

## CHAPTER IV

### RESULTS AND DISCUSSION

#### 4.1 Method optimization

##### 4.1.1 Types of organic extracting solvent

From flux equation in section 2.1.4, diffusion coefficient ( $D_i$ ) of the analyte in SLM is one of parameters that affect the flux of analyte, which is controlled by type of solvent used as the SLM [9]. The specific properties for solvent to enable electrokinetic migration are required. First, the organic solvent should be immiscible in water to prevent losing from the pores of the hollow fiber membrane and prevent dissolving in the donor solution during agitation [10]. Second, the polarity of solvent should be similar to polypropylene fiber for properly immobilization in the porous wall [52]. Third, the solvent should have higher boiling point to avoid losses during extraction by Joule heating, which is the result of high current flowing in the system leading to unstable EME system [53]. Fourth, the solvent should have high resistance to support a relatively low current flow in the system and to prevent bubble formation at the two electrodes [21]. In addition, it should have suitable viscosity that allows analyte to go through SLM easily.

Based on these considerations, four types of organic solvents including nitrophenyloctylether (NPOE), 1-octanol, 1-heptanol and 1-octanol/NPOE were studied based on their polarity and viscosity properties. Besides, addition of ionic carrier as anionic exchanger; i.e., methyltrioctylammonium chloride (Aliquat 336) in organic solvents were also studied.

The enrichment factors of Cr(VI) obtained from the electrical field assisted LPME are shown in Figure 4.1. 1-heptanol yielded slightly better enrichment factor compared to 1-octanol probably because 1-heptanol is slightly less viscous than 1-octanol. Surprisingly, NPOE, which had been working very well in the EME systems for organic acid or basic compounds, did not work for extraction of metal ions. The reason might be that NPOE is too viscous to restrict the ionic transfer. Moreover,

NPOE has higher log P value than 1-heptanol and 1-octanol. So, NPOE is highly hydrophobic that the ionic metal would hardly go through. Enrichment factors obtained from using 1-heptanol/Aliquat336 (90/10%), 1-octanol/Aliquat336 (90/10%) and 1-octanol/NPOE/Aliquat336 (90/5/5%) were considerably higher than those obtained from using pure organic solvent. 1-heptanol/Aliquat336 gave the highest enrichment factor; hence, it was chosen for the SLM. The concentration of Aliquat336 in 1-heptanol was studied in section 4.1.2.

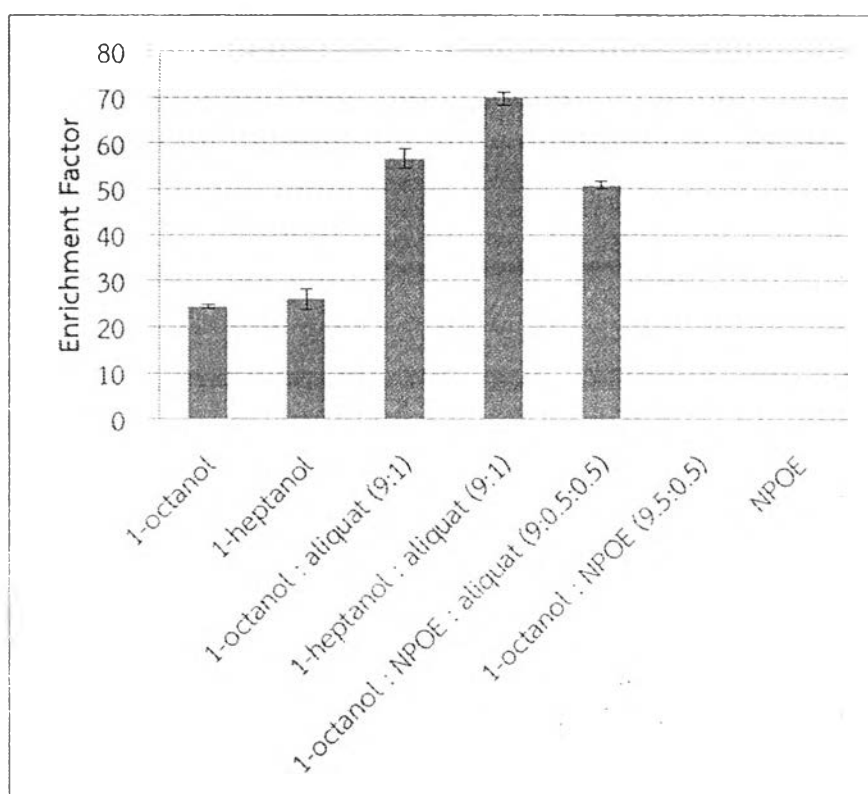


Figure 4.1 Effect of types of organic extracting solvent on enrichment factor for electrical field assisted LPME of Cr(VI) in aqueous donor solution ( $\text{Cr(VI)} 30 \mu\text{g L}^{-1}$ ;  $n=3$ ; acceptor solution: NaOH 0.5 M; extraction time: 5 min; applied voltage: 30 V; stirring speed: 1000 rpm)

Table 4.1 Properties of organic solvent

Solvent	Boiling point (°C)	Viscosity (mPa-s)	Dielectric constant	Log P (hydrophobicity)	Dipole moment (Debye)
1-heptanol	176.45 <sup>A</sup>	5.81 <sup>A</sup>	11.75 <sup>A</sup>	2.62 <sup>A</sup>	1.71 <sup>B</sup>
1-octanol	195.16 <sup>A</sup>	7.29 <sup>A</sup>	10.30 <sup>A</sup>	3.07 <sup>A</sup>	1.80 <sup>A</sup>
NPOE	198 <sup>C</sup>	13.8 <sup>D</sup>	24.0 <sup>E</sup>	5.80 <sup>E</sup>	4.33 <sup>F</sup>
Aliquat336	225 <sup>G</sup>	1450 <sup>H</sup>	-	5.33 <sup>B</sup>	-

References A: [54], B: [55], C: [56], D: [57], E: [58], E: [59], F: [60], G: [61], H: [62]

#### 4.1.2 Concentration of Aliquat336

Aliquat336 was orientated in the electrical field with partly ionized ammonium group direct towards the aqueous sample-membrane interface and the hydrophobic chains into the SLM. Ion-pairing formation occurs at these positive sites with anionic metal that facilitates the metal entrance into the SLM; therefore, it promotes their transports into the organic phase [9].

Since addition of Aliquat336 in organic solvent offered improvement in EME efficiency, concentrations of Aliquat336 in organic solvent at various ratios; i.e., 0, 1, 5, 10 and 20% (v/v) were optimized. Figure 4.2 illustrated that the enrichment factors of Cr(VI) increased with increased concentrations of Aliquat336 in 1-heptanol from 0 to 1% (v/v) and were steady at the concentrations of Aliquat336 in 1-heptanol from 1 to 5% (v/v). Nevertheless, the enrichment factor decreased when the concentrations of Aliquat336 in 1-heptanol were more than 5%. It appears that increasing in Aliquat336 increases its viscosity; consequently the ionic transfer might have been impeded. Furthermore, addition of high concentration of Aliquat336 leads to decrease the electrical resistance in SLM and increase the current flow in the system that may generate Joule heating resulting in unsatisfactory extraction efficiency. Moreover, Aliquat336 must provide excessive active sites for exchanging

with the Cr(VI) ion at the working concentration range. Thus, 5% Aliquat336 in 1-heptanol was chosen as the SLM.

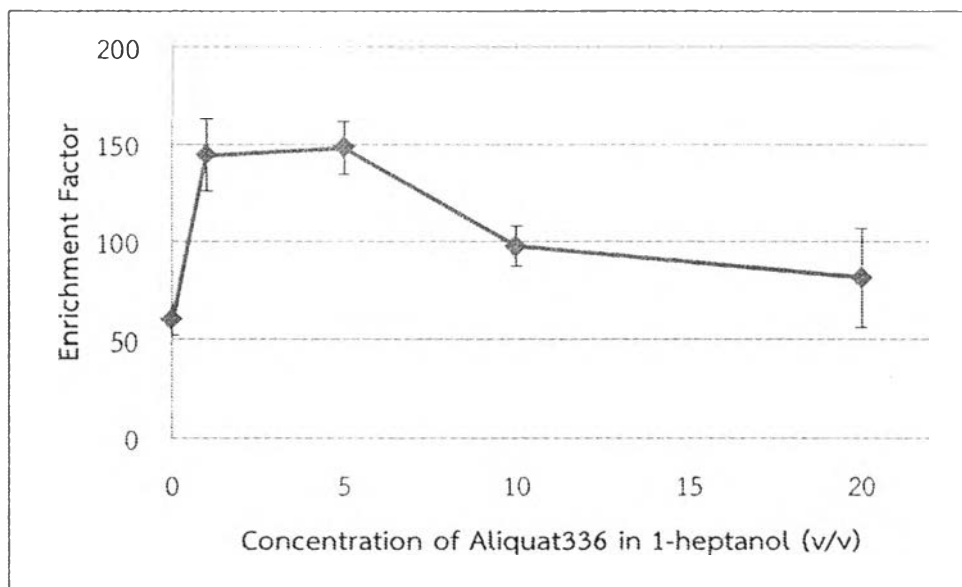


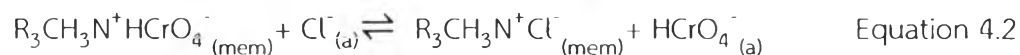
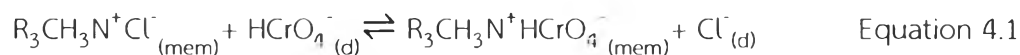
Figure 4.2 Effect of concentration of Aliquat336 in 1-heptanol on enrichment factor for electrical field assisted LPME of Cr(VI) in aqueous donor solution. (Cr(VI)  $10 \mu\text{g L}^{-1}$ ;  $n=3$ ; acceptor solution: NaOH 0.5 M; extraction time: 5 min; applied voltage: 30 V; stirring speed: 1000 rpm)

#### 4.1.3 Type and concentration of acceptor solution

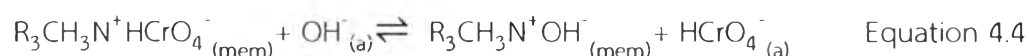
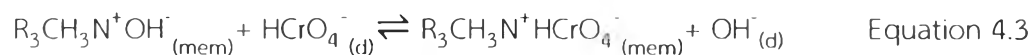
Since the transfer of Cr(VI) was based on ion-exchange mechanism, the driving force of mass transfer through SLM could be promoted by the gradient of counter ion from the acceptor to the donor phase [63]. Therefore, concentrations and type of acceptor solution were studied for high extraction efficiency.

Types of acceptor solution studied in this work were NaOH (pH 13) and NaCl (pH 7). As seen in Figure 4.3, using NaOH as the acceptor solution gave higher enrichment factor for extraction of Cr(VI) than using NaCl. The reason might be the disturbance of chemical equilibrium. The extraction equilibria were shown below.

In case of NaCl as acceptor (pH 7)



In case of NaOH as acceptor (pH 13)



Where  $R_3CH_3N^+Cl^-$  represents the Aliquat 336 and subscripts  $_{(mem)}$ ,  $_{(d)}$ ,  $_{(a)}$  indicate the location of the species in membrane, donor solution and acceptor solution, respectively.

In the case of NaOH as the acceptor solution, where the pH was 13,  $HCrO_4^-$  was completely turned to  $CrO_4^{2-}$  as seen in Equation 4.5 and the equilibrium of Equation 4.4 was driven forward resulting in the more  $HCrO_4^-$  being transferred from the donor solution [64].



In the case of NaCl as the acceptor solution, where the pH was 7,  $HCrO_4^-$  was not completely turned to  $CrO_4^{2-}$  because the pH of the acceptor solution was around pKa of  $HCrO_4^-$  (Equation 4.6). Thus, the equilibrium of Equation 4.2 in NaCl was driven forward less than in NaOH as the acceptor solution.

Then NaOH was chosen as the acceptor solution. NaOH with various concentrations from 0 M to 1.5 M were studied. According to Figure 4.4, no extraction occurred at 0 M NaOH. The enrichment factor increased as increased NaOH concentrations in the acceptor phase. However, at the concentration of NaOH more than 1.0 M, the enrichment factor was slightly dropped. The saturation of the analyte and hydroxide ions in the acceptor solution may lead to mass transfer resistance causing boundary layer of ions at the interface of the acceptor solution and the SLM resulting in back-extraction based on passive transport [19].

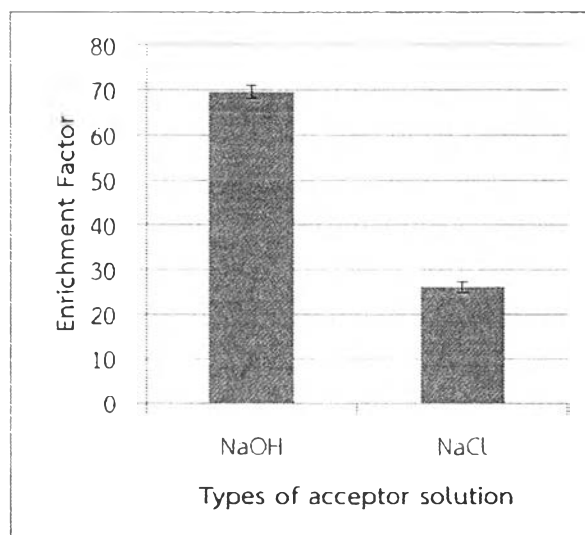


Figure 4.3 Effect of types of counter ion in acceptor solution on enrichment factor for electrical field assisted LPME of Cr(VI) in aqueous donor solution. (Cr(VI)  $10 \mu\text{g L}^{-1}$ ;  $n=3$ ; organic membrane: 5% Aliquat336 in 1-heptanol; extraction time: 5 min; applied voltage: 30 V; stirring speed: 1000 rpm)

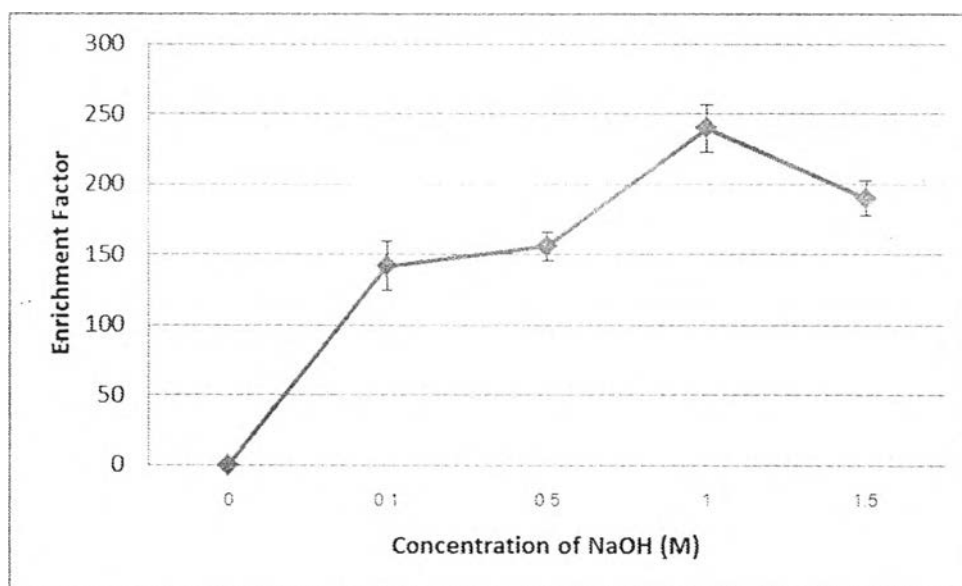


Figure 4.4 Effect of Concentration of NaOH on enrichment factor for electrical field assisted LPME of Cr(VI) in aqueous donor solution. (Cr(VI)  $10 \mu\text{g L}^{-1}$ ;  $n=3$ ; organic membrane: 5% Aliquat336 in 1-heptanol; extraction time: 5 min; applied voltage: 30 V; stirring speed: 1000 rpm)

#### 4.1.4 Extraction time and applied voltage

The electrical field promotes the EME process in two important ways; first is enhancing the analyte distribution over the aqueous sample-membrane interface and second is improving mass transfer of the analyte across the SLM by electrokinetic migration, which could be described by Nernst-Planck flux equation [41] that described in section 2.1.4 [65].

In this work, applied voltages and extraction times were studied simultaneously. Due to reducing energy consumption and safety reason, voltages in the lower range were studied. Thus, the voltage applied across the SLM was optimized in the range of 0-50 volt and the extraction time was varied from 1-30 min.

Figure 4.5 shows that the enrichment factor of Cr(VI) increased considerably with increased voltages and reached highest at 50 volt. In addition, the enrichment factor of Cr(VI) at all voltages increased with increased extraction times but after reaching equilibrium, the enrichment factors decreased gradually. There are two assumptions that may be the reason why the enrichment factor declined moderately at the longer extraction time. One is the saturation of the analyte in the acceptor phase that could lead to mass transfer resistance causing boundary layer of ions at the interfaces of the acceptor solution and SLM resulting in back-extraction based on passive transport. Another reason is the high current level that could generate heat due to Joule heating and cause electrolysis occurring at the electrode resulting in losses of solvent and acceptor solution due to heat and bubble formation at the surface of electrodes, respectively [8, 11, 19, 31].

For the relation between voltage and time, at 0 and 10 volt, EF of Cr(VI) went up gradually and reached equilibrium at 15 min whereas at 20, 30 and 50 volt, EF rose dramatically and reached equilibrium at 10 min. The result showed that extraction at lower voltage took longer time to reach equilibrium. Even though, 10 min extraction time yielded the highest sensitivity and enrichment factor but the repeatability was not so good for extraction in real samples. Accordingly, extraction

time of 5 min, which provided limit of detection (LOD) below the MCL of Cr(VI) recommended by WHO was chosen for extraction time with satisfactory precision.

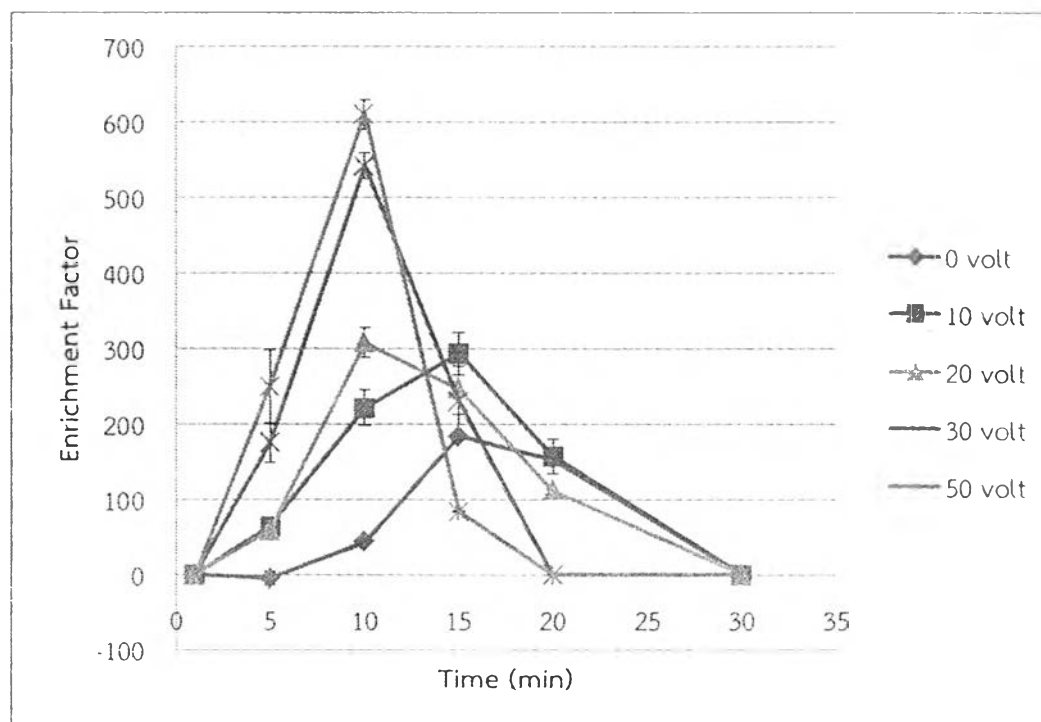


Figure 4.5 Effect of extraction time and applied voltage on enrichment factor for electrical field assisted LPME of Cr(VI) in aqueous donor solution. (Cr(VI)  $5 \mu\text{g L}^{-1}$ ;  $n=3$ ; organic membrane: 5% Aliquat336 in 1-heptanol; acceptor solution: NaOH 0.5 M; stirring speed: 1000 rpm)

#### 4.1.5 Stirring speed

The mass transfer of analyte could be enhanced by stirring in the donor phase during the extraction process to improve the physical movement of the ionic analyte in the bulk solution and to decrease the thickness of the boundary layer at the interface between the donor phase and the SLM. In this work, the stirring rates in the donor phase were studied in the range of 0-1000 rpm.

As seen in Figure 4.6, the enrichment factor went up dramatically with increased stirring rates from 0 rpm to 100 rpm then remained steady after 100 rpm to 500 rpm and decreased slowly at higher stirring rates after 500 rpm. At the high



stirring rate, the magnetic bar moved vigorously and sometimes hit the fiber that affect precision of the extraction because the acceptor solution may be lost during the extraction. The enrichment factor in the stagnant sample solution (0 rpm) system was around 350, which was a result of only electrokinetic migration. The enrichment factor in the agitated solution was around 650, which was a result of both electrokinetic migration and convection. The results showed that the stirring could potentially enhance the extraction efficiency. Hence, the stirring rate of 500 rpm was chosen for the experiments providing satisfactory repeatability.

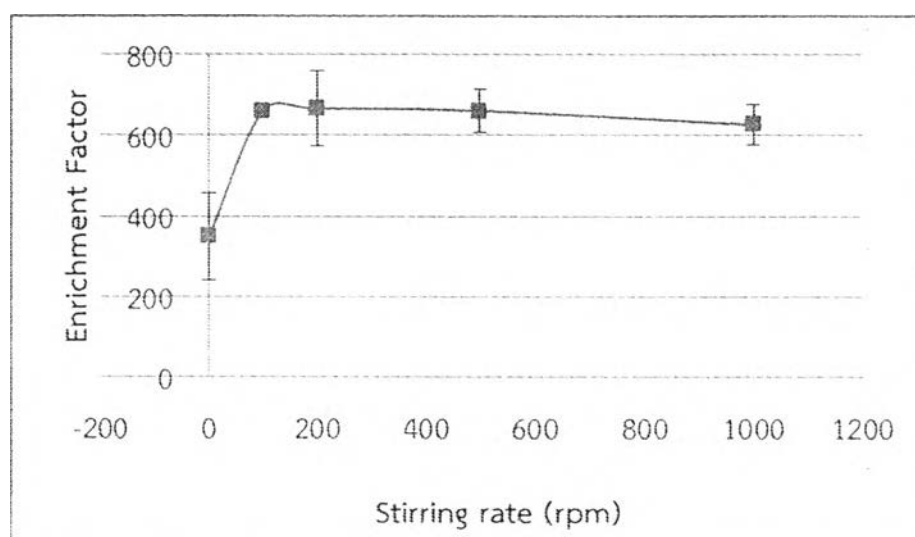


Figure 4.6 Effect of stirring speed on enrichment factor for electrical field assisted LPME of Cr(VI) in aqueous donor solution. (Cr(VI)  $3 \mu\text{g L}^{-1}$ ;  $n=3$ ; organic membrane: 5% Aliquat336 in 1-heptanol; acceptor solution: NaOH 1.0 M; extraction time: 10 min; applied voltage: 30 V)

The optimal parameters of electrical field assisted LPME for extraction and determination of Cr(VI) ion are summarized in Table 4.1. The total analysis time was about 10 minutes per 1 sample.

Table 4.2 Optimum conditions of electrical field assisted LPME for determination of chromate ion

Parameters	Optimum
Length of hollow fiber membrane	8 cm
Extracting solvent (organic membrane)	5% (v/v) Aliquat336 in 1-heptanol
Acceptor solution	1.0 M NaOH
Extraction time	5 min
Applied voltage	30 V
Sample volume	28 mL
Stirring rate	500 rpm
Total analysis time	10 min

## 4.2 Method evaluation

### 4.2.1 Evaluation of extraction in pure milli Q water

The method was evaluated with respect to parameters including linearity, precision, accuracy, limits of detection (LOD), recovery and matrix effect.

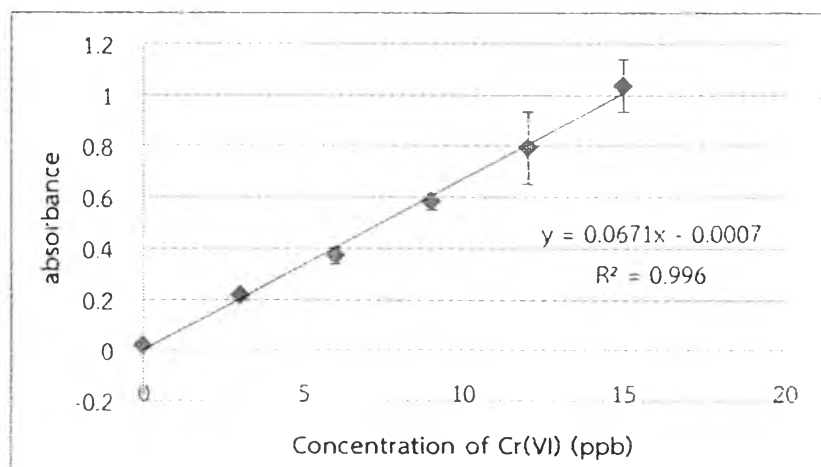


Figure 4.7 Working range of Cr(VI) determined by electrical field assisted LPME in milli Q water

The calibration curve for determination of Cr(VI) was prepared based on extraction of Cr(VI) spiked milli Q water. The linear working range was established in the range of 3 to 15  $\mu\text{g L}^{-1}$ . From Figure 4.7, good linearity was obtained with coefficient of determination ( $R^2$ ) = 0.9960. The linear regression equation was  $y = 0.0671x - 0.0007$ .

Repeatability or intra-day precision of the method was investigated by replicate extraction and determination of spiked Cr(VI) at 10  $\mu\text{g L}^{-1}$  in milli Q water in the same day under the optimal conditions. Reproducibility or inter-day precision of the method was assessed by replicate extraction and determination of spiked Cr(VI) at 10  $\mu\text{g L}^{-1}$  in milli Q water for five consecutive days under the optimal conditions. The relative standard deviations are shown in Table 4.3. It indicates that the method provides acceptable precision [66].

Table 4.3 Analytical merits of electrical field assisted LPME of Cr(VI) spiked milli Q water

Method evaluation	Milli Q water
Linear equation	$y = 0.0671x - 0.0007$
Correlation coefficient ( $R^2$ )	0.9960
Enrichment factor (EF)	216
LOD ( $\mu\text{g L}^{-1}$ )	1.4
Precision	
%RSD Intra-day (n=3)	4
%RSD Inter-day (n=5)	12

Furthermore, the enrichment factor of the method for extraction of Cr(VI) was approximately 216 (Table 4.3), calculated based on the ratio of the final concentration of Cr(VI) in the acceptor phase to the initial concentration of Cr(VI) in the donor solution (Equation 3.4).

#### 4.2.2 Matrix effect

The effect of other ions that may interfere the extraction efficiency was investigated. There were three common species including  $\text{Cl}^-$ ,  $\text{SO}_4^{2-}$  and  $\text{HPO}_4^{2-}$  that were studied under the optimized conditions. Cr(VI) solutions of  $3 \mu\text{g L}^{-1}$  were mixed with each species at various concentrations from 0 to  $30,000 \mu\text{g L}^{-1}$  and proceeded the electrical field assisted LPME.

As illustrated in Figure 4.8, the EF of Cr(VI) were not different in the presence of the common ions at the concentrations ranging from 0 to  $300 \mu\text{g L}^{-1}$ . The EF went down significantly in the presence of the common ions at the concentrations more than  $300 \mu\text{g L}^{-1}$  and Cr(VI) was not detected at the concentrations of the common ions greater than  $30,000 \mu\text{g L}^{-1}$ . The results showed that tolerances of common ions for the extraction of Cr(VI) were in the range  $0.03 - 300 \mu\text{g L}^{-1}$  for  $\text{Cl}^-$  and in the range of  $0.03 - 3 \mu\text{g L}^{-1}$  for  $\text{SO}_4^{2-}$  and  $\text{HPO}_4^{2-}$ .

In addition, the presence of ionic salts in high concentration in the donor solution could lead to bubble formation at the electrodes during extraction affecting the stability of the system. There may be due to two assumptions. One is that the high concentration of ionic salts; i.e., NaCl could increase the conductance of the donor solution and allow the electrolysis to occur at the electrodes. The other is high concentration of ionic salts led to high ion balance ( $\chi$ ) shown in equation 2.14 in section 2.1.4), which is the ratio of total ionic concentration in the donor solution to that in the acceptor solution and led to low flux of analyte across the SLM into acceptor phase [52].

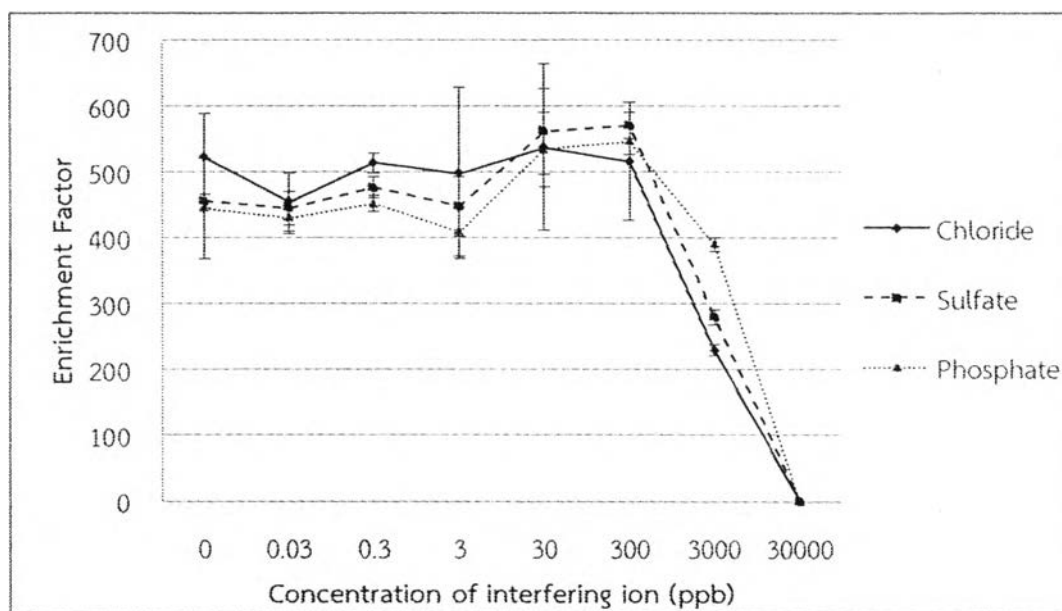


Figure 4.8 Effect of concentration of interfering ion ( $\text{Cl}^-$ ,  $\text{SO}_4^{2-}$  and  $\text{HPO}_4^{2-}$ ) on enrichment factor for electrical field assisted LPME of Cr(VI) in aqueous donor solution. (Cr(VI)  $3 \mu\text{g L}^{-1}$ ;  $n=3$ ; organic membrane: 5% Aliquat336 in 1-heptanol; acceptor solution: NaOH 1.0 M; extraction time: 10 min; applied voltage 30 V; stirring rate: 500 rpm)

#### 4.2.3 Evaluation of extraction in water samples

The proposed method was applied to CU pond water and four drinking waters; drinking water A, B, C and D.

Calibration curves were established in drinking waters A and B in the range of 3 to  $15 \mu\text{g L}^{-1}$ . The results are summarized in Table 4.4. According to ANOVA, the slopes of the linear equations obtained using drinking water were not significantly different from the slope obtained using milli Q water (see Appendix Figure A.1-A.2, Table A.3-A.5). It indicates that matrix in drinking water A and B do not affect the extraction efficiency. Good linearity was obtained with coefficient of determination ( $R^2$ ).

The relative standard deviations of both drinking waters were in the acceptable range recommended by AOAC International [66]. The enrichment factor

obtained from extraction of drinking water A and B were similar to that of milli Q water.

Moreover, the accuracy was investigated by recovery study. The recovery was determined by the analysis of spiked drinking water sample containing known amounts of Cr(VI) at  $10 \mu\text{g L}^{-1}$ . The recoveries obtained from drinking water A and B shown in Table 4.4 were in satisfactory values recommended by AOAC International [66] indicating that the method provides good accuracy.

Drinking water C, drinking water D and CU pond were extracted by the electrical field assisted LPME method. All of these samples encountered extraction problems. The common problem was a bubble formation during extraction. It was expected that there were lots of ionic ions acted as an electrolyte in such samples that could cause high current across the liquid membrane resulting in electrolysis to occur at the electrodes as described in section 4.2.2.

From the different results, they were divided into two groups; first is promising extraction which the samples are drinking water A and B. The other group is unworked extraction that the samples are drinking water C, D and CU pond water. The difference of these two group samples is conductance of each sample. The first group has low conductivity. The other has high conductivity. Table 4.5 showed the conductivity determined in such samples. It may suggest that samples with high conductivity may cause a problem with the electrical field assisted LPME method. Further investigation may be needed.

Table 4.4 Analytical merits of electrical field assisted LPME of Cr(VI) spiked samples

Method evaluation	Drinking water A	Drinking water B	Drinking water C	Drinking water D	CU pond water
Linear equation	$y = 0.0708x + 0.0149$	$y = 0.0579x + 0.0269$	N/D	N/D	N/D
Correlation coefficient ( $R^2$ )	0.9809	0.9946	N/D	N/D	N/D
Enrichment factor (EF)	185	220	N/D	N/D	N/D
RSD (%), (n=3)	< 14.4	< 13.9	N/D	N/D	N/D
LOD ( $\mu\text{g L}^{-1}$ )	3.5	1.0	N/D	N/D	N/D
%Recovery (spiked 10 $\mu\text{g L}^{-1}$ )	104.3%	86.7%	N/D	N/D	N/D

Table 4.5 Conductivity ( $\mu\text{s cm}^{-1}$ ) of sample solution

Sample solution	Conductivity ( $\mu\text{s cm}^{-1}$ )
Milli Q	2.70-3.00
Drinking water 1	19.3-19.4
Drinking water 2	20.8-21.1
Drinking water 3	640-643
Drinking water 4	302-304
CU pond	476-477

#### 4.2.4 Comparison between the proposed method and the standard method (ICP-OES) for extraction and determination of Cr(VI)

The method for determination of Cr(VI) in drinking water samples were compared between our proposed method and the standard method (U.S. EPA method 200.7) using inductively coupled plasma optical emission spectrometry (ICP-OES). The drinking water samples were purchased from local markets in Bangkok. Cr(VI) was spiked to the samples at  $50 \mu\text{g L}^{-1}$ . The results are shown in Table 4.6. According to the t-test, there were no significant differences observed between the results obtained from both methods ( $P > 0.05$ ) (see Appendix Table A.1-A.2).

Table 4.6 Comparison of the amount of Cr(VI) in drinking water samples determined by the proposed method and the standard method (U.S. EPA method 200.7)

Sample	Spiked Cr(VI) $50 \mu\text{g L}^{-1}$	
	Proposed method	ICP-OES (U.S.EPA method 200.7)
	Found Cr(VI) $\mu\text{g L}^{-1} \pm \text{SD}$ (n=3)	Found Cr(VI) $\mu\text{g L}^{-1} \pm \text{SD}$ (n=3)
Drinking water A	$54.0 \pm 6.0$	$58.5 \pm 0.1$
Drinking water B	$56.5 \pm 2.9$	$59.6 \pm 1.4$