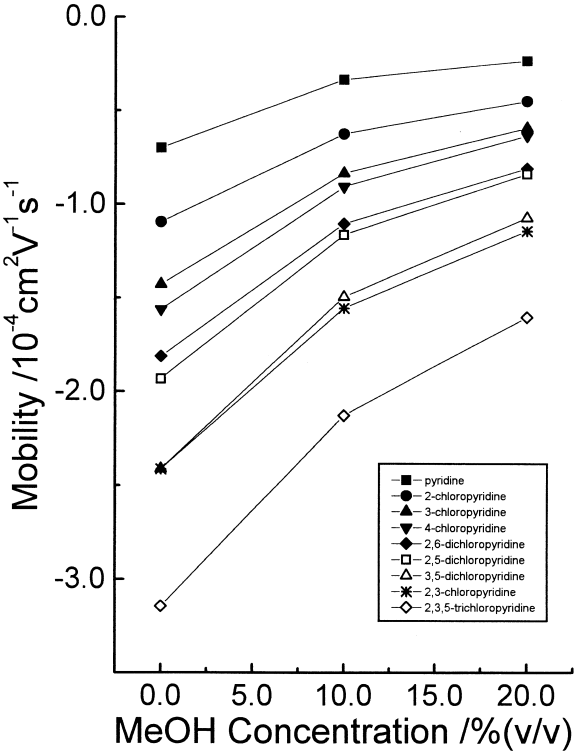


Fig. 4. Variations of the electrophoretic mobility of pyridine and chloropyridines as a function of methanol content using SDS (30 m*M*)–phosphate (10 m*M*) buffer system at pH 7.0. Other operating conditions as for Fig. 2.

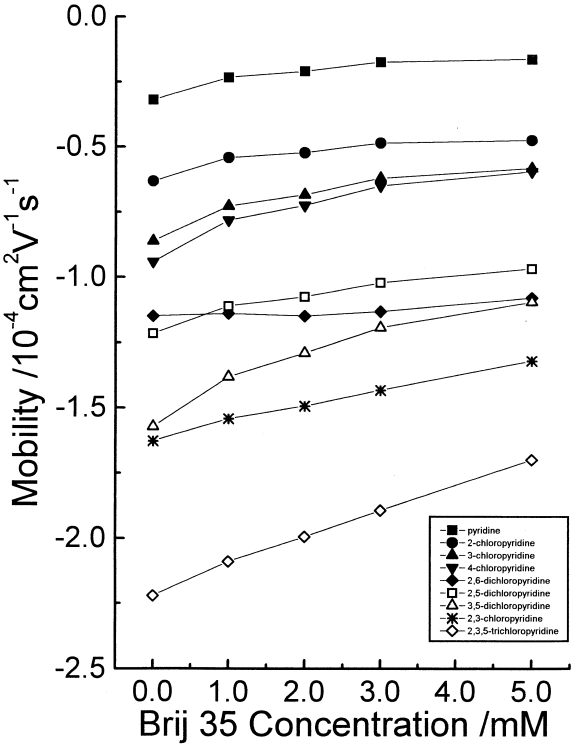


Fig. 5. Variations of the electrophoretic mobility of pyridine and chloropyridines as a function of Brij 35 concentration using SDS (30 m*M*)–phosphate (10 m*M*) buffer system at pH 7.0. Other operating conditions as for Fig. 2.

[24]. Fig. 5 shows the variations of the electrophoretic mobility of pyridine and chloropyridines as a function of Brij 35 concentration in a phosphate buffer (10 m*M*) containing 30 m*M* SDS 10% (v/v) methanol, and varied concentration of Brij 35 in the range 0–5 m*M* at pH 7.0. As can be seen, the resolution of the peaks of 2,3- and 3,5-dichloropyridine is greatly enhanced with further addition of Brij 35 to the SDS–phosphate buffer system. With addition of Brij 35, the electrophoretic mobility and the selectively of the analytes studied, except 2,3-dichloropyridine, are considerably affected. The migration of 2,6-dichloropyridine behaves differently from the other analytes, thus causing the reversal of the migration order of the peaks of 2,5- and 2,6-dichloropyridine. Moreover, the peaks of 3- and 4-chloropyridine become even unresolvable when the concentration of Brij 35 exceeds 3 m*M*.

As a result, the separation of these chloropyridines is optimally achieved with addition of 2–3 m*M* Brij 35 in the SDS–Brij 35 mixed micellar buffer system. Fig. 6 shows the electropherogram of a mixture of chloropyridines obtained with addition of 3 m*M* Brij 35 to the SDS–phosphate buffer system.

### *3.5. Binding constants versus migration order*

In MEKC, the effective electrophoretic mobility ($\mu_{\text{eff}}$) of a neutral solute can be expressed by the following equation [25,26]:

$$\mu_{\text{eff}} = \frac{K_{\text{B}\cdot\text{S}}(\text{CMC})\mu_{\text{B}\cdot\text{S}} + K_{\text{B}\cdot\text{M}}[\text{M}]\mu_{\text{M}}}{1 + K_{\text{B}\cdot\text{S}}(\text{CMC}) + K_{\text{B}\cdot\text{M}}[\text{M}]} \quad (3)$$

where $\mu$ and $K$ denote the electrophoretic mobility and binding constant, B·S represents the complexes formed between neutral analytes (B) and surfactant monomers (S), and B·M represents the complexes

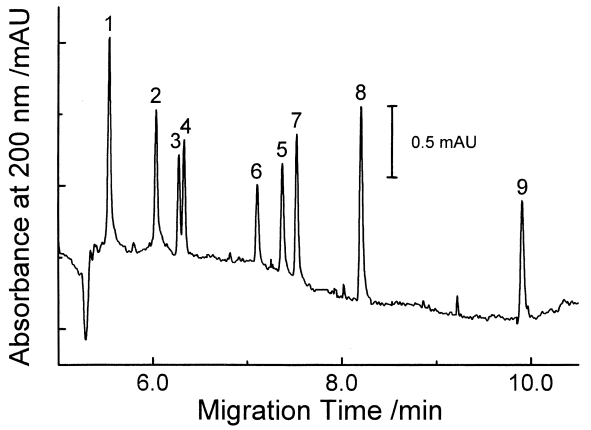


Fig. 6. An electropherogram of pyridine and eight chloropyridines obtained in a phosphate buffer (10 m*M*) containing 30 m*M* SDS, 3 m*M* Brij 35 and 10% (v/v) methanol at pH 7.0. Detection wavelength, 200 nm. Other operating conditions and peak identification are the same as for Fig. 3.

formed between neutral analytes (B) and surfactant micelles (M), [M] is the concentration of surfactant micelles which is defined as the total concentration of surfactant molecules minus the critical micelle concentration (CMC). The CMC value of SDS in 10 m*M* phosphate buffer containing 10% (v/v) methanol in the presence of chloropyridines is determined to be 4.8 m*M* [27]. As the magnitude of the term involving $K_{B\cdot S}$ is very small because the binding between chloropyridines and SDS monomers is very weak, Eq. (3) can be simplified to

$$\mu_{eff} = \frac{K_{B\cdot M}[M]\mu_M}{1 + K_{B\cdot M}[M]} \quad (4)$$

Accordingly, the binding constant of each individual solute to SDS micelles can be calculated by varying the parameter ($K_{B\cdot M}$) through the utilization of Excel software until the simulated mobility curve is best fitted to the observed data points. Table 1 lists the magnitudes of the binding constants evaluated for these chloropyridines, together with the log $P_{ow}$ (logarithm of octanol–water partition coefficient) values available in the literature.

As the log $P_{ow}$ values of pyridine, monochloropyridines, dichloropyridines, 2,3,5-trichloropyridine, 2,3,5,6-tetrachloropyridine, and pentachloropyridine reported in the literature [28,29] are in the range

Table 1
Binding constants evaluated for pyridine and eight chloropyridines in a SDS–phosphate buffer system at pH 7.0[a]

| Peak no. | Analytes | Binding constant ($M^{-1}$) | Log $P_{ow}$[b] |
|---|---|---|---|
| 1 | Pyridine | 3.2 | 1.04 |
| 2 | 2-Chloropyridine | 7.5 | 1.45 |
| 3 | 3-Chloropyridine | 11.2 | 1.43 |
| 4 | 4-Chloropyridine | 13.0 | 1.28 |
| 5 | 2,6-Dichloropyridine | 17.0 | 2.15 |
| 6 | 2,5-Dichloropyridine | 18.5 | 2.40 |
| 7 | 3,5-Dichloropyridine | 28.5 | 2.56 |
| 8 | 2,3-Dichloropyridine | 30.0 | 2.11 |
| 9 | 2,3,5-Trichloropyridine | 58.0 | 3.11 |

[a] Binding constants are evaluated according to Eq. (4) with the CMC value of SDS equal to 4.8 m*M*.
[b] Refs. [28,29].

1.04, 1.28–1.45, 2.11–2.56, 3.11, 3.32, and 3.53, respectively, the hydrophobicity of chloropyridines increases with increasing the number of chlorine substituents on the pyridine ring. Moreover, as indicated in Table 1, the binding constants of pyridine, monochloropyridines, dichloropyridines and 2,3,5-trichloropyridine evaluated are in the range 3.2, 7.5–13.0, 17.0–30.0, and 58.0 $M^{-1}$, respectively. The binding constant of chloropyridines increases as the number of chlorine substituents on the pyridine ring increases. The results clearly indicate that the migration order of these chlopopyridines depends primarily on their hydrophobicity. However, the magnitudes of the binding constants of positional isomers of monochloropyridines and those of dichloropyridines do not follow the same order as for their hydrophobicity. The results reveal that the migration behavior of those chloropyridines is not solely governed by the hydrophobic interaction. It also implies that electrostatic interactions may play a significant role in the determination of the migration order.

### *3.6. Reproducibility and detection limits*

The migration times of these analytes were quite reproducible, with relative standard deviation (RSD) varying in the range 0.6–0.8% ($n = 6$). The limits of detection (LOD) at a signal-to-noise ratio ($S/N$) equal to 3 determined with UV detection at 214 nm

for 2- and 3-chloropyridine, 2,5- and 3,5-dichloropyridine, and 2,3,5-trichloropyridine were in the range 0.5~1.1 μg/ml, depending on the molar absorbtivity of each individual analyte. It is believed that much lower levels of the LOD values of these analytes can be obtained with UV detection by using sweeping technique [30–32]. Currently, on-line concentration of chloropyridines by sweeping in MEKC is undertaken and the results of the analysis will be reported later.

## 4. Conclusion

The separation of pyridine and eight chloropyridines is effectively achieved by MEKC with the use of either SDS micelles or SDS–Brij 35 mixed micelles. Various separation parameters are optimized. In addition to buffer pH and SDS concentration, methanol content and Brij 35 concentration also affect the selectivity of analytes considerably. The migration order of those analytes depends primarily on the hydrophobic interaction of each analyte with the SDS micelles. However, electrostatic interactions may also play a significant role.

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