CHAPTER IV

RESULTS AND DISCUSSION

4.1 Choices of CE Conditions

4.1.1 pH and concentration of buffer

Since avermectins are highly hydrophobic compounds, MEEKC with suppressed EOF should be carried out, in order to obtain faster analysis time as previously mentioned in Section 2.5 [Altria *et al.* 2000b, Pedersen-Bjergaard *et al.* 2000, Nhujak *et al.* 2006]. In this work, a low pH buffer was used to eliminate EOF due to simplicity and low cost. The pH of 2.5 is the lowest value typically used in CE. The phosphate buffer was chosen because it contains weak acid (H_3PO_4 or HA) and conjugated base ($H_2PO_4^-$ or A^-) which can be expressed by a dissociation equilibrium in Equation 4.1.

$$H_3PO_4 \rightleftharpoons H_2PO_4^- + H^+ \quad : pK_{a1} = 2.12 \tag{4.1}$$

The pH of the solution is given by the Henderson-Hasselbalch's equation [Chang 2005]

$$pH = pK_a + \log \frac{[A^-]}{[HA]} \tag{4.2}$$

The buffer pH of 2.5 is in a range of $pK_{a1} \pm 1.0$ and provides high buffering capacity, resulting in high precision in the suppressed EOF and migration times of analytes.

In MEEKC, the concentration of the phosphate buffer at 50 mM is usually used. The lower concentration of the buffer results in poor precision in migration time, while the higher concentration of the buffer generates high current and Joule heating which may limit the use of high voltage and temperature [Altria *et al.* 2000b, Nhujak *et al.* 2006].

In addition, the stability of avermectins stored in the acidic buffer for 2 hours and 7 days was investigated by measuring UV absorption of abamectin in the microemulsion buffer containing 50 mM phosphate buffer at pH 2.5, 1.1 %v/v *n*-octane, 890 mM 1-butanol and 180 mM SDS. An insignificant change of UV-absorbance of abamectin at 245 nm within 2 hours and 7 days was obtained as shown in Figure 4.1a and 4.1b, respectively, indicating high stability of avermectins in the acidic buffer used in this work. Therefore, the 50 mM phosphate buffer at pH 2.5 was selected in this work, which is widely used in MEEKC at low pH.

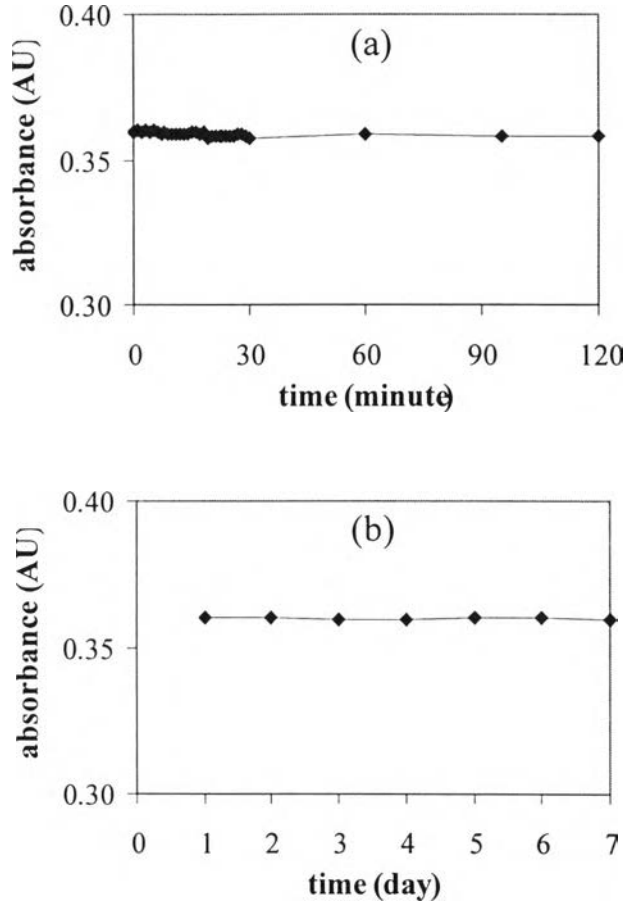

Figure 4.1 UV absorption of abamectin in the microemulsion buffer containing 50 mM phosphate buffer at pH 2.5, 1.1 %v/v *n*-octane, 890 mM 1-butanol and 180 mM SDS, at 245 nm within (a) 2 hours and (b) 7 days.

4.1.2 Diameter and length of capillary

An uncoated fused silica capillary with 50 μm internal diameter (I.D.) × 40.2 cm in length (30 cm to detector) was selected for analysis of avermectins. The most commonly used capillaries are 50 and 75 mm μm I.D. [Chankvetadze 1997]. The 75 μm I.D. capillary provides higher sensitivity and lower interaction between analytes and capillary wall, but may cause low resolution due to greater peak broadending caused by Joule heating. In addition, it has limitation of the use of high BGE concentration and high voltage. Therefore, 75 μm I.D. capillary is usually used to improve detection sensitivity and to decrease wall interaction when the separation has no problem of resolution. Typically, the 50 μm I.D. capillary is used for many applications due to its compromise among resolution, sensitivity, heat dissipation and wall interaction. This work does not involve analysis of trace levels of avermectins, and therefore, the 50 μm I.D. capillary was selected. For the length of the capillary, the 40.2 cm (30 cm to detector) capillary was used to give fast analysis time. In MEEKC, the mostly used internal diameter and length of capillary are 50 μm and 40.2 cm, respectively [Altria 2000a].

4.1.3 Detection wavelength

Avermectins, such as abamectin, ivermectin and doramectin, was found to absorp UV light with maximum absorbance at the wavelength of 245 nm. However, dodecylbenzene (DB) used as a microemulsion marker has very low absorption near 245 nm. Therefore, UV detection of avermectins was scanned from 190 to 400, and electropherograms were monitored at 214 nm for determining migration time of DB and 245 nm for quantitative analysis of avermectins in this work.

4.2 MEEKC Optimisation

MEEKC optimisation for separation of avermectins were carried out by varying types and concentrations of organic co-solvents, SDS concentrations, types of co-surfactants, temperature and applied voltage. The effects of these factors on migration time (t_m), electrophoretic mobility (μ), retention factor (k), selectivity (α), efficiency

(N) and resolution (R_s) in Equation 2.42 were discussed, where R_s of analytes depends on α, k and N. The migration times of analytes and the microemulsion marker (DB) were observed from the electropherogram. The electrophoretic mobilities were obtained using Equations 2.8 to 2.10, where electroosmotic mobility was negligible as reported in previous work of our group [Nhujak *et al.* 2006]. The values of k were determined using Equation 2.16, where t_{mc} was obtained from migration of DB used as microemulsion marker [Altria *et al.* 2000b, Nhujak *et al.* 2006]. The selectivity is defined as the ratio of retention factor as previously mentioned in Section 2.4.3. In this work, $\alpha_{I/D}$ and $\alpha_{D/A}$ refer to k_I/k_D and k_D/k_A, respectively. The peak efficiency was determined using Equation 2.22, where w_b was measured from the electropherogram. It should be noted that N and R_s were obtained from the electropherograms at 245 nm, due to high sensitivity and low background noise.

4.2.1 Effect of types and concentration of organic co-solvents

The organic co-solvent in MEEKC is one of the important factors affecting separation. In previous work, 2-propanol was found to be suitable co-solvent in MEEKC for separation of neutral water-insoluble compounds, such as vitamin [Pedersen-Bjergaard *et al.* 2000], polymer additives [Hilder *et al.* 2001] and curcuminoids [Nhujak *et al.* 2006], while acetonitrile for MEEKC separation of capsaicin and dihydrocapsaicin [Jungmanotham *et al.* 2004]. Therefore, the suitable organic solvent in MEEKC should be investigated for separation of particular analytes.

In initial work, MEEKC analysis of avermectins was carried out using a microemulsion buffer containing a 50 mM phosphate buffer at pH 2.5, 1.1 % v/v *n*-octane as oil droplet phase, 180 mM SDS as negatively charged surfactant and 890 mM 1-butanol as co-surfactant. No resolution of avermectins was obtained, and co-retention of these compounds and DB was observed using the microemulsion buffer without organic co-solvent as seen from the electropherogram in Figure 4.2a. This indicates that these compounds strongly and completely partition into the oil droplet phase. Organic solvents at 0 to 30 % v/v, such as acetonitrile, methanol, ethanol and 2-propanol, were separately added in the microemulsion buffer in order to obtain the

difference in partitioning of these analytes into the aqueous phase. Figures 4.2b-4.2e show an example of electropherograms of avermectins using 30 % v/v organic solvent in the MEEKC buffer. Addition of organic co-solvent resulted in the separation of avermectins from DB, and the retention factor of analytes (Figure 4.3c) decreased with an increase in the concentration of organic co-solvent due to partitioning of analytes in organic-aqueous phase [Altria *et al.* 2000b]. In comparison with other organic co-solvents, methanol gave higher retention factors of analytes possibly due to its higher hydrophobicity than other organic co-solvents. Separation of two analytes can be described by α or R_s. In MEEKC without EOF, the analytes having greater retention factor or stronger partitioning of the analytes into the microemulsion phase migrate with a smaller migration time as previously discussed in Section 2.5 and shown in Figure 2.7b. In MEEKC buffer containing 30 % v/v organic co-solvent, the migration time order for avermectins (Figures 4.2 and 4.3a) in acetonitrile ~ methanol < ethanol < 2-propanol was not consistent with the retention factor order for abamectin B_{1a} (Figure 4.3c) in methanol > ethanol > 2-propanol ~ acetonitrile. This is because an increase in the migration time is also due to two additional effects caused by an increase in the viscosity, 2-propanol > ethanol > methanol > acetonitrile, and the smaller charge to size ratio caused by solvation of organic solvent [Altria *et al.* 2000b]. α is used to indicate the difference in the retention of analytes, while R_s is used to measure the quantity of separation of analytes, the distance of center peaks divided by the average peak width at base. Over a range of 0 to 15 % v/v organic co-solvent in the MEEKC system, no separation was found for avermectins, which is not shown in Figures 4.3. In a range of 20 to 35 % v/v organic co-solvents, except methanol, gave a slight change of α. An increase in α was observed using 20 to 35 % v/v methanol as shown in Figures 4.3d. In the presence of 25 to 35 % v/v acetonitrile or 2-propanol in the MEEKC buffer, the values of α, N, and R_s were slightly changed, like ethanol (Figures 4.3d, 4.3e and 4.3f). In the presence of 30 to 35 % v/v co-solvent in the MEEKC buffer, the selectivity of D and A, $\alpha_{D/A}$, was obtained to be in order methanol > ethanol > acetonitrile > 2-propanol added in the MEEKC buffer. Although acetonitrile, ethanol and 2-propanol gave slight difference in $\alpha_{D/A}$ and k, ethanol provided significantly greater resolution of A and D, (Figure 4.3f). This is due to the greater N of analytes in MEEKC containing ethanol (Figure 4.3e), $R_s \propto N^{1/2}$ as shown in Equation 2.42. In previous work on MEKC and MEEKC, the organic modifiers can

either enhance or reduce efficiency due to several effects such as an increase in viscosity and a change in micellar structure and different diffusion in organic solvent [Altria *et al.* 2000b, Nhujak *et al.* 2006]. In most cases, simple explanation for the change in *N* is not clear [Khaledi 1998]. From Figure 4.3f, 30 and 35 % v/v ethanol gave slight difference in the resolution of D and A, but longer analysis time was obtained at the higher concentration of ethanol. Therefore, 30 % v/v ethanol was chosen as organic co-solvent in this work.

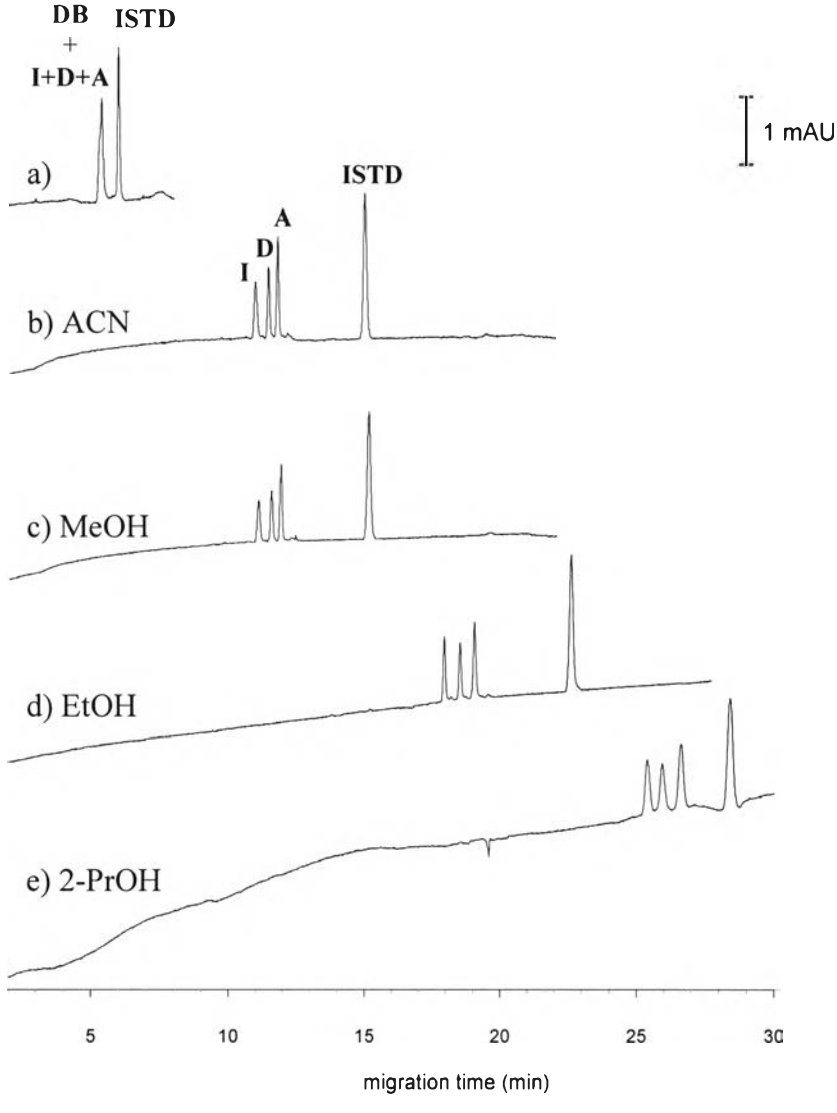

Figure 4.2 MEEKC separation of avermectins using (a) no organic co-solvent and 30% v/v organic co-solvent as (b) acetonitrile (ACN), (c) methanol (MeOH), (d) ethanol (EtOH), and (e) 2-propanol (2-PrOH) in a pH 2.5 50 mM phosphate buffer containing 1.1 % v/v *n*-octane, 180 mM SDS and 890 mM 1-butanol. CE conditions: uncoated fused silica capillary 50 μm I.D. × 40.2 cm (30 cm to detector), temperature 25 °C, voltage –15 kV, 0.5 psi pressure injection for 3 s and UV detection at 214 nm, DB = dodecyl benzene used as microemulsion marker, I = ivermectin, D = doramectin, A = abamectin B_{1a}, and ISTD = internal standard.

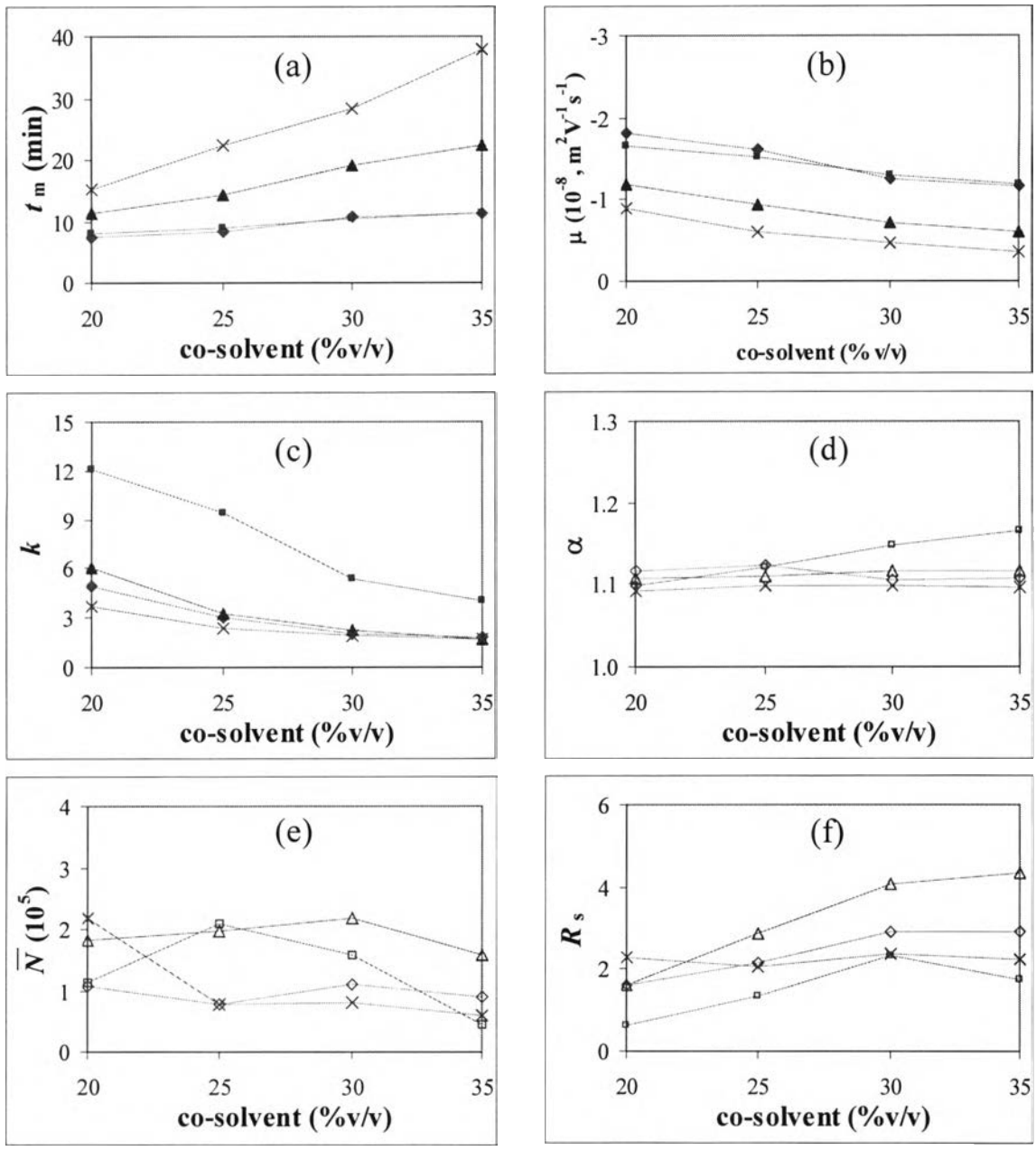

Figure 4.3 Effect of types and concentration of organic co-solvents on (a) retention factor of A, (b) selectivity of D and A, (e) average efficiency of D and A, and (f) resolution of D and A. Acetonitrile(◊), methanol (□), ethanol (Δ), and 2-propanol (×). Other CE conditions as shown in Figure 4.2. Each symbol is the average value obtained from duplicate runs.

4.2.2 Effect of SDS concentration

In MEEKC, sodium dodecyl sulphate (SDS) is mostly used as anionic surfactant because it is cheap and available in highly purified form. Therefore, SDS was selected in this work The C_{12} alkyl chain of the SDS surfactant penetrates into the oil droplet, while the negatively charged hydrophilic sulphate groups reside in the surrounding aqueous phase. The effect of SDS concentration in a range of 130 to 200 mM on the separation of avermectins was investigated as shown in Figure 4.4.

The faster migration time (Figure 4.5a) and higher electrophoretic mobility of microemulsion marker (Figure 4.5b) were observed with increasing the concentration of SDS, due to an increase in surface charge density of oil droplet [Altria *et al.* 2000b]. From Figure 4.5c, the retention factor increased with an increase in the SDS concentration due to a decrease in the phase ratio ($k \propto \phi$ as shown in Equation 2.11). Therefore, an increase in migration time of analytes is due to an increase in both electrophoretic mobility of charged oil and retention factor. Over a range of 130 to 200 mM SDS, the selectivity was insignificantly different due to the independent K ($\alpha = k_2/k_1 = K_2/K_1$) with the SDS concentration as shown in Figure. 4.5d. Besides, increasing SDS concentration provided an increase in N which may be influenced by reduction of H_{aq} and H_{pd}, similarly explained in MEKC and MEEKC at high EOF [Terabe *et al.* 1989 and 1992]. In addition, an increase in μ_{mc} with increasing the SDS concentration results in a decrease in H_l (Equation 2.30). However, an increase in the SDS concentration may cause higher Joule heating, leading to an increase in H_t and a decrease in N, which was observed for D and A in Figure 4.5e. In the range of investigated SDS concentrations, the resolution of avermectins (Figure 4.5f) was slightly different, due to insignificant difference in α and the value of $N^{1/2}/(1+k_2)$ (see Figures 4.5c and 4.5e, and Equation 2.42). The 180 mM SDS concentration was chosen for investigation other effects on the separation of avermectins, due to the faster migration time with the higher concentration of SDS.

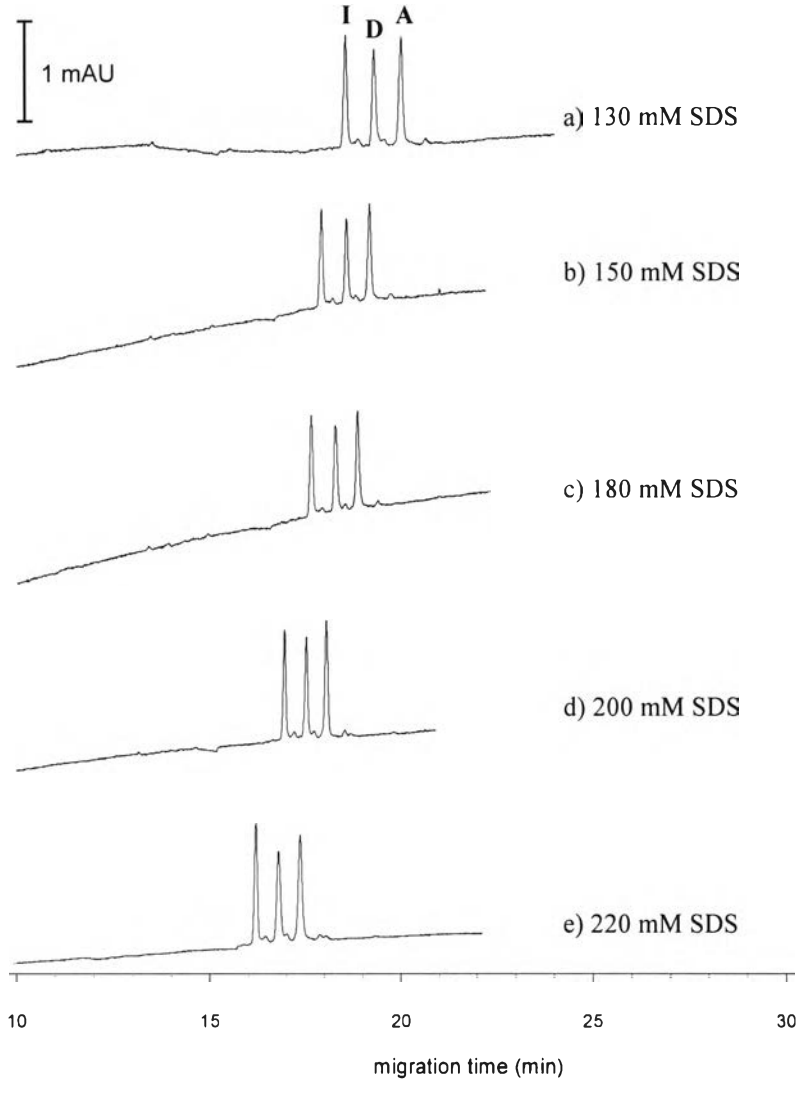

Figure 4.4 Electropherograms of avermectins in MEEKC using different concentration of SDS (a) 130, (b) 150, (c) 180, (d) 200 and (e) 220 mM. Other CE conditions as shown in Figure 4.2d.

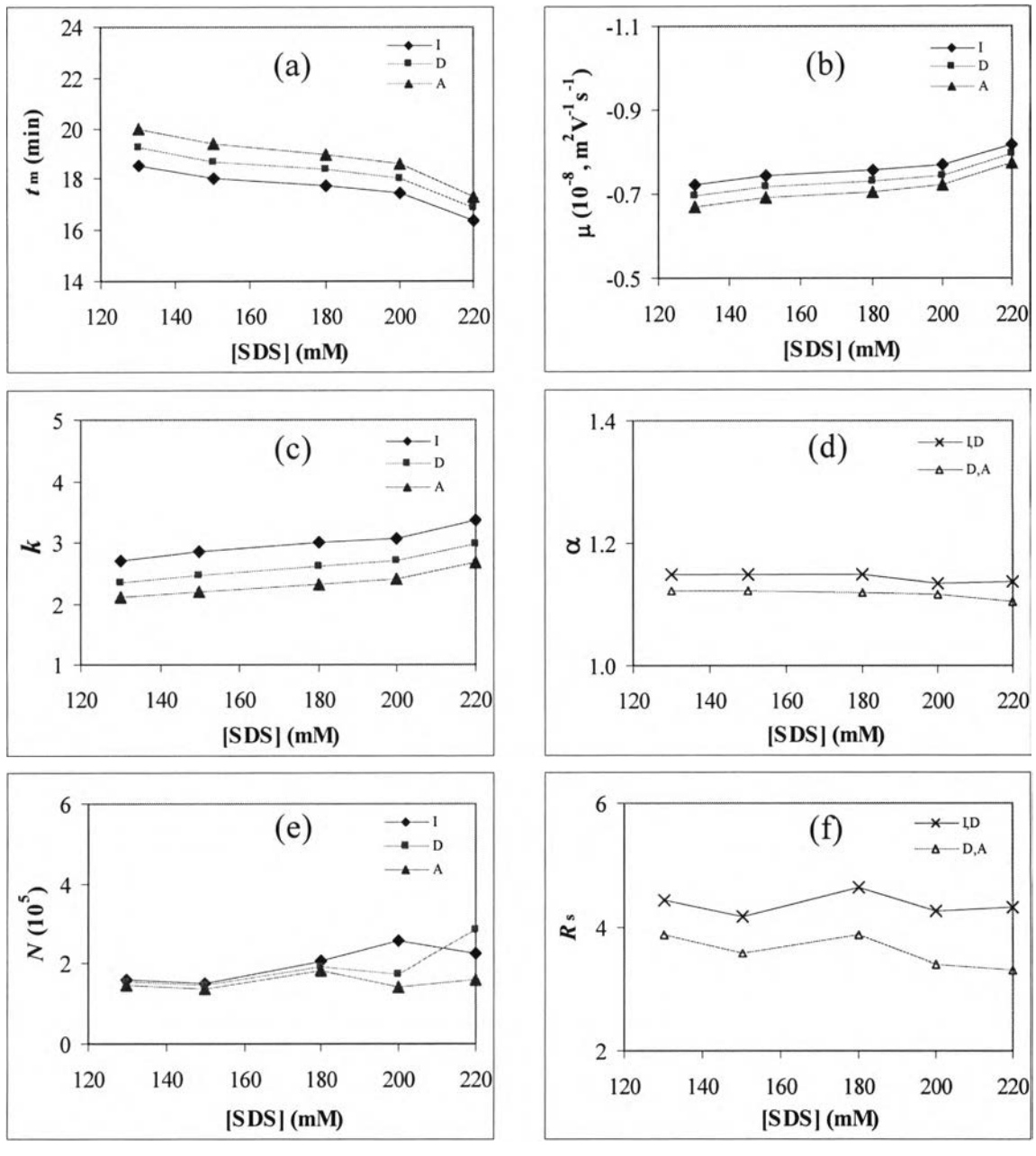

Figure 4.5 Effect of SDS concentration on (a) migration time, (b) retention factor, (c) selectivity, (d) efficiency, and resolution of avermectins. Other CE conditions as shown in Figure 4.2d. Each symbol is the average value obtained from duplicate runs.

4.2.3 Effect of co-surfactant types

Various alcohols, C_4 to C_6, at 890 mM were used as co-surfactant in MEEKC for separation of avermectins. The resulting electropherograms are shown in Figure 4.6. Poor resolution and baseline in Figures 4.6d-4.6f indicate that 4-methyl-2-pentanol or 1-hexanol used as co-surfactant gave unstable microemulsion. Baseline resolution was achieved, $R_s > 1.5$, with the co-surfactant as 1-butanol, 2-butanol, *tert*-butanol or isoamyl alcohol. From Figure 4.6c, the greater retention factor of analytes was found with increasing the number of C-atoms of the aliphatic alcohols, possible due to the larger microemulsion diameter with the larger size of alcohols [Pomponio *et al.* 2003]. Figures 4.6d and 4.6f, similar selectivity and resolution of avermectins were obtained with butanols used as organic co solvent in the microemulsion buffer, which was also found in previous work on MEEKC separation of curcuminoids [Nhujak *et al.* 2006]. However, significant difference in selectivity, resolution and migration time order was observed with different C_3 to C_6 alcohols, especially butanols, in the MEEKC buffer for saparation of catechins [Pomponio *et al.* 2003]. This may be due to the different numbers of hybrophobic phenolic group in catechins or different concentration of co-organic solvent used in their work. Therefore, in this work, 1-butanol was chosen as co-surfactant, which is widely used in the MEEKC separation and also our previous works [Jungmanotham *et al.* 2004, Nhujak *et al.* 2006].

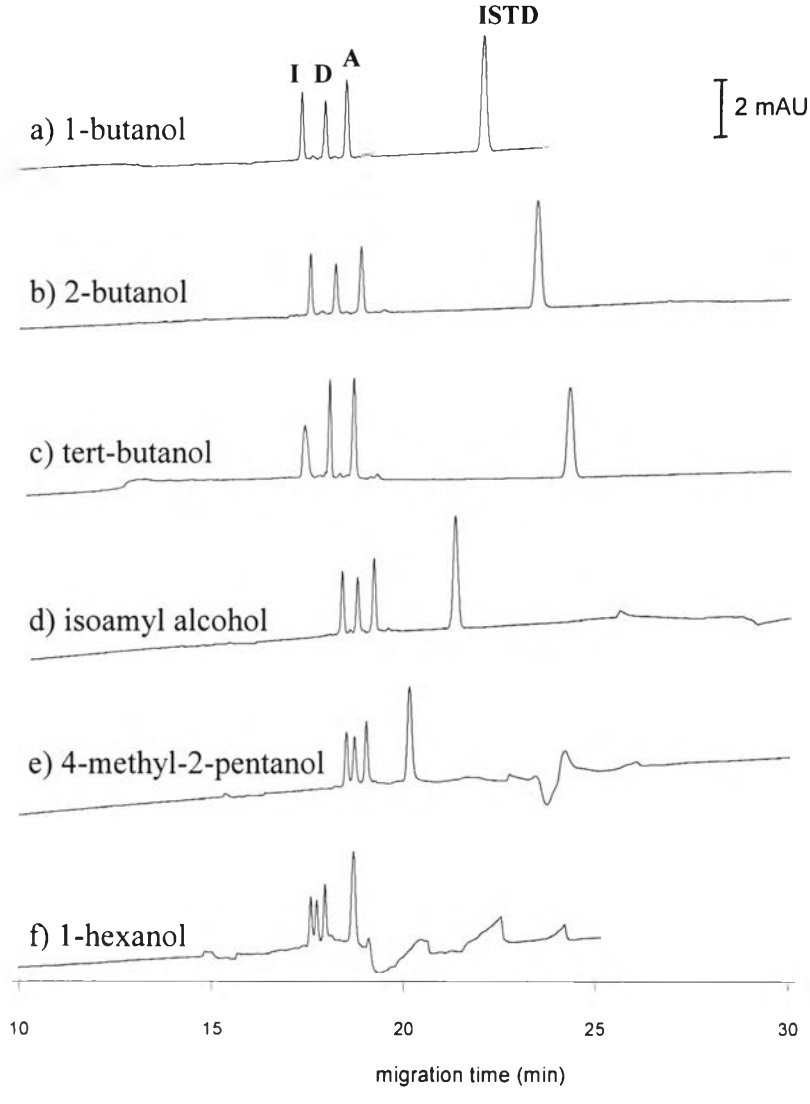

Figure 4.6 Electropherograms of avermectins in MEEKC using different types of 890 mM co-surfactants as (a) 1-butanol, (b) 2-butanol, (c) tert-butanol, (d) isoamyl alcohol, (e) 4-methyl-2-pentanol, and (f) 1-hexanol. Other CE conditions as shown in Figure 4.2d.

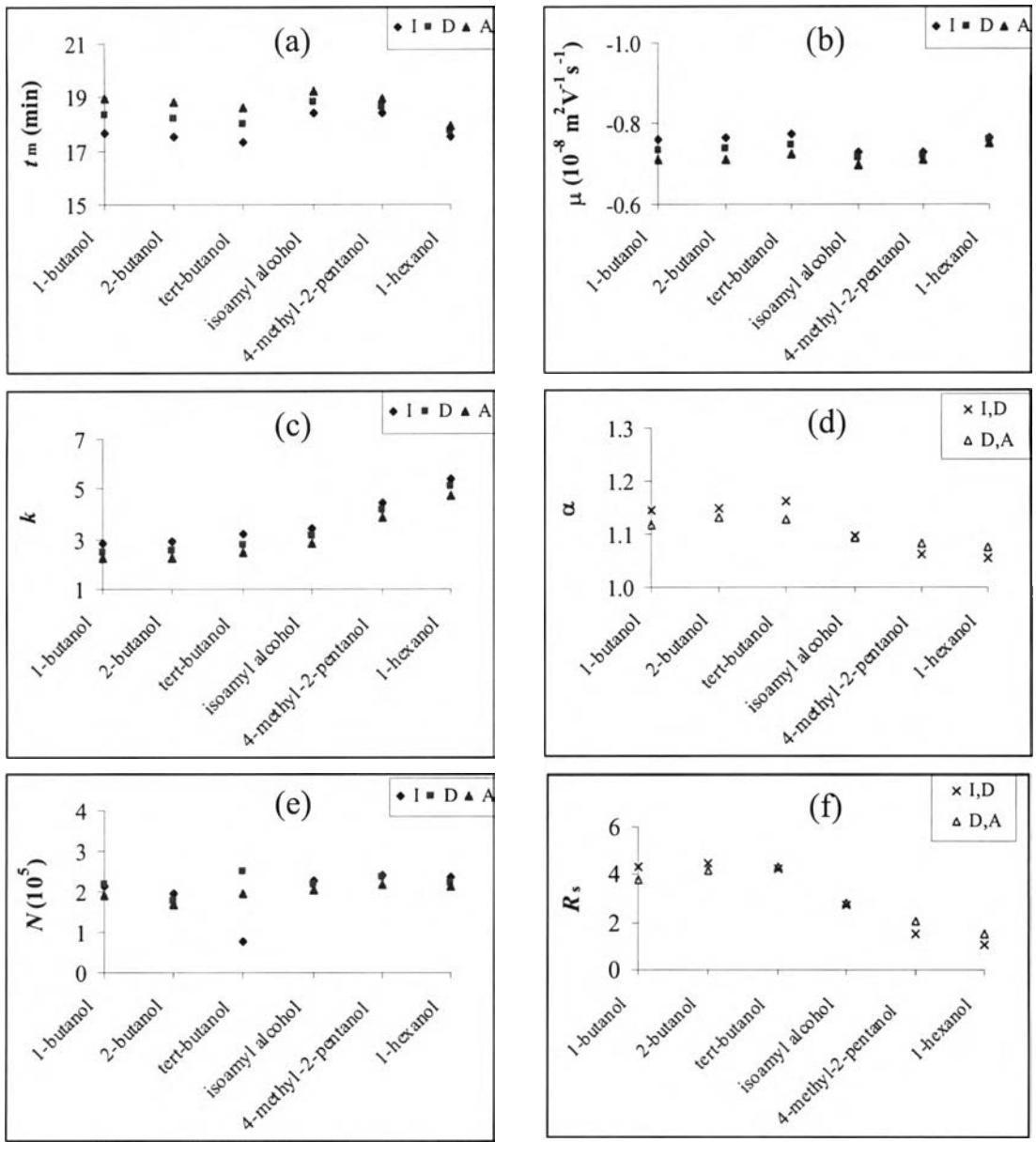

Figure 4.7 Effect of types of co-surfactants on (a) migration time, (b) electrophoretic mobility, (c) retention factor, (d) selectivity, (e) efficiency, and (f) resolution of avermectins. Other CE conditions as shown in Figure 4.2d. Each symbol is the average value obtained from duplicate runs.

4.2.4 Effect of temperature

MEEKC was carried out using separation temperatures in a range of 25 to 40 °C. From Figures 4.8, 4.9a and 4.9b, an increase in separation temperature resulted in a decrease in t_m and an increase in μ, due to a decrease in the buffer viscosity. The value of k decreased with an increase in the temperature (Figure 4.9c), due to an increase in the solubility of water-insoluble compounds in the organic-water phase [Altria *et al.* 2000b], which is consistent with vant Hoff equation, where ln$K$ or ln k decrease with an increase in temperature. From Figure 4.9d, α of analytes slightly changed when the temperature increased. From 25 to 40 °C in Figure 4.9e, a decrease in N with increasing the temperature may be due to an increase in H_t caused by Joule heating, resulting worse R_s above 25 °C (Figure 4.9f). Therefore, the temperature at 25 °C near the room temperature was chosen in this work due to

4.2.5 Effect of applied voltage

Electrophoregrams of avermectins in MEEKC using applied voltage in a range of –15 kV to –25 kV are illustrated in Figure 4.10. An increase in applied voltage gave faster analysis time, (Figure 4.11a) due to the fact that v_{ep} is proportional to E as shown in Equation 2.2. Theoretically, μ and k are independent with the voltage. With increasing applied voltage, a slightly decrease in k (Figure 4.11c) and an increase in μ (Figure 4.11b) were obtained possibly due to Joule heating. At -25 kV, a decrease in N and R_s (Figures 4.11e and 4.11f) may be also caused by Joule heating. Similar result was found in previous work on MEEKC separation of curcuminoids [Nhujak *et al.* 2006]. In this work, the applied voltage of -15 kV was chosen due to worse resolution at higher voltage.

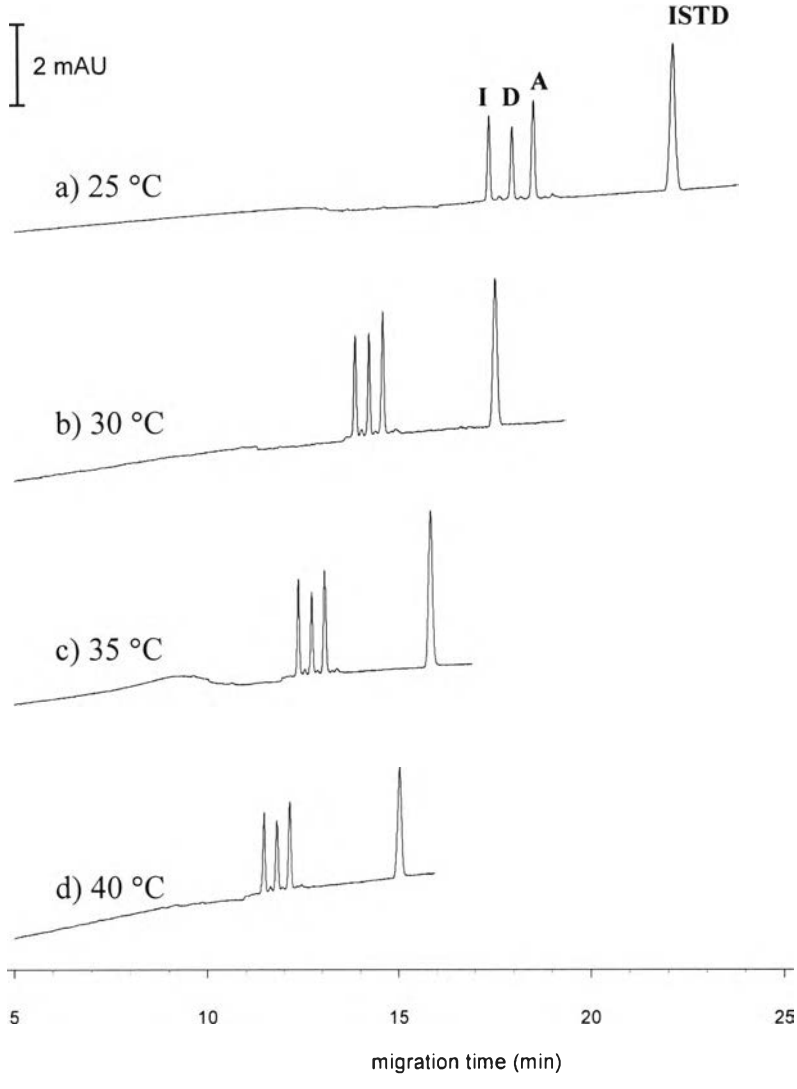

Figure 4.8 Electropherograms of avermectins in MEEKC using different temperatures (a) 25, (b) 30, (c) 35, and (d) 40°C. Other CE conditions as shown in Figure 4.2d.

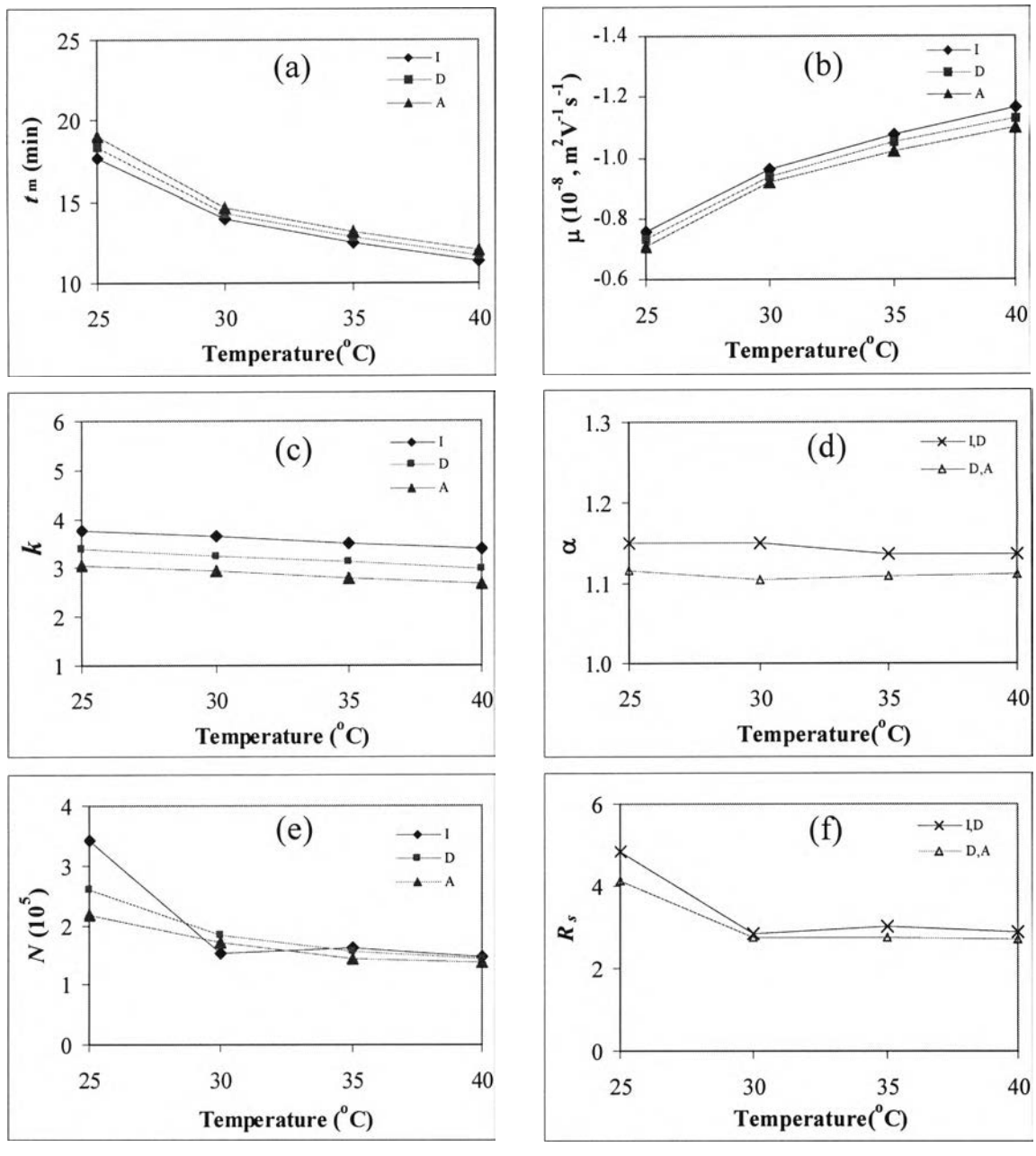

Figure 4.9 Effect of temperature on (a) migration time, (b) electrophoretic mobility, (c) retention factor, (d) selectivity, (e) efficiency, and (f) resolution of avermectins. Other CE conditions as shown in Figure 4.2d. Each symbol is the average value obtained from duplicate runs.

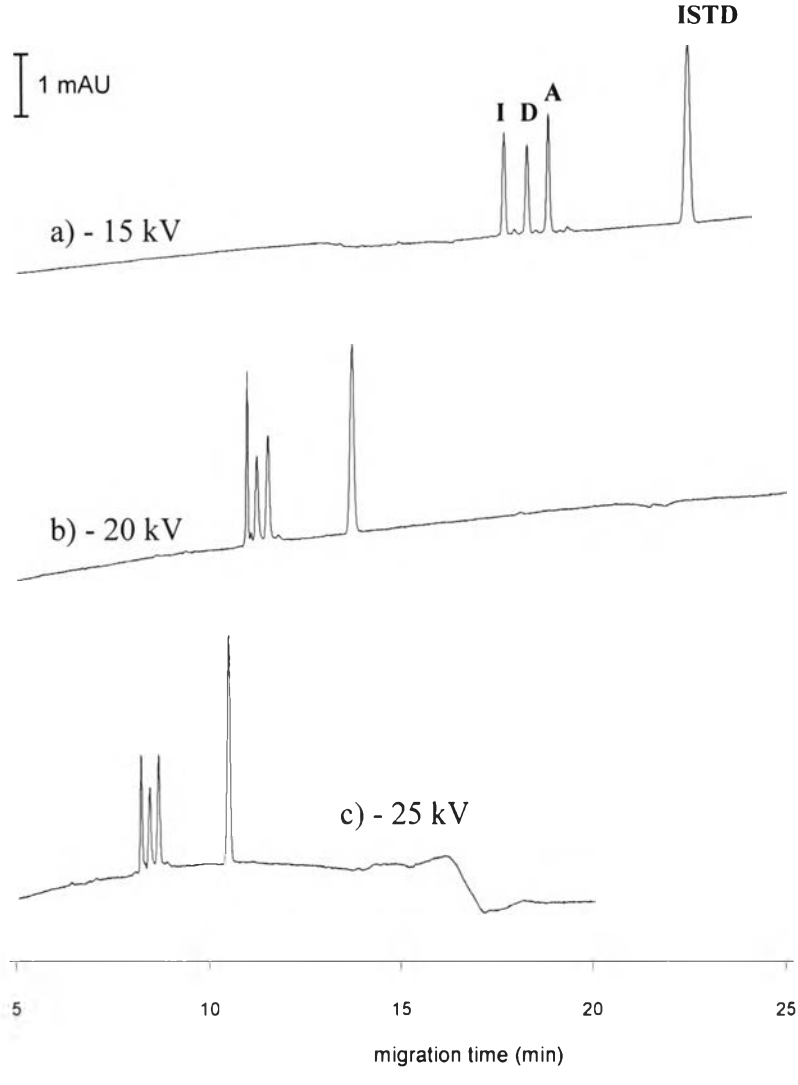

Figure 4.10 Electropherograms of avermectins in MEEKC using different applied voltages (a) -15, (b) -20, and (c) -25 kV. Other CE conditions as shown in Figure 4.2d.

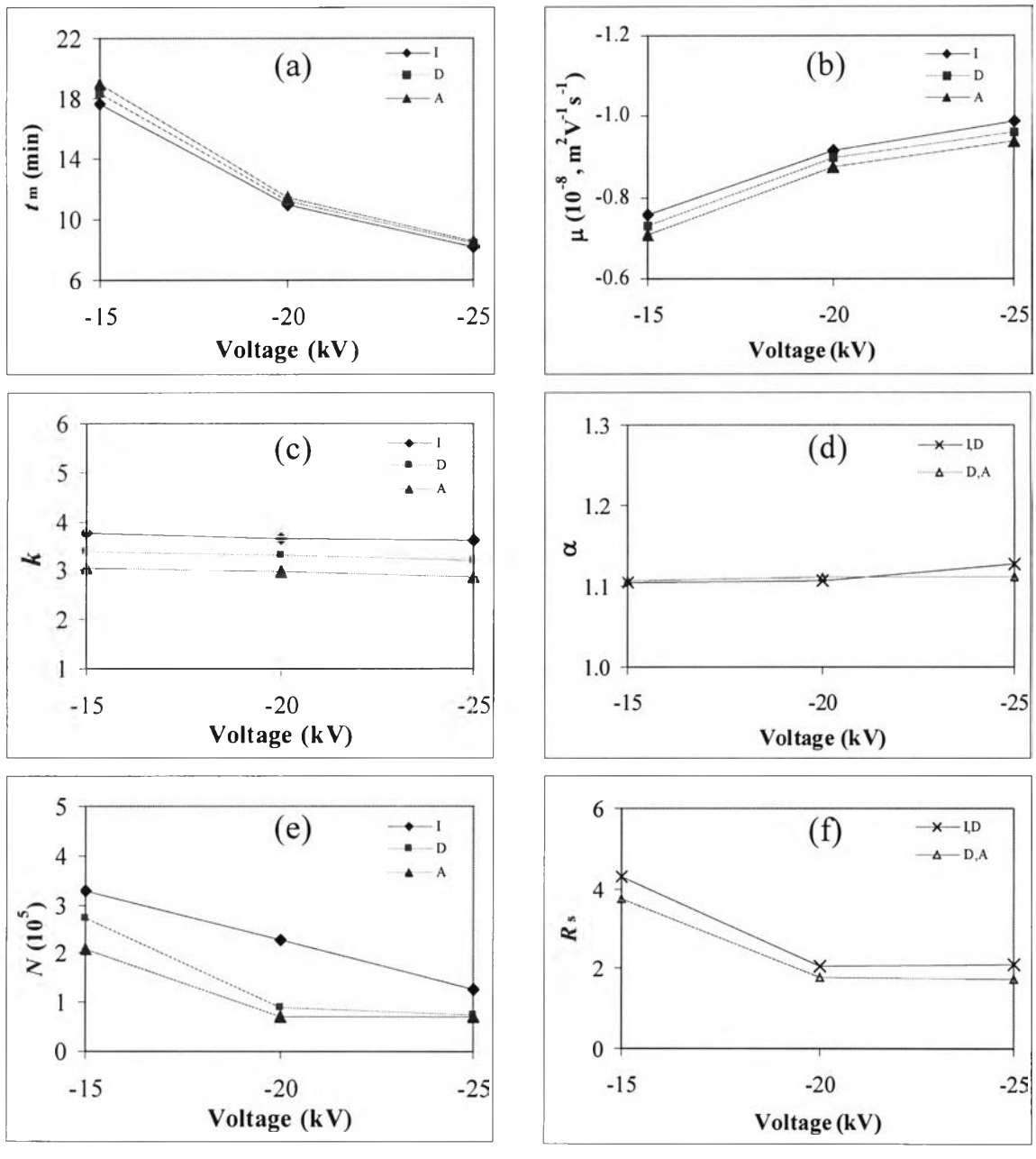

Figure 4.11 Effect of applied voltage on (a) migration time, (b) electrophoretic mobility, (c) retention factor, (d) selectivity, (e) efficiency, and (f) resolution of avermectins. Other CE conditions as shown in Figure 4.2d. Each symbol is the average value obtained from duplicate runs.

4.2.6 Effect of pressure

Another way to reduce analysis time in CE is application of pressure at the inlet buffer vial during application of voltage. Therefore, MEEKC was carried out using the applied voltage of −15 kV and additional pressure in a range of 0.1 to 1.0 psi. From Figure 4.12, an increase in applied pressure resulted in a decrease in t_m due to an increase in the velocity of analytes, but worse resolution was obtained due to an in crease in parabolic flow profile generated by pressure.

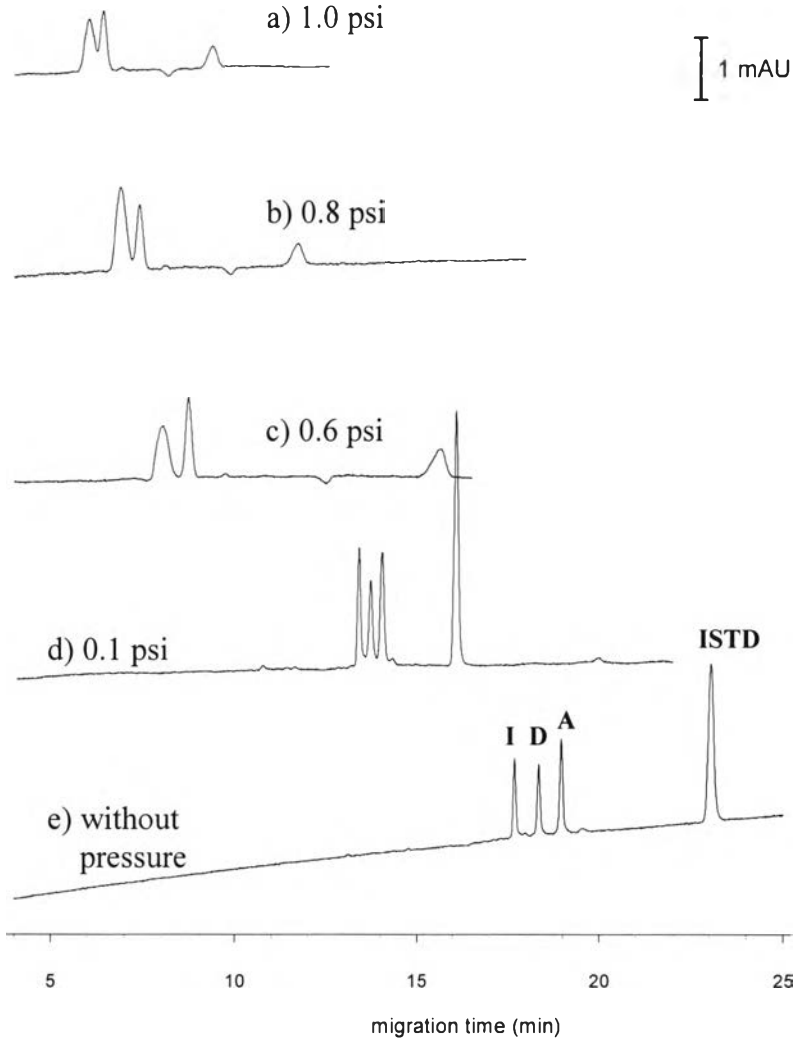

Figure 4.12 Electropherograms of avermectins in MEEKC using -15 kV and different applied pressure (a) 1.0, (b) 0.8, (c) 0.6, (d) 0.1 psi and (e) without pressure. Other CE conditions as shown in Figure 4.2d.

4.3 Validation of MEEKC Method

From Section 4.2, the following suitable MEEKC conditions were used for quantitative analysis of avermectins in this work; the microemulsion buffer containing 50 mM phosphate buffer at pH 2.5, 1.1 % v/v *n*-octane, 180 mM SDS and 890 mM 1-butanol and 30 %v/v ethanol, temperature 25 °C, voltage –15 kV, UV detection at 245 nm.

4.3.1 Limit of detection (LOD) and limit of quantitation (LOQ)

LOD and LOQ are defined as the analyte concentration giving signal-to-noise of 3 and 10, respectively. Table 4.1 shows the values of LOD and LOQ for I, D and A. This work does not involve analysis of trace levels of avermectins. Therefore, LOD and LOQ in this work are sufficient for quantitative analysis of avermectins in commercial products.

Table 4.1 LOD and LOQ of individual avermectin

Analyte	LOD (ppm)	LOQ (ppm)
I	5.3	16.8
D	6.0	18.2
A	4.5	14.6

4.3.2 Calibration plots

Calibration plots were established from the ratio of corrected peak area of the analyte to that of internal standard, $A_{corr,ratio}$, against the analyte concentration for six levels in a range of 25 to 400 mM, where corrected peak area is defined as the peak area divided by migration time [Mayer 2001]. A reason using $A_{corr,ratio}$ for quantitative analysis is to correct any error and variation of sample injection as given in Section 2.8. The standard solutions were prepared as previously mentioned in Section 3.4.2. Ethyl 4-hydroxybenzoate was chosen as an internal standard because it has UV absorption in the region of 245 nm, is stable in the acidic buffer used and separates

from avermectins and other compounds in commercial formulations. High linear relationship of $A_{corr,ratio}$ and the concentration of each avermectin was obtained with $r^2 \approx 0.999$, as summarised in Table 4.2.

Table 4.2 Concentration ranges and linear equations for calibration plots

Analyte	Concentration range (ppm)	Linear equation		
		Slope	Intercept	r^2
I	25 to 400	0.00330	0.00133	0.9985
D		0.00311	-0.00172	0.9993
A		0.00468	-0.02884	0.9993

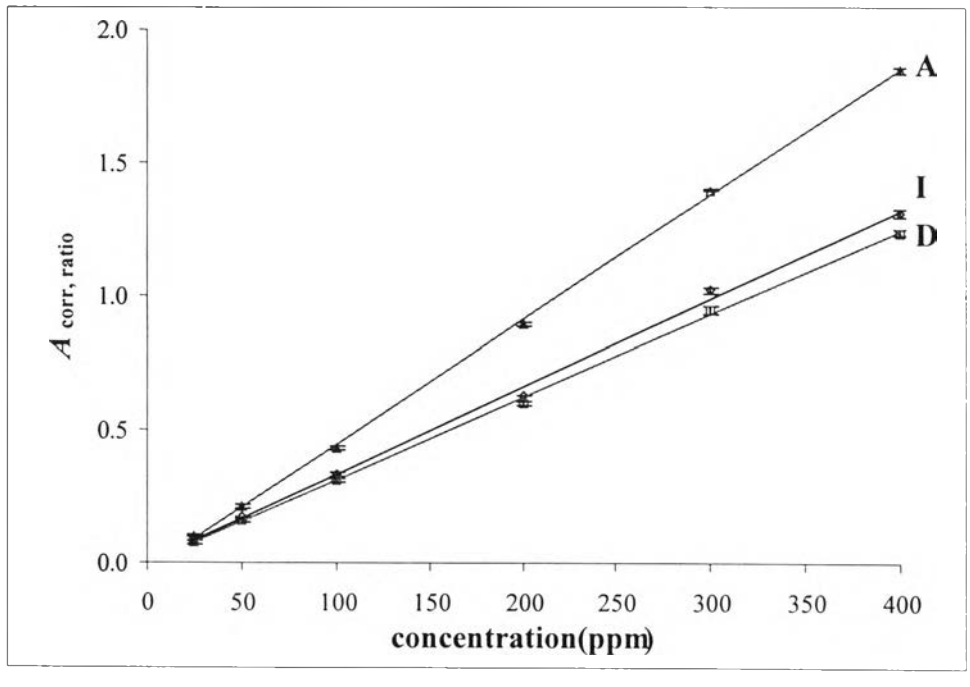

Figure 4.13 Calibration plots between $A_{corr,ratio}$ and the concentration of ivermectin (I), doramectin (D) and abamectin B_1 (A). Each point was run in triplicate and with RSD < 4%.

4.3.3 Accuracy and precision

The effect of sample matrix on accuracy of the method was investigated by spiking a mixture of avermectin standards with known amounts in the microemulsion and separately spiking each avermectin with known amounts in the diluted solution of samples containing an interested avermectin. Each MEEKC experiment was carried out for five runs as previously mentioned in Section 3.4.3. The recovery of the amount of avermectins spiked is calculated using Equation 4.3.

$$\text{Recovery } (\%) = \frac{Q_{\text{determined}} - Q_{\text{sample}}}{Q_{\text{spiked}}} \times 100 \qquad (4.3)$$

where Q_{spiked}, Q_{sample}, and $Q_{\text{determined}}$ are the amount of spiked standard, the determined amount of analyte in the diluted sample solution before spiking standard, and the determined amount of analyte in the diluted sample after spiking standard, respectively. In the case of the matrix as the microemulsion, Q_{sample} is equal to zero.

Results in Table 4.3 show high accuracy of the method with the recoveries for spiked standards ranging from 98.7 to 103.4 % with RSD < 2.0 %. In addition, the sample matrix was found to give no effect on the accuracy and precision due to the similar range of the recovery and RSD for standards spiked in the microemulsion and diluted samples. The values of recovery and RSD for standards in this work are obtained to be in the acceptable range according to AOAC Peer-Verified methods, where acceptable AOAC recoveries are in a range of 93.2 to 106.8 % for 50 ppm, and 94.6 to 105.4 % for 200 ppm [Horwitz 1982, AOAC 1993].

Table 4.3 MEEKC analysis of the amounts of standard avermectins spiked in the microemulsion and the diluted solution of samples, $n = 5$ runs.

Matrix	Avermectins	Spiked (ppm)	Mean recovery (%)	RSD (%)
Microemulsion[a]	I	50	99.7	1.1
		200	99.0	1.3
	D	50	101.3	1.7
		200	98.7	1.1
	A	50	99.3	1.0
		200	99.6	1.3
A diluted solution of the sample[b]	I	50	99.2	1.4
		200	98.7	1.5
	D	50	103.4	1.3
		200	100.4	1.8
	A	50	99.2	1.0
		200	102.6	1.4

[a]Microemulsion contained 180 mM SDS, 1,1 % v/v *n*-octane, 890 mM 1-butanol, 30 % v/v ethanol and water.
[b]An appropriate amount of the sample was diluted with microemulsion.

The intraday and interday precision in migration time (t_m) and $A_{corr,ratio}$ was determined using a same batch of 100 ppm avermectin standard each. For the intraday precision, the values of the mean and RSD were obtained from five separate runs each day, while five days for the interday precision. Results in Table 4.4 indicate high precision in t_m and $A_{corr,ratio}$ for the intraday, with RSD < 2.0 %. In order to achieve RSD for interday precision, treating all $A_{corr,ratio}$ data, obtained from 5 days analyses, as one large sample can be done due to non-significant result of variance, calculated from single factor ANOVA [Miller *et al.* 2000]. On the other hand, the single factor ANOVA test result of t_m for 5 days gives significant difference of variance, and therefore, RSD for interday precision is obtained using the estimation of variance as described in [Miller *et al.* 2000: pp. 77-78].

Table 4.4 Intraday and interday precision in migration time and $A_{corr,ratio}$ of 100 ppm avermectin each.

Precision		RSD (%) and mean of t_m (min)			RSD (%) and mean of $A_{corr,ratio}$		
		I	D	A	I	D	A
[a]Intraday	Day 1	0.7 (18.07)	0.7 (18.77)	0.8 (19.42)	2.0 (0.341)	1.9 (0.319)	0.5 (0.437)
	Day 2	1.2 (17.54)	1.3 (18.20)	1.3 (18.82)	1.8 (0.339)	1.0 (0.313)	0.7 (0.434)
	Day 3	0.4 (17.59)	0.4 (18.26)	0.4 (18.88)	1.8 (0.337)	1.9 (0.313)	0.9 (0.435)
	Day 4	0.9 (18.44)	1.0 (19.17)	1.1 (19.85)	0.6 (0.346)	0.3 (0.313)	1.2 (0.432)
	Day 5	0.7 (18.52)	0.7 (19.26)	0.8 (19.95)	1.2 (0.345)	1.3 (0.316)	1.4 (0.437)
[b]Interday	Overall	2.5 (18.03)	2.6 (18.73)	2.7 (19.38)	1.8 (0.342)	1.5 (0.315)	1.0 (0.435)

The values of mean are in parentheses. [a]n = 5 runs for each day and [b]n = 5 days.

The values of RSD for interday precision were found to be ≤ 2.7 % for t_m, and ≤ 1.8 % for $A_{corr,ratio}$, indicating high interday precision, which less than the acceptable RSD of 5.3% for 100 ppm analyte [Horwitz 1982, AOAC 1993]. Slight poorer precision in t_m for intraday than interday was observed possibly due to variation in chemical properties of capillary wall surface each day, resulting in a change in electroosmotic flow and migration time of analytes. In addition, the high precision in $A_{corr,ratio}$ for intraday and interday indicate high stability of sample solutions stored for five days, which is consistent with stability test in Section 4.1.

4.4 Application to Real Samples

Five samples were used in this work such as abamectin formulations (A1, A2 and A3), ivermectin (I1) and doramectin (D1) formulations using MEEKC and HPLC. The procedure for sample preparation and quantitative analysis are mentioned in Section 3.6. Figures 4.14 and 4.15 show MEEKC electropherograms and HPLC chromatograms, respectively. Peaks of analytes in samples were identified by comparing their UV spectra in a range of 190 to 400 nm with the UV spectra of standards and using a spiking technique. From the electrophoregram of UV detection at 214 nm as shown in Fig. 4.14a, several peaks of highly hydrophobic compounds in abamectin formulations A1, A2 and A3 were observed to overlap and to be near the

DB peak. This is a reason why direct HPLC analysis of avermectin formulations requires long time to remove these compounds from an HPLC column. It should be noted that the migration time order of avermectins in MEEKC is reversed to the retention time order in HPLC. This is because the analyte with higher retention factor has the faster migration time in MEEKC with suppressed EOF but the longer retention time in reversed phase HPLC. The better resolution of analytes in HPLC than MEEKC was found because the higher amounts of organic solvents with elution gradient (60 to 90 % v/v) were used in HPLC, while 30 % v/v ethanol in MEEKC.

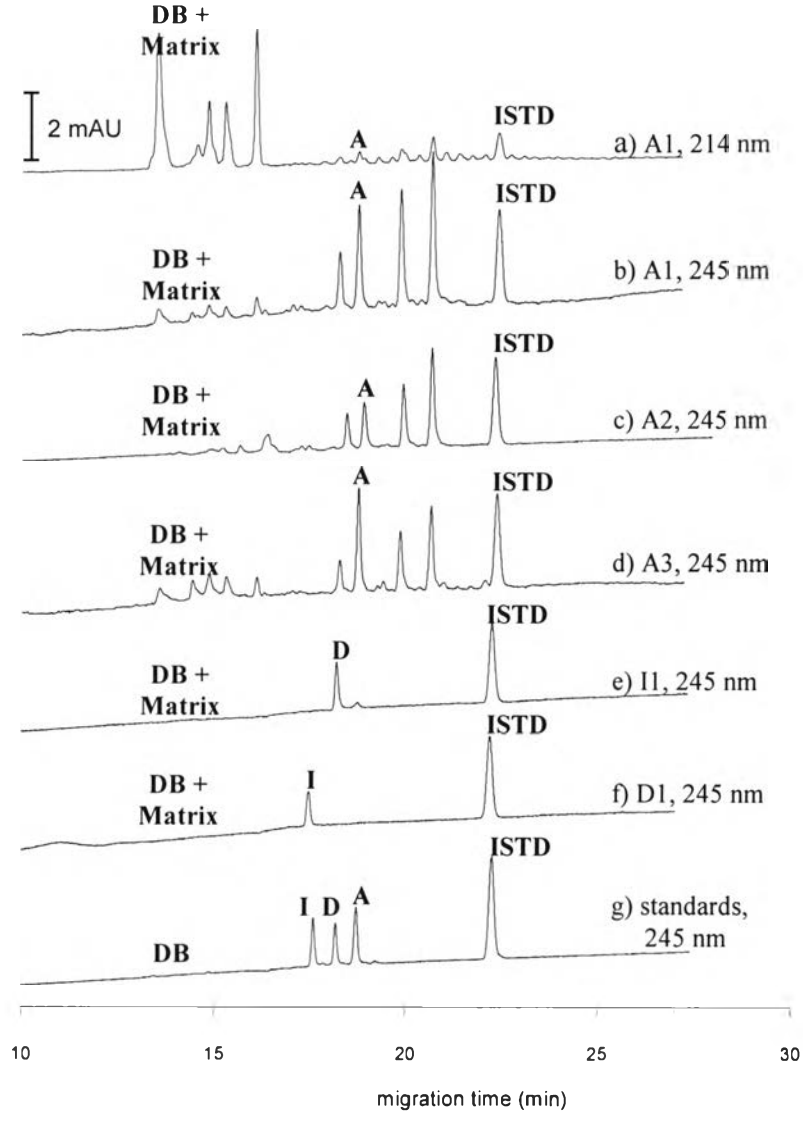

Figure 4.14 An example of electropherograms of avermectins formulations of samples A1 (a) and (b), A2 (c), A3 (d), D1 (e), I1 (f) and avermectin standards (g). UV detection at 214 nm (a) and 245 nm (b-g). Other CE conditions as shown in Figure 4.2d.

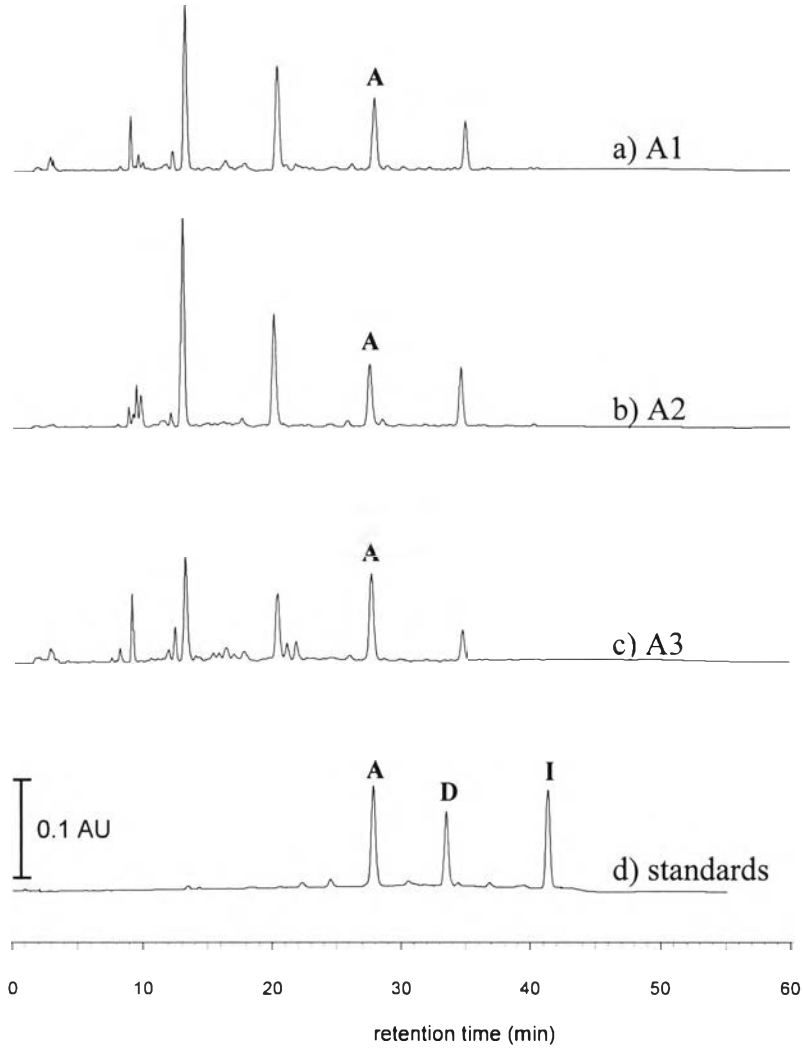

Figure 4.15 An example of HPLC chromatograms of (a) mixed standards and avermectins formulations of samples A1 (b), A2 (c) and A3 (d) using HPLC conditions as described in Section 3.5.1.

The amounts of individual avermectin in commercial formulations determined by MEEKC were obtained using calibration plots as previously mentioned in Section 4.3.2. For HPLC analysis, the calibration plots were established from the peak area of the analyte (y) against the analyte concentration (c) for six levels in a range of 10 to 100 ppm, giving linear equations $y = 19377c\text{-}14816$ with r^2 of 0.9992 for abamectin, $y = 14217c\text{-}17991$ with r^2 of 0.9987 for doramectin, and $y = 16446c\text{-}72765$ with r^2 of 0.9986 for ivermectin. It should be noted that internal standard is not used in HPLC analysis due to high precision in the amount of analyte injected by a fixed volume injection loop.

The percentage of weight by volume (%w/v) of individual avermectin in each product is obtained from Equation 4.4,

$$P(\%\text{w/v}) = \frac{f_d \times c(\text{ppm}) \times V(\text{l}) \times d(\text{g/ml})}{10 \times w(\text{g})} \qquad (4.4)$$

where w is the desired sampling weight of commercial formulations (mg), V is the initial volume of the sample solution (l), f_d is the dilution factor, c is the concentration of individual avermectin in the final solution (ppm) obtained from the calibration plots, and d is the density of each commercial formulation of avermectins (g/ml).

Table 4.5 lists a comparison of the contents of avermectins in commercial products determined by MEEKC and HPLC. Using paired t-test analysis at 95 % confidence interval of the mean, MEEKC and HPLC gives non-significant difference in the determined amount of each avermectin in each sample, which is obtained from the statistic t-test value less than the critical t-test value.

Good agreement was obtained for determined and labeled amounts of avermectins in each sample, except for Sample I1 where the determined amount of ivermectin was found to be approximately a half of the labeled amount. The experiment for sample I1 was repeated, and same results were obtained. This difference for sample I1 is not known. Moreover, the amounts of abamectin B_1 in all abamectin formulations were found to be in a range of 1.53 to 2.07 %w/v, which are acceptable according to the regulation of the Department of Agriculture, the Ministry of Agricultural and

Cooperatives. Therefore, MEEKC may be used to be an alternative method for quantitative determination of individual avermectin in commercial samples or formulations. Advantages of MEEKC over HPLC include fast analysis time and lower amount of organic solvent consumption.

Table 4.5 The amounts of avermectins in samples determined by MEEKC and HPLC.

Sample	Avermectins	Content (% w/v)			Paired *t*-test	
		Labeled	Determined		statistic	critical
			MEEKC	HPLC		
A1	A	1.8	1.70 ± 0.01	1.71 ± 0.01	0.67	2.78
A2	A	1.8	1.58 ± 0.01	1.56 ± 0.01		
A3	A	1.8	1.76 ± 0.01	1.77 ± 0.02		
D1	D	1.0	0.99 ± 0.01	0.98 ± 0.01		
I1	I	1.5	0.77 ± 0.01	0.76 ± 0.01		