CHAPTER III

RESULTS AND DISCUSSION

3.1 Preliminary Studies of Spectrophotometric Determination of Selenium by Using Variamine Blue as Complexing Agent

3.1.1 Absorption spectra

The absorption spectra of VB, Se(IV)-HCl-KI, Se(IV)-HCl-KI-VB complexes against water were scanned over a range from 300 – 800 nm, using JENWAY 6400 spectrophotometer in conjunction with the laboratory developed software connected to laptop computer as shown in Figure 3.1.

VB in 30% (v/v) ethanol, Se(IV)-HCl-KI and Se(IV)-HCl-KI-VB give pale blue, yellow and purple colors respectively and the complex between Se(IV)-HCl-KI-VB showed a maximum absorbance at 546 nm.



Figure 3.1 Absorption Spectra of VB, Se(IV)-HCl-KI, Se(IV)-HCl-KI-VB complexes against water

3.1.2 Chemical reaction

This method was based on reaction between selenium(IV) in acid solution and potassium iodide solution. Se(IV) ion was reduced by iodide ion to Se^0 and iodine. Then the liberated iodine oxidized Variamine Blue selectively in the presence of sodium acetate to form purple-colored complex product. The maximum absorbance of the product was measured at 546 nm.

$$SeO_{3}^{2-} + 4I^{-} + 6H^{+} \longrightarrow Se^{0} + 2I_{2} + 3H_{2}O$$

$$I_{2} + CH_{3}O \longrightarrow NH \longrightarrow NH_{2} \longrightarrow CH_{3}O \longrightarrow NH_{2}^{+}$$



3.2 FIA Spectrophotometric Determination of Selenium Using Variamine Blue as Complexing Agent

3.2.1 Optimization of the Flow System by Univariate Method

The conditions for the determination of selenium were optimized by studying the influences of the various parameters, such as wavelength (nm), concentration of KI (% w/v), ethanol (% v/v), concentration of VB (mg L⁻¹), concentration of HCl (mol L⁻¹), flow rate (mL min⁻¹), reaction coil(I), reaction coil(II) and sample injection loop(μ L), respectively. The optimum conditions obtained by means of the univariate optimization procedure (changing one variable in turn and keeping the others at their optimum values). To optimize the conditions, the FIA manifold in Figure 2.1 and the preliminary experimental conditions (Table 2.2) were used.

3.2.1.1 Optimum wavelength

The optimum wavelength for selenium determination was studied over the range 537 - 555 nm by the proposed FIA system (Figure 2.3) using the experimental conditions as shown in Table 2.2. The results shown in Table 3.1 and Figure 3.3 indicated that the highest Δ peak height (Δ P.H.) was obtained when the absorbance was measured at 546 nm. Δ Peak height (Δ P.H.) was the difference between peak height of blank and peak height of analyte. The analytical wavelength at 546 nm was selected for the further studies because of its highest sensitivity (defined as slope of calibration graph) with the reasonable correlation coefficient (r^2).

$\Delta P.H.*$ (the stand		(AU) obtai lard Se(IV	ned from) (mg L ⁻¹)	$\mathbf{v} = \mathbf{m}\mathbf{x} + \mathbf{c}$	r^2
(nm)	0.10	0.20	0.30		
537	0.0224	0.0407	0.0587	y = 0.0181x + 0.0043	0.9999
540	0.0191	0.0387	0.0558	y = 0.0183x + 0.0120	0.998
543	0.0197	0.0413	0.0571	y = 0.0186x + 0.0200	0.991
546	0.0210	0.0426	0.0614	y = 0.0201x + 0.0135	0.998
549	0.0200	0.0410	0.0569	y = 0.0184x + 0.0024	0.993
552	0.0224	0.0430	0.0593	y = 0.0182x + 0.0476	0.995
555	0.0199	0.0250	0.0559	y = 0.0179x + 0.0170	0.999

Table 3.1 Peak heights at various wavelengths



Figure 3.3 ΔPeak heights at various wavelengths

3.2.1.2 Effect of potassium iodide concentration

Effect of KI concentrations on the determination of Se(IV) $(0.10 - 0.90 \text{ mg L}^{-1})$ was studied over the range 1.0 - 4.5% (w/v) which was mixed with selenium solution. The results obtained are shown in Table 3.2 and Figure 3.4. The results indicated that the sensitivity increases with increases in the potassium iodide concentration and reaches the maximum value when the potassium iodide concentration is 3.0% (w/v) when the concentration of potassium iodide below 2.5 or above 3.5% (w/v) the sensitivity (slope of the calibration curve) decreased significantly. So, 3.0% (w/v) was chosen because it provided the greatest sensitivity.

	$\Delta P.H.*$ (the stand	AU) obtai ard Se(IV	ned from) (mg L ⁻¹)		y = mx + c	r ²	
0.10	0.30	0.50	0.70	0.90			
0.0098	0.0114	0.0134	0.0144	0.0164	y = 0.0081x + 0.0090	0.9923	
0.0134	0.0160	0.0174	0.0198	0.0210	y = 0.0095x + 0.0128	0.9861	
0.0164	0.0186	0.0230	0.0260	0.0294	y = 0.0167x + 0.0143	0.9928	
0.0166	0.0200	0.0244	0.0286	0.0314	y = 0.0191x + 0.0147	0.9951	
0.0104	0.0134	0.0182	0.0234	0.0290	y = 0.0240x + 0.0068	0.9924	
0.0164	0.0194	0.0236	0.0292	0.0324	y = 0.0209x + 0.0138	0.9900	
0.0220	0.0250	0.0288	0.0324	0.0364	y = 0.0181x + 0.0199	0.9978	
0.0214	0.0232	0.0270	0.0314	0.0334	y = 0.0161x + 0.0192	0.9807	
	0.10 0.0098 0.0134 0.0164 0.0166 0.0104 0.0164 0.0220 0.0214	AP.H.* (the stand0.100.300.00980.01140.01340.01600.01640.01860.01660.02000.01040.01340.01640.01940.02200.02500.02140.0232	AP.H.* (AU) obtain the stand-WI Set(IV)0.100.300.500.00980.01140.01340.01340.01600.01740.01640.01860.02300.01660.02000.02440.01040.01340.01820.01640.01940.02360.02200.02500.02880.02140.02320.0270	AP.H.* (J. obtained from the standwork Se(IV) (mg L ⁻¹)0.100.300.500.700.00980.01140.01340.01440.01340.01600.01740.01980.01640.01860.02300.02600.01660.02000.02440.02860.01040.01340.01820.02340.01640.01940.02360.02920.02200.02500.02880.03240.02140.02320.02700.0314	AP.H.* (AU) obtained from the standword Se(IV) (mg L ⁻¹)0.100.300.500.700.900.00980.01140.01340.01440.01640.01340.01600.01740.01980.02100.01640.01860.02300.02600.02940.01660.02000.02440.02360.02140.01040.01340.01820.02340.02900.01640.01940.02360.02920.03240.02200.02500.02880.03240.03640.02140.02320.02700.03140.0334	AP.H.* (AU) obtained from the stand=Y Se(IV) (mg L^{-1}) $y = mx + c$ 0.100.300.500.700.900.00980.01140.01340.01440.0164 $y = 0.0081x + 0.0090$ 0.01340.01600.01740.01980.0210 $y = 0.0095x + 0.0128$ 0.01640.01860.02300.02600.0294 $y = 0.0167x + 0.0143$ 0.01660.02000.02440.02860.0314 $y = 0.0191x + 0.0147$ 0.01040.01340.01820.02340.0290 $y = 0.0240x + 0.0068$ 0.01640.01940.02360.02920.0324 $y = 0.0181x + 0.0199$ 0.02140.02320.02700.03140.0334 $y = 0.0161x + 0.0192$	

 Table 3.2
 Effect of KI concentrations on the sensitivity

*average of triplicate results



Figure 3.4 Relationship between concentrations of KI and sensitivity for Se(IV) determination $(0.10 - 0.90 \text{ mg L}^{-1})$

3.2.1.3 Effect of hydrochloric acid concentration

Effect of HCl concentrations in the standard and/or sample solution containing Se(IV) on the determination of Se(IV) $(0.10 - 0.90 \text{ mg L}^{-1})$ was studied over the range $0.1 - 0.6 \text{ mol L}^{-1}$. The results are shown in Table 3.3 and Figure 3.5. It was found that sensitivity (defined as slope of calibration curve) increased very rapidly from the HCl concentration of 0.1 to 0.2 mol L⁻¹. After that, the sensitivities decreased rather rapidly. The results indicated that selectivity for selenium determination using Variamine Blue as reagent could be achieved in a 0.2 mol L⁻¹ hydrochloric acid solution.

 Table 3.3 Effect of HCl concentrations on the sensitivity

HCl concentration	t	ΔΡ.Η.* (A he standa	AU) obtai ard Se(IV	ined from 7) (mg L ⁻¹	n ¹)	y = mx + c	r ²
(mol L ⁻¹)	0.10	0.30	0.50	0.70	0.90		
0.1	0.0130	0.0148	0.0168	0.0190	0.0218	y = 0.0109x + 0.0116	0.9924
0.2	0.0122	0.0176	0.0220	0.0260	0.0294	y = 0.0214x + 0.0107	0.9924
0.3	0.0160	0.0190	0.0220	0.0274	0.0306	y = 0.0188x + 0.0136	0.9904
0.4	0.0172	0.0202	0.0228	0.0262	0.0304	y = 0.0162x + 0.0153	0.9915
0.5	0.0170	0.0190	0.0220	0.0250	0.0284	y = 0.0144x + 0.0151	0.9925
0.6	0.0150	0.0180	0.0210	0.0224	0.0250	y = 0.0122x + 0.0142	0.9862

average of triplicate results

hiang Mai University



Figure 3.5 Relationship between concentrations of HCl and sensitivity for Se(IV) determination $(0.10 - 0.90 \text{ mg L}^{-1})$

3.2.1.4 Effect of ethanol concentration in Variamine Blue solution

The effect of concentration of ethanol in VB solution was studied in the range of 15 - 50% (v/v). The results are shown in Table 3.4 and Figure 3.6. It was shown that the greater sensitivity was obtained when the concentration of ethanol in VB solution up till 30% (v/v). Thus, a concentration of ethanol in VB solution of 30% (v/v) was chosen as optimum concentration and used throughout the experimental.

Ethanol concentration	t	∆P.H.* (A he standa	AU) obtai ard Se(IV	ined fron /) (mg L ⁻	n ¹)	y = mx + c	r ²
(% v/v)	0.10	0.30	0.50	0.70	0.90		
15	0.0058	0.0068	0.0076	0.0086	0.0102	y = 0.0106x + 0.0046	0.9822
20	0.0060	0.0070	0.0086	0.0100	0.0110	y = 0.0130x + 0.0046	0.9937
25	0.0058	0.0072	0.0090	0.0100	0.0114	y = 0.0140x + 0.0045	0.9935
30	0.0058	0.0078	0.0100	0.0118	0.0136	y = 0.0196x + 0.0039	0.9983
35	0.0060	0.0070	0.0090	0.0108	0.0124	y = 0.0166x + 0.0041	0.9915
40	0.0050	0.0064	0.0074	0.0094	0.0110	y = 0.0150x + 0.0033	0.9889
45	0.0044	0.0064	0.0074	0.0090	0.0110	y = 0.0146x + 0.0034	0.9853
50	0.0520	0.0064	0.0070	0.0082	0.0090	y = 0.0094x + 0.0043	0.9915

Table 3.4 Effect of concentrations of ethanol on the sensitivity

*average of triplicate results





3.2.1.5 Effect of Variamine Blue concentration

Effect of VB concentrations on the determination of Se(IV) $(0.10 - 0.90 \text{ mg L}^{-1})$ was studied in the range of 6 – 14 mg L⁻¹. The results are shown in Table 3.5 and Figure 3.7. It was found that sensitivity increased very rapidly from the VB concentration of 6 to 10 mg L⁻¹. After that, the sensitivities decreased slightly. This is due to the fact that increasing of the VB concentration gives rise to the increase in the amounts of Se(IV)-VB complexation which results in a higher sensitivity. However, beyond the VB concentration of 10 mg L⁻¹, the amount of Se(IV)-VB complex became constant so as the Δ peak height. Consequently, a concentration of 10 mg L⁻¹ of VB was chosen as optimum.

Variamine Blue concentration	t	AP.H.* (A he standa	AU) obtai ard Se(IV	ined fron /) (mg L ⁻¹	n ¹)	y = mx + c	r ²
(mg L ⁻¹)	0.10	0.30	0.50	0.70	0.90		
6	0.0030	0.0046	0.0060	0.0070	0.0080	y = 0.0124x + 0.0020	0.9987
7	0.0050	0.0074	0.0074	0.0086	0.0102	y = 0.0128x + 0.0036	0.9961
8	0.0050	0.0068	0.0080	0.0100	0.0110	y = 0.0152x + 0.0036	0.9911
9	0.0052	0.0072	0.0092	0.0104	0.0126	y = 0.0180x + 0.0035	0.9936
10	0.0058	0.0082	0.0100	0.0114	0.0136	y = 0.0188x + 0.0042	0.9928
	0.0062	0.0074	0.0092	0.0114	0.0134	y = 0.0184x + 0.0040	0.9897
A 12	0.0062	0.0076	0.0090	0.0110	0.0130	y = 0.017x + 0.0043	0.9914
13	0.0070	0.0084	0.0104	0.0120	0.0134	y = 0.0164x + 0.0053	0.9964
14	0.0068	0.0088	0.0104	0.0116	0.0130	y = 0.0152x + 0.0056	0.9904
		1	1				

Table 3.5 Effect of concentrations of Variamine Blue on the sensitivity

^{*}average of triplicate results



Figure 3.7 Relationship between concentrations of VB solution and sensitivity of the proposed method

3.2.1.6 Effect of sodium acetate concentration

The Effect of CH₃COONa concentration on the determination of Se(IV) $(0.10 - 0.90 \text{ mg L}^{-1})$ was studied at different values in the range of $0.1 - 0.9 \text{ mol L}^{-1}$. The results are shown in Table 3.6 and Figure 3.8. It was found that sensitivity increased very rapidly from the CH₃COONa concentration of $0.1 - 0.4 \text{ mol L}^{-1}$. After that, the sensitivities were quite constant. This is due to the fact that increasing the CH₃COONa concentration leading to the increase in the amounts of Se(IV)-VB complexation which results in a higher sensitivity because sodium acetate acts as buffer in the reaction solution stream so that the optimum pH for the complex formation is achieved. However, beyond the CH₃COONa concentration of 0.4 mol L^{-1} .

the amount of Se(IV)-VB complexation became constant so as the Apeak height.

Consequently, a concentration of $0.4 \text{ mol } L^{-1}$ of CH₃COONa was chosen as optimum.

CH ₃ COONa concentration	t	∆P.H.* (∦ he standa	\P.H.* (AU) obtained fr he standard Se(IV) (mg			$\mathbf{v} = \mathbf{m}\mathbf{x} + \mathbf{c}$	r^2
(mol L ⁻¹)	0.10	0.30	0.50	0.70	0.90		
0.1	0.0068	0.0080	0.0090	0.0104	0.0118	y = 0.0124x + 0.0055	0.9959
0.2	0.0072	0.0086	0.0100	0.0112	0.0130	y = 0.0142x + 0.0057	0.9962
0.3	0.0086	0.0104	0.0124	0.0146	0.0168	y = 0.0206x + 0.0064	0.9982
0.4	0.0088	0.0112	0.0134	0.0156	0.0178	y = 0.0224x + 0.0066	0.9997
0.5	0.0098	0.0120	0.0138	0.0160	0.0190	y = 0.0224x + 0.0074	0.9915
0.6	0.0102	0.0120	0.0146	0.0164	0.0192	y = 0.0224x + 0.0078	0.9946
0.7	0.0102	0.0120	0.0142	0.0168	0.0192	y = 0.0224x + 0.0077	0.9965
0.8	0.0100	0.0114	0.0134	0.0158	0.0178	y = 0.0200x + 0.0077	0.9929
0.9	0.0098	0.0120	0.0138	0.0150	0.0174	y = 0.0182x + 0.0081	0.9906
*average of triplic	cate result	ts	Jι	INI	VER	5	1

Table 3.6 Effect of concentrations of CH₃COONa on the sensitivity



Figure 3.8 Relationship between concentrations of CH₃COONa and sensitivity of the proposed method

3.2.1.7 Effect of flow rate

Effects of flow rate of KI and VB solutions were studied, by determination of various concentrations of standard selenium solutions $(0.10 - 0.90 \text{ mg L}^{-1})$ which were flowed into the FIA manifold as shown in Figure 2.1. The flow rates were varied from 1.0 to 3.5 ml min⁻¹. The effect of flow rate on the sensitivity is shown in Table 3.7 and Figure 3.9. It can be seen that the optimum flow rate was 1.5 ml min⁻¹. In addition, the sensitivity of the calibration curve decreased, when the flow rate was lower than 1.5 ml min⁻¹. This is because the low flow rate increased dispersion. On the other hand, the sensitivity of the calibration curve was lower, when the flow rate was higher than 1.5 ml min⁻¹. This is due to the fact that the higher flow rate reduced the reaction time and hence reduced complex formation.

flow rate	∆P.H.* (A the standa		U) obtain rd Se(IV)	ned from) (mg L ⁻¹)		y = mx + c	r ²
(ml min)	0.10	0.30	0.50	0.70	0.90		
1.0	0.0100	0.0154	0.0210	0.0252	0.0286	y = 0.0235x + 0.0083	0.9899
1.5	0.0096	0.0138	0.0208	0.0242	0.0296	y = 0.0252x + 0.0070	0.9913
2.0	0.0118	0.0152	0.0200	0.0244	0.0286	y = 0.0214x + 0.0093	0.9977
2.5	0.0134	0.0162	0.0202	0.0256	0.0292	y = 0.0204x + 0.0107	0.9910
3.0	0.0122	0.0156	0.0192	0.0242	0.0280	y = 0.0201x + 0.0098	0.9954
3.5	0.0144	0.0170	0.0210	0.0252	0.0296	y = 0.0193x + 0.0118	0.9920

*average of triplicate results



Figure 3.9 Relationship between flow rates and sensitivity of the proposed method

3.2.1.8 Effect of reaction coil (I) length

The effect of reaction coil (I) length was investigated on the determination of $Se(IV) (0.10 - 0.90 \text{ mg L}^{-1})$ was studied by using Tygon tubing with diameter of 1.07 mm i.d. and length of reaction coil (I) length were varied from 0 to 60 cm. The results are shown in Table 3.8 and Figure 3.10. The sensitivity increased to a maximum at a reaction coil (I) length of 40 cm. It can be explained that increasing the reaction coil (I) length up to 40 cm give rise to an increase in the residence time allowing well mixing between selenium in acidic solution and KI. On the other hand, the sensitivity of the calibration curve decreased when the reaction coil (I) length was longer than 40 cm. This is due to dispersion occurred at the reaction coil (I) length longer than 40 cm. The reaction coil (I) length 40 cm was chosen as optimum since it provided the greatest sensitivity.

	Reaction coil(I)	t	∆P.H.* (A he standa	AU) obtai ard Se(IV	ined from ') (mg L ⁻¹	y = mx + c	r ²	
	length (cm)	0.10	0.30	0.50	0.70	0.90		
5	0	0.0170	0.0190	0.0214	0.0232	0.0254	y = 0.0105x + 0.016	0.9986
	20	0.0168	0.0208	0.0238	0.0272	0.0318	y = 0.0182x + 0.015	0.9950
	30	0.0162	0.0210	0.0256	0.0312	0.0348	y = 0.0237x + 0.013	0.9968
	40	0.0160	0.0220	0.0268	0.0318	0.0360	y = 0.0249x + 0.014	0.9963
A	50	0.0160	0.0210	0.0262	0.0308	0.0352	y = 0.0214x + 0.013	0.9988
	60	0.0188	0.0232	0.0270	0.0318	0.0350	y = 0.0205x + 0.016	0.9971

Table 3.8 Effect of reaction coil (I) lengths on the sensitivity

average of triplicate results



Figure 3.10 Relationship between reaction coil (I) lengths and sensitivity of the proposed method

3.2.1.9 Effect of reaction coil (II) length

The effect of reaction coil (II) length on the determination of Se(IV) $(0.10 - 0.90 \text{ mg L}^{-1})$. The 1.07 mm i.d. Tygon tubing was examined for using as a reaction coil (II). The reaction coils (II) lengths were varied from 75 to 200 cm. The results are shown in Table 3.9 and Figure 3.11. The sensitivity increased to a maximum at a reaction coil (II) length of 125 cm. It can be explained that increasing the reaction coil (II) length up to 125 cm an increase in the residence time is obtained, allowing well mixing between Se(IV)-KI and VB. On the other hand, the sensitivity decreased when the reaction coil (II) length was longer than 125 cm. This is due to the fact that dispersion occurred at the reaction coil (II) length longer than 125 cm. The sensitivity.

Reaction coil (II)	t	∆P.H.* (A he standa	AU) obtai ard Se(IV	ined fron /) (mg L ⁻	n ¹)	y = mx + c	r ²
length (cm)	0.10	0.30	0.50	0.70	0.90		
75	0.0240	0.0284	0.0328	0.0358	0.0390	y = 0.0187x + 0.0227	0.9917
100	0.0216	0.0248	0.0288	0.0326	0.0366	y = 0.0190x + 0.0194	0.9986
125	0.0180	0.0234	0.0264	0.0318	0.0360	y = 0.0222x + 0.0160	0.9942
150	0.0182	0.0224	0.0260	0.0300	0.0348	y = 0.0204x + 0.0161	0.9977
175	0.0232	0.0266	0.0298	0.0332	0.0376	y = 0.0177x + 0.0212	0.9961
200	0.0200	0.0234	0.0272	0.0304	0.0332	y = 0.0167x + 0.0185	0.9972

Table 3.9 Effect of reaction coil (II) lengths on the sensitivity

*average of triplicate results



Figure 3.11 Relationship between reaction coil (II) lengths and sensitivity of the proposed method

3.2.1.10 Effect of sample injection volume

The sample volume injected into the selenium stream has a significant effect on peak height. The effect of sample volume on the determination of $0.10 - 0.90 \text{ mg L}^{-1}$ selenium was studied by varying sample volume of 50, 75, 100, 125 and 150 µL. As shown in Table 3.10 and Figure 3.12, the sensitivity increases with increasing sample volume up to 75 µL. The highest slope was obtained in the rang of 75 to 150 µL, but the better linearity of calibration curve was obtained when the injection volume was 75 µL. The sample throughputs were stable with injection volume. A sample volume of 75 µL was chosen as a compromise between good sensitivity and reagent consumption.

 $\Delta P.H.*$ (AU) obtained from Sample injection the standard Se(IV) (mg L^{-1}) r^2 y = mx + cvolume (µL) 0.30 0.10 0.50 0.70 0.90 50 0.0178 0.9994 0.0120 0.0148 0.0206 0.0232 y = 0.0141x + 0.010675 0.0154 0.0196 0.0240 0.0268 0.0300 y = 0.0182x + 0.01410.9915 100 0.0224 0.0258 0.0300 0.0328 0.0358 y = 0.0169x + 0.02090.9949 125 0.0246 0.0284 0.0330 0.0358 0.0386 y = 0.0177x + 0.02320.9892 150 0.0256 0.0302 0.0350 0.0390 0.0440 y = 0.0228x + 0.02340.9992

 Table 3.10 Effect of sample injection volumes on the sensitivity

^{*}average of triplicate results

reserved



Figure 3.12 Relationship between sample injection volumes and sensitivity of the proposed method

A diagram in the FIA manifold is displayed in Figure 2.1. Table 3.11 shows the ranges over which the variables involved in the FIA system were studied and their optimum values.

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่ Copyright[©] by Chiang Mai University All rights reserved

Variable	Studied range	Optimum value
Wavelength (nm)	537 - 555	546
Concentration of KI (% w/v)	1.0 - 4.5	3.0
Concentration of HCl (mol L ⁻¹)	0.1 - 0.6	0.2
Concentration of ethanol in VB solution (% v/v)	15 - 50	30
Concentration of VB (mg L ⁻¹)	6 - 14	10
Concentration of CH ₃ COONa (mol L ⁻¹)	0.1 - 0.9	0.4
Flow rate (mL min ⁻¹)	1.0 - 3.5	1.5
Reaction coil (I) length (cm)	0-60	40
Reaction coil (II) length (cm)	75 - 200	125
Sample injection volume (µL)	50 - 150	75

 Table 3.11 Optimum conditions for selenium determination

3.2.2 Analytical Characteristics of the method

3.2.2.1 Linear range

The linear range of the proposed method was studied by flowing selenium standard solution into FIA system under the suitable conditions as shown in Table 3.12, linear range of the calibration graph was obtained for selenium standards solution at the concentration ranging from 0.05 to 2.50 mg L^{-1} . All measurements were made in five replicate injections. The results obtained are shown in Table 3.12 and Figure 3.13.

Selenium	Peak height (AU)								
(mg L ⁻¹)	1	2	3	4	5	x	(AU)		
0	0.006	0.006	0.006	0.006	0.006	0.0060	0		
0.05	0.010	0.011	0.011	0.011	0.011	0.0108	0.0048		
0.06	0.011	0.011	0.011	0.011	0.012	0.0112	0.0052		
0.07	0.011	0.011	0.011	0.012	0.012	0.0114	0.0054		
0.08	0.011	0.011	0.012	0.012	0.012	0.0116	0.0056		
0.09	0.011	0.012	0.012	0.012	0.012	0.0120	0.0060		
0.10	0.012	0.012	0.012	0.013	0.013	0.0124	0.0064		
0.15	0.013	0.013	0.013	0.013	0.013	0.0130	0.0070		
0.25	0.014	0.015	0.015	0.015	0.015	0.0148	0.0088		
0.75	0.022	0.021	0.021	0.022	0.022	0.0216	0.0156		
1.00	0.025	0.025	0.026	0.026	0.026	0.0256	0.0196		
1.25	0.029	0.030	0.030	0.030	0.030	0.0298	0.0238		
1.50	0.034	0.034	0.034	0.035	0.035	0.0344	0.0284		
1.75	0.038	0.038	0.038	0.039	0.039	0.0384	0.0324		
2.00	0.042	0.042	0.042	0.043	0.043	0.0424	0.0364		
2.50	0.049	0.049	0.049	0.050	0.050	0.0494	0.0434		
3.00	0.055	0.054	0.055	0.056	0.057	0.0554	0.0494		
4.00	0.067	0.067	0.067	0.067	0.067	0.0670	0.0610		
5.00	0.078	0.078	0.078	0.078	0.078	0.0780	0.0720		
6.00	0.087	0.087	0.087	0.088	0.088	0.0874	0.0814		
7.00	0.091	0.092	0.092	0.092	0.093	0.0920	0.0860		
8.00	0.094	0.093	0.095	0.095	0.095	0.0944	0.0884		
9.00	0.096	0.096	0.096	0.098	0.098	0.0968	0.0908		

 Table 3.12
 Linearity of selenium determination



Figure 3.13 Relationship between Apeak height and concentration of selenium

3.2.2.2 Precision of the flow injection system

The precision of the proposed method was verified by 11 replicated determination of 0.20 mg L^{-1} selenium standard solution, under the optimum conditions listed in Table 3.11. The relative standard deviation was found to be 0.31% (Table 3.13).

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่ Copyright[©] by Chiang Mai University All rights reserved

Experimental number	Peak height (AU)
1	0.0138
29129	0.0137
3	0.0138
4 1,0	0.0138
5	0.0138
6	0.0137
7	0.0138
8	0.0138
9	0.0138
10	0.0138
11	0.0138
x	0.0138
SD	4.40×10 ⁻⁵
% R.S.D.	0.31

Table 3.13 Precision verification using 0.20 mg L⁻¹ selenium standard solution

average of five replicate results

ลิขสิทธิ์มหาวิทยาลัยเชียงไหม่ Copyright[©] by Chiang Mai University All rights reserved

3.2.2.3 Calibration curve

As depicted in Figure 2.1, the standard solutions containing $0.05 - 2.50 \text{ mg L}^{-1}$ were flowed into the FIA system under the established optimum conditions (Table 3.11). The results are shown in Table 3.14. The calibration curve as shown in Figures 3.14 and 3.15 were established by plotting Δ peak heights versus the various selenium concentrations. Correlation coefficients (r²) and the regression equation are as follows:

 $y = 0.0158x + 0.0044 (r^2 = 0.9993)$

Where y is Apeak height in AU

x is concentration of selenium in mg L^{-1}

Table 3.14 Δ Peak height for calibration curve

Selenium	Peak height (AU)						
(mg L ⁻¹)	1	2	3	4	5	Ī	(AU)
0	0.006	0.006	0.006	0.006	0.006	0.006	0
0.05	0.010	0.011	0.011	0.011	0.011	0.0108	0.0048
0.06	0.011	0.011	0.011	0.011	0.012	0.0112	0.0052
0.07	0.011	0.011	0.011	0.012	0.012	0.0114	0.0054
0.08	0.011	0.011	0.012	0.012	0.012	0.0116	0.0056
0.10	0.012	0.012	0.012	0.013	0.013	0.0124	0.0064
0.15	0.013	0.013	0.013	0.013	0.013	0.0130	0.0070
0.25	0.014	0.015	0.015	0.015	0.015	0.0148	0.0088
0.50	0.018	0.018	0.018	0.019	0.018	0.0182	0.0122
1.00	0.025	0.025	0.026	0.026	0.026	0.0256	0.0156
1.25	0.029	0.030	0.030	0.030	0.030	0.0298	0.0196
1.50	0.034	0.034	0.034	0.035	0.035	0.0344	0.0238
1.75	0.038	0.038	0.038	0.039	0.039	0.0384	0.0324
2.00	0.042	0.042	0.042	0.043	0.043	0.0424	0.0364
2.50	0.049	0.049	0.049	0.050	0.050	0.0494	0.0434

^{*}average of five replicate results



Figure 3.14 Calibration signal of FIA spectrophotometric determination of selenium $0.05 - 2.50 \text{ mg L}^{-1}$



Figure 3.15 The Calibration curve of FIA spectrophotometric determination of selenium $0.05 - 2.50 \text{ mg L}^{-1}$

3.2.2.4 Detection limit [61]

For investigation of detection limit, the detection limit of the proposed method was investigated by preparing various standard solutions containing low concentration of selenium which were introduced into the proposed FIA system. The detection limit (defined as the concentration of the analyte which give the signal equivalent to three time of the standard deviation of the blank signal) was found to be 0.008 mg L⁻¹ from selenium 0.05 – 2.50 mg L⁻¹. The limit of quantitation was 0.0253 mg L⁻¹. The concentration at limit of detection (S_m) can be calculated from equation 2.2.

$$S_m = S_{bl} + kSD_{bl}$$

Where

Sm is the minimum analytical signal

S_{bl} is the mean blank signal

SD_{bl} is the standard deviation of blank

k is the confidence level of detection : 3

The linear regression equation is y = 0.0158x + 0.0044

Sm, y = 0.0044 + 3(0.00004)= 0.0045y = 0.0158x + 0.0044x, LOD = $0.008 \text{ mg L}^{-1} \text{Se(IV)}$ LOQ = 0.0044 + 10(0.00004)= 0.0048x, LOQ = $0.0253 \text{ mg L}^{-1} \text{Se(IV)}$

3.2.2.5 Interference studies

The interference effects of some possible foreign ions in FIA system for selenium determination were studied by the proposed FIA procedure under the optimum conditions obtained (Table 3.11). A systematic study to check for the effects of some possible foreign ions on the determination of selenium was undertaken for the maximum w/w ratio of selenium to foreign ions up to 1 : 5000. The solutions of a 1.00 mg L⁻¹ selenium standard solution containing varying concentrations of diverse ions were determined using the FIA system. The tolerance is defined as the largest foreign-ion concentration causing % error between 90 – 110% for determining the analyte of interest. The tolerance values for the ions studied are given in Table 3.15.



Interference	Selenium : Interference	Peak height [*] (AU)	% Error
Cu ²⁺	1:0	0.0196	-
0	1: 0.2	0.0207	105
	1: 0.5	0.0216	111
	1: 0.8	0.0224	114
Fe ²⁺	1:0	0.0196	- 6
G	1: 0.1	0.0205	104
	1: 0.5	0.0216	110
525	1: 1.0	0.0227	115
Fe ³⁺	1: 0	0.0196	500
	1: 0.1	0.0211	107
	1: 0.3	0.0217	110
	1: 0.5	0.0225	114
NO ₂ ⁻	1: 0	0.0196	
	1: 0.1	0.0208	106
	1: 0.5	0.0215	109
	1: 1.0	0.0232	118
Na ⁺	1: 0	0.0196	-
8.5	1: 500	0.0202	100
Jansi	1: 2500	0.0184	96
nuight (1 : 5000	0.0176	96
K ⁺		0.0196	IIVEISI
II ri	1 : 500	0.0209	106
	1: 1000	0.0214	109
	1: 2500	0.0217	110

Table 3.15 Interference studies for 1.00 mg L⁻¹ selenium standard solution by FIA method

Table 3.15 (Continued)

Interference	Selenium : Interference	Peak height [*] (AU)	% Error
Mg ²⁺	1:091	0.0196	-
	1: 300	0.0197	100
9	1: 1500	0.0204	104
	1: 4000	0.0191	98
Ca ²⁺	1: 0	0.0196	3
6	1: 500	0.0200	102
	1: 2500	0.0206	105
No.	1: 5000	0.0210	107
Ba ²⁺		0.0196	795
	1: 100	0.0210	107
G	1: 500	0.0212	108
E	1: 1000	0.0218	111
Al ³⁺	1: 0	0.0196	
	1: 10	0.0206	105
	1: 50	0.0210	107
	1: 100	0.0217	110
Mn ²⁺	1: 0	0.0196	-
¢*	1: 10	0.0206	105
angı	1: 50	0.0212	108
	1: 80	0.0217	110
Co ²⁺	by 1Chiang	0.0196	niversit
	1: 10	0.0200	103
I II	1: 50	e _{0.0216} e	110
	1: 80	0.0220	112

Table 3.15 (Continued)

Interference	Selenium : Interference	Peak height [*] (AU)	% Error
Zn ²⁺	1:09	0.0196	-
0	1: 500	0.0208	106
	1: 1500	0.0214	109
	1: 2000	0.0217	110
Ni ²⁺	1:0	0.0196	63 -
	1: 10	0.0199	101
	1: 100	0.0210	107
	1: 200	0.0216	111
Cr ³⁺	1:0	0.0196	502
	1: 5	0.0189	97
9	1: 10	0.0199	101
	1: 100	0.0214	109
Cd ²⁺	1:0	0.0196	
	1: 10	0.0201	102
	1: 50	0.0209	106
	1: 100	0.0214	109
F	1: 0	0.0196	-
	1: 500	0.0197	100
Jansi	1: 1500	0.0190	97
	1: 2000	0.0178	91
PO ₄ ³⁻	1:0	0.0196	IIVEISI
ll ri	g h 1: 10	0.0205	104
	1: 500	0.0212	108
	1: 1000	0.0215	109

Interference	Selenium : Interference	Peak height [*] (AU)	% Error
NO ₃ -	1:0	0.0196	-
0	1: 100	0.0200	102
	1: 2500	0.0200	102
	1: 5000	0.0201	102
SO4 ²⁻	1:0	0.0196	3-
	1: 500	0.0204	104
	1: 2500	0.0192	98
502	1: 5000	0.0190	97
CO3 ²⁻	1:0	0.0196	<u>20</u> 6
	1: 300	0.0197	100
E	1: 1000	0.0206	105
	1: 3000	0.0218	111
Br⁻	1:0	0.0196	· / -
	1: 500	0.0210	107
	1: 2500	0.0204	104
	1: 5000	0.0190	97
Cl	1: 0	0.0196	-9
ิปสิทธา	1: 100	0.0197	100
	1 : 800	0.0206	105
opyrights	1: 2500	0.0218	111

The interference effects of some possible foreign ions on selenium determination using FIA system for are summarized in Table 3.16. It was found that Fe^{2+} , Fe^{3+} , NO_2^{-} and Cu^{2+} interfered determination of selenium in surface waters, which referred to standard quality of surface water defined by Pollution Control Department Ministry of Natural Resources and Environment (Appendix A).

Table 3.16 Summary of interference effects of some ions on the response obtainedfrom selenium 1.00 mg L^{-1}

Interference ion	Tolerable concentration ratio [*]
Na ⁺ , Ca ²⁺ , Cl ⁻ , NO ₃ ⁻ , SO ₄ ²⁻ , Br ⁻	5000
Mg ²⁺	4000
CO3 ²⁻	3000
K ⁺	2500
Zn ²⁺ , F	2000
PO ₄ ³⁻ , Ba ²⁺	1000
Ni ²⁺	200
Cr ³⁺ , Al ³⁺ , Cd ²⁺	100
Mn ²⁺	
Co ²⁺ ght ^O by Chian	ng Mai Loniversit
Cu^{2+}, Fe^{2+}, NO_2^-	
Fe ³⁺	0.3

The concentration of interference ion is considered to interfered when causing % error less than 90% and more than 110% with respect of the signal of selenium alone

3.2.2.6 Effects of masking agents and interference

The effect of masking agents and interference were studied by the proposed FIA procedure under the optimum conditions. In order to overcome interference effects of Fe^{2+} , Fe^{3+} , NO_2^{-} and Cu^{2+} on Se(IV) determination various masking agents were investigated. The results are shown in Table 3.17. It was found that Fe^{2+} and Fe^{3+} were masked with 0.1% (w/v) of NaF and 0.5% (w/v) of NaF respectively [63]. Cu^{2+} was masked with 0.3% (w/v) of EDTA [64] and NO_2^{-} was masked with 0.005 % (w/v) NH₃SO₃[64].

Table 3.17 Effect of masking agents for mask Fe^{2+} , Fe^{3+} Cu^{2+} and NO_2^{-} on the response obtained from selenium 1.00 mg L⁻¹

Interference	Concentration of masking agent	Selenium :Interference	Peak height [*] (AU)	% Error
Fe ²⁺		1:0	0.0196	_
	0.05% (w/v) NaF	1:1	0.0202	103
	AI	1:5	0.0207	105
		1: 10	0.0213	108
	~	1: 0	0.0196	-
alans	0.1% (w/v) NaF	1:5	0.0204	104
UCIII		1: 10	0.0215	109
opyrigh	t [©] by (hiarig 15 Aai	0.0222	113
	ight	1: 0	0.0196	
	0.5% (w/v) NaF	1:5 8 5	0.0201	102
		1: 10	0.0209	106
		1: 15	0.0221	112

^{*}average of five replicate results

NaF was sodium fluoride

Table	3.17	(continued)
			/

Interference	Concentration of masking agent	Selenium :Interference	Peak height [*] (AU)	% Error
Fe ³⁺	-12	1:0	0.0196	-
		1: 0.5	0.0203	103
	0.1% (w/v) NaF	1; 1	0.0218	111
4		1:-5	0.0290	147
		1:0	0.0196	-
	0.20/ (/) N.F.	() 1: 1	0.0197	100
	0.3% (W/V) NaF	1: 5	0.0218	101
525		1: 10	0.0245	125
305	ſ	1: 0	0.0196	
CH	0.5% (w/v) NaF	1:1	0.0182	93
		1: 5	0.0203	103
		1: 10	0.0211	107
Cu ²⁺	1	1:0	0.0196	_
	0.1% (w/v) EDTA	1:5	0.0208	106
		1: 10	0.0220	112
	In	1: 20	0.0242	123
		1: 0	0.0196	-
9	0.20/(m/m) EDTA	1: 10	0.0203	103
ana	0.3% (W/V) EDTA	1: 20	0.0206	105
		1: 50	0.0217	110
Jyrigh			0.0196	:rs <u>i</u> ty
	0.59/(m/z) EDTA	S 1: 100 S	0.0202	103
	0.3% (W/V) EDIA	1: 20	0.0216	100
		1: 50	0.0186	95

*average of five replicate results

NaF was sodium fluoride EDTA was ethylenediaminetetraacetic acid

Table 3.17 (continued)

Interference	Concentration of masking agent	Selenium :Interference	Peak height [*] (AU)	% Error
NO ₂ -	0.0010/0/09	0 1:0	0.0196	-
	0.001% (w/v)	1: 5	0.02023	103
	NH ₃ SO ₃	1: 10	0.0219	111
6		1: 20	0.0228	116
	0.0050/(())	1:0	0.0196	-
	0.005% (w/v) NH ₃ SO ₃	1: 10	0.0202	103
		1: 20	0.0216	110
582		1: 50	0.0223	113
500	0.010/ (/)	1: 0	0.0196	2 -
	0.01% (W/V)	1: 10	0.0202	103
	NH ₃ SO ₃	1: 20	0.0216	110
		1: 50	0.0220	112

*average of five replicate results NH₃SO₃ was sulfamic acid

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่ Copyright[©] by Chiang Mai University All rights reserved

3.2.2.7 Determination of selenium in waters

The proposed FIA spectrophotometric method was applied to the simultaneous determination of selenium in surface water samples which were collected from Chiang Mai canal and Chiang Mai University reservoir. The peak heights from each sample were compared with standard calibration curve. The results are given in Table 3.18.

224	Peak heights (AU)				Selenium	0/2		
Water samples	1	2	3	x	SD	concentration [*] (mg L ⁻¹)	Recovery*	
Suan Dok Gate	ND**	ND**	ND**) _# - /	-	ND**	-	
Hua Lin Corner	0.016	0.017	0.018	0.017	0.001	0.017 ± 0.001	102.20	
Chang Phuak Gate	ND ^{**}	ND ^{**}	ND ^{**}	-1	-	ND**	-	
Sri Poom Corner	0.050	0.052	0.051	0.051	0.001	0.051 ± 0.001	102.33	
Thaphae Gate	ND**	ND ^{**}	ND ^{**}	XT.	R	ND**	-	
Khatum Corner	0.024	0.023	0.025	0.024	0.001	0.024 ± 0.001	100.10	
Chiang mai Gate	0.030	0.028	0.029	0.029	0.001	0.029 ± 0.001	99.78	
Khu Huang Corner	ND ^{**}	ND ^{**}	ND**	<u>าส</u>	81	ND**	11	
Ang-Keaw I	ND**	ND ^{**}	ND**	-	-	ND ^{**}		
Ang-Keaw II	ND**	ND ^{**}	ND ^{**}	<u>Ig</u> /	nai	ND** ers	sit <u>y</u>	
ri	o h		S			erve	4 0	

Table 3.18 Determination of selenium in water samples by FIA method

*average of five replicate results

**not detected

The selenium contents in the water samples were in the range of 0.017 - 0.051mg L^{-1} and 0.016 – 0.050 mg L^{-1} using the proposed method and ICP-MS respectively. The results obtained by the proposed FIA spectrohotometric method compared favorably with those obtained by ICP-MS using the student t-test (Table 3.19 and Appendix B in Table B.1). It was evident that the t-value for Se(IV) contents in water samples determined by comparison the results obtained by FIA spectrohotometric method with those obtained by ICP-MS was 0.581. It was seen that the experimental t-value for Se(IV) assay(0.581) which was smaller than the theoretical t-value at a confidence interval of 95% (3.18) indicating that results obtained by both methods were in excellent agreement.

Table 3.19 Comparative determination of selenium in water samples by proposed FIA method and ICP-MS

Water samples	Concentrations (mg L ⁻¹)		
	FIA [*]	ICP-MS*	xd
Suan Dok Gate	ND***	ND**	-
Hua Lin Corner	0.017	0.016	0.001
Chang Phuak Gate	ND ^{**}	ND ^{**}	-
Sri Poom Corner	0.051	0.050	0.001
Thaphae Gate	ND ^{**}	ND ^{**}	- 7
Khatum Corner	0.024	0.024	0.000
Chiang mai Gate	0.029	0.028	0.001
Khu Huang Corner	ND**	ND ^{**}	lniver
Ang-Keaw I	ND** 5	ND ^{**}	
Ang-Keaw II	ND ^{**}	ND***	P - 1/
δ			0.003
Sd			0.00258
Т			0.581
3.3 SIA Spectrophotometric Determination of Selenium Using Variamine Blue as Complexing Agent

The conditions for the determination of selenium were optimized by studying the influences of the various parameters, such as sample and reagent volumes, reagent/carrier flow rates, holding time and reagent concentrations of the respective measurements. The optimum conditions obtained by means of the univariate optimization procedure (changing one variable in turn and keeping the others at their optimum values). The optimal value for each parameter was judged from maximum response of the detector, minimum noise of the baseline and relative standard deviation. To optimize the conditions, the SIA manifold in Figures 2.2 and 2.3 and preliminary experimental conditions in Table 2.5 were used. The range of variables studied and the optimal values chosen are shown in Table 2.4.

3.3.1 Study aspiration order

The complexation of Se(IV)-VB was studied at different aspiration orders. The sensitivities obtained are shown in Table 3.20. It was found that the aspiration order of first series provides a highest sensitivity. So, aspiration order of first series was chosen for further optimization of SIA method.

Copyright[©] by Chiang Mai University All rights reserved

Series	Aspiration order	Sensitivity (AU/mg L ⁻¹)
1	A-B-C	0.0755
2	А-С-В	0.0180
3	B-A-C	0.0460
4	B-C-A	ND*
5	C-A-B	0.0130
6	C-B-A	0.0150

T-LL 2 20	C	- 4		1
1 able 3.20	Sensitivity	at various	aspiration	orders

^{*}not detected

A was 0.1 mg L^{-1} selenium standard solution B was 0.4 % (w/v) potassium iodide C was 50 mg L^{-1} Variamine Blue (VB)

3.3.2 Optimization of the sequential injection system by univariate method

To optimize the experimental conditions, the SIA manifold in Figures 2.2-2.3 was employed and the preliminary experimental conditions (Table 2.5) were re-investigated.

Copyright[©] by Chiang Mai University All rights reserved

3.3.2.1 Effect of potassium iodide concentration

The effect of potassium iodide concentration on the reactions of selenium were studied in the range of 1.0 - 4.0% (w/v). The results are shown in Table 3.21 and Figure 3.16. It was found that the sensitivity of the calibration curve increased very rapidly from potassium iodide concentration of 1.0 - 2.0% (w/v). After that, the sensitivities were decreasing when increases potassium iodide concentration. Therefore, a concentration of 2.0% (w/v) was chosen as optimum.

KI		CD CD			
(% w/v)	1	2	3	<u>x</u> *	SD SD
1.0	0.0246	0.0261	0.0242	0.0249	0.001002
1.5	0.0245	0.0282	0.0230	0.0252	0.002676
2.0	0.0320	0.0335	0.0327	0.0327	0.000751
2.5	0.0272	0.0299	0.0293	0.0288	0.001418
3.0	0.0234	0.0284	0.0276	0.0264	0.002686
3.5	0.0235	0.0253	0.0237	0.0241	0.000987
4.0	0.0223	0.0219	0.0223	0.0221	0.000023
*average of triplica	ate results	BUG	360	BAI	OIK

Table 3.21 Effect of various concentrations of KI on the sensitivity



Figure 3.16 Relationship between various concentrations of KI and sensitivity of the SIA method

3.3.2.2 Effect of hydrochloric acid concentration

Effect of HCl concentrations on the determination of Se(IV) $(0.10 - 0.90 \text{ mg L}^{-1})$ was studied with the following SIA system in Figure 2.2 or 2.3, the concentration of HCl solutions were varied from $0.2 - 1.0 \text{ mol L}^{-1}$. The results are shown in Table 3.22 and Figure 3.17. The results indicated that the concentration of HCl acid below 0.3 or above 0.5 mol L⁻¹ the sensitivity (slop of the calibration curve) decreased significantly. So, 0.4 mol L⁻¹ was chosen because it provided the greatest sensitivity.

HCl		CD			
(mol L ⁻¹)	1	2	3	x *	50
0.2	0.0012	0.0011	0.0016	0.0013	0.000265
0.3	0.0018	0.0021	0.0024	0.0021	0.000300
0.4	0.0028	0.0030	0.0033	0.0030	0.000252
0.5	0.0026	0.0027	0.0029	0.0027	0.000153
0.6	0.0025	0.0024	0.0025	0.0024	0.000057
0.7	0.0022	0.0024	0.0024	0.0023	0.000115
0.8	0.0020	0.0020	0.0022	0.0020	0.000115
0.9	0.0020	0.0016	0.0021	0.0019	0.000265
1.0	0.0017	0.0018	0.0012	0.0015	0.000321

 Table 3.22
 Effect of various concentrations of HCl on the sensitivity

*average of triplicate results



Figure 3.17 Relationship between various concentrations of HCl and sensitivity of the SIA method

3.3.2.3 Effect of ethanol concentration in Variamine Blue solution

The effect of ethanol concentration in VB solution was studied in the range of 15 - 45% (v/v). The results are shown in Table 3.23 and Figure 3.18. It was shown that the greater slope was obtained when the concentration of ethanol in VB solution up to 30% (v/v). Thus, a concentration of ethanol in VB solution of 30% (v/v) was chosen as optimum % ethanol in VB solution.

Ethanol					
(% v/v)	1	2	3	<u>x</u> * 3	5.50
15	0.0080	0.0063	0.0078	0.0073	0.000929
20	0.0096	0.0105	0.0099	0.0100	0.000458
25	0.0131	0.0100	0.0131	0.0120	0.001790
30	0.0132	0.0138	0.0150	0.0140	0.000917
35	0.0110	0.0121	0.0136	0.0122	0.001305
40	0.0106	0.0092	0.0103	0.0100	0.000737

 Table 3.23 Effect of various concentrations of ethanol on the sensitivity

^{*}average of triplicate results

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่ Copyright[©] by Chiang Mai University All rights reserved



Figure 3.18 Relationship between various concentrations of ethanol in VB solution and sensitivity of the SIA method

3.3.2.4 Effect of Variamine Blue concentration

Effect of VB concentrations on the determination of Se(IV) $(0.10 - 0.90 \text{ mg L}^{-1})$ was studied in the range of 50 – 200 mg L⁻¹. The results are shown in Table 3.24 and Figure 3.19. It was found that sensitivity of calibration curve increased very rapidly from the VB concentration of 50 to 100 mg L⁻¹. After that, the sensitivities were decreased. This is due to the fact that increase of the VB concentration gives rise to the increase in the amounts of Se(IV)-VB complexation which results in a higher sensitivity of calibration curve obtained. However, beyond the VB concentration of 100 mg L⁻¹, the amount of Se(IV)-VB complex became constant so as the Δ peak height. Consequently, a concentration of 100 mg L⁻¹ of VB was chosen as optimum.

VB		CD			
(mg L ⁻¹)	1	2	3	x *	SD
50	0.0080	0.0070	0.0070	0.0073	0.000577
75	0.0120	0.0120	0.0150	0.0130	0.001732
100	0.0140	0.0160	0.0180	0.0160	0.002000
125	0.0140	0.0120	0.0120	0.0126	0.001154
150	0.0110	0.0140	0.0110	0.0120	0.001732
175	0.0110	0.0120	0.0120	0.0116	0.000577
200	0.0100	0.0100	0.0110	0.0103	0.000577

 Table 3.24
 Effect of various concentrations of VB in 30% ethanol on the sensitivity

*average of triplicate results



Figure 3.19 Relationship between various concentrations of VB in 30% ethanol solution and sensitivity of the SIA method

3.3.2.5 Effect of sodium acetate concentration

The Effect of CH₃COONa concentration on the determination of Se(IV) $(0.10 - 0.90 \text{ mg L}^{-1})$ was studied at different values in the range of $0.1 - 0.6 \text{ mol L}^{-1}$. The results are shown in Table 3.25 and Figure 3.20. It was observed that the intensity of the colored complex decreased with increase in the concentration of CH₃COONa, which may be due to the dissociation of the complex at high base concentration. Therefore the 0.2 mol L⁻¹ CH₃COONa concentration was selected to the subsequent investigations.

Table 3.25 Effect of various concentrations of CH₃COONa on the sensitivity

CH ₃ COONa					
(mol L ⁻¹)	1	2	3	x [*]	SD
0.1	0.0300	0.0306	0.0327	0.0311	0.001418
0.2	0.0400	0.0403	0.0409	0.0404	0.000458
0.3	0.0370	0.0388	0.0368	0.0375	0.001102
0.4	0.0317	0.0330	0.0342	0.0329	0.001250
0.5	0.0291	0.0288	0.0288	0.0289	0.000173
0.6	0.0253	0.0258	0.0269	0.0260	0.000819
*average of triplicat	e results	DNU	188	1080	Dinu



Figure 3.20 Relationship between concentrations of CH₃COONa on the sensitivity of the SIA method

3.3.2.6 Effect of aspiration volumes of KI

The aim for optimization of this parameter is to minimize the consumption of reagent while maintaining the best sensitivity, accuracy and reproducibility of the procedure for the analyte of interest. The procedure adopted for optimizing this parameter was to keep the volumes of other reagents and the sample at constant values (chosen by trial and error) while varying the reagent to be optimized at different volumes. This was done by changing the period during which the specific sample and/or reagent volume was aspirated into the holding coil. In this investigation, the effect of aspirated volumes of the 2.0 %(w/v) of KI was studied over the range 75 – 200 µL at every 25 µL interval (Table 3.26 and Fig 3.21). It was found that the sensitivity increased when the aspiration volume of 2.0 %(w/v) of KI was increased and reached a maximum sensitivity at 125 µL, above which the

sensitivity started to decline. So, a volume of 125 μ L was chosen as an optimum for subsequent measurements.

Aspiration KI	No /	CD			
volume (μL)		2	3	x *	SD
75	0.0015	0.0018	0.0017	0.0016	0.000153
100	0.0021	0.0021	0.0023	0.0021	0.000115
125	0.0024	0.0028	0.0026	0.0026	0.000200
150	0.0023	0.0021	0.0020	0.0021	0.000153
175	0.0014	0.0016	0.0020	0.0016	0.000306
200	0.0010	0.0012	0.0012	0.0011	0.000115

Table 3.26 Effect of various aspiration volumes of 2.0 %(w/v) of KI on the sensitivity

*average of triplicate results





3.3.2.7 Effect of aspiration volumes of HCl

The effect of the HCl aspirated volume was considered. When the volumes of HCl solution were varied from 75 to 175 μ L at every 25 μ L interval, maximum sensitivity was obtained at a volume of 125 μ L (Table 3.27 and Figure 3.22). Thus, a 125 μ L of HCl solution was chosen as an optimum volume for subsequent measurements.

Aspirate HCl					
μL)	10	2 2	3	x *	SD SD
75	0.0012	0.0011	0.0011	0.0011	0.000057
100	0.0014	0.0013	0.0014	0.0013	0.000057
125	0.0022	0.0019	0.0020	0.0020	0.000152
150	0.0015	0.0016	0.0017	0.0016	0.000100
175	0.0011	0.0012	0.0013	0.0012	0.000100

Table 3.27 Effect of various aspiration volumes of 0.4 mol L⁻¹ of HCl on the sensitivity

*average of triplicate results

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม Copyright[©] by Chiang Mai University All rights reserved



Figure 3.22 Relationship between various aspiration volumes of 0.4 mol L^{-1} of HCl on the sensitivity of the SIA method

3.3.2.8 Effect of aspiration volumes of VB

To minimize the consumption of reagent volume, the Effect of aspiration volumes of VB on the determination of Se(IV) $(0.10 - 0.90 \text{ mg L}^{-1})$ was studied at different concentrations in the range of 75 – 200 µL at every 25 µL interval. The results are shown in Table 3.28 and Figure 3.23. It was found that the sensitivity decreased, when the aspiration volume of VB lower and higher than 100 µL. Thus, the optimum aspiration volume of VB was 100 µL because it provided highest sensitivity.

Aspirate VB		SD			
μL)	19	2	3	X *	50
75	0.0015	0.0016	0.0015	0.0015	0.000057
100	0.0020	0.0019	0.0017	0.0018	0.000152
125	0.0016	0.0014	0.0014	0.0014	0.000115
150	0.0013	0.0015	0.0013	0.0013	0.000115
175	0.0013	0.0011	0.0011	0.0011	0.000115
200	0.0008	0.0010	0.0010	0.0009	0.000115

Table 3.28 Effect of various aspiration volumes of 100 mg L^{-1} of VB in 30% ethanol on the sensitivity

*average of triplicate results



Figure 3.23 Relationship between various aspiration volumes of 100 mg L^{-1} of VB in 30% ethanol on the sensitivity of the SIA method

3.3.2.9 Effect of aspiration volumes of sample

The influence of the sample volumes was examined between 50 to 175 μ L at every 25 μ L interval and it was found that the sensitivity increased markedly up to 100 μ L, above which the sensitivity started to decline (Table 3.29 and Figure 3.24). So, a volume of 100 μ L was chosen as an appropriate sample volume for further investigations.

Aspirate		SD			
sample volume (μL)	1	2	3	<u>x</u> *	55D
50	0.0022	0.0022	0.0025	0.0023	0.000173
75	0.0029	0.0027	0.0029	0.0028	0.000115
100	0.0041	0.0040	0.0039	0.0040	0.000100
125	0.0034	0.0034	0.0031	0.0033	0.000173
150	0.0029	0.0029	0.0030	0.0029	0.000057
175	0.0025	0.0028	0.0027	0.0026	0.000153

Table 3.29 Effect of various aspiration volumes of sample on the sensitivity

*average of triplicate results

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่ Copyright[©] by Chiang Mai University All rights reserved



Figure 3.24 Relationship between various aspiration volumes of sample on the sensitivity of the SIA method

3.3.2.10 Effect of flow rate

The flow rate is one of the most important parameters to be optimized because it regulates the amount of final product (purple colored product as chromophore) formed and hence the sensitivity together with the sample throughput. The effect of the flow rate on the absorbance was investigated from $50 - 175 \ \mu L \ s^{-1}$. The sample, K1, HCl and VB volumes aspirated were kept constant at their optimum values by changing the flow rate over the range $50 - 175 \ \mu L \ s^{-1}$. The effect of flow rate is shown in Table 3.30 and Figure 3.25. Maximum absorbance was obtained at a flow rate of $100 \ \mu L \ s^{-1}$. Therefore, a flow rate of $100 \ \mu L \ s^{-1}$ does not significantly enhance the sensitivity, but they increase the pressure in the tubing, resulting in the more consumption of the reagents.

flow rate		Sensitivity	(AU/mg L ⁻¹)		CD
(µL s ⁻¹)	1	2	3	x *	- SD
50	0.0029	0.0028	0.0029	0.0028	0.000057
75	0.0032	0.0031	0.0034	0.0032	0.000152
100	0.0036	0.0038	0.0037	0.0037	0.000100
125	0.0028	0.0027	0.0027	0.0027	0.000057
150	0.0024	0.0026	0.0024	0.0024	0.000115
175	0.002	0.0019	0.0018	0.0019	0.000100
Sensitivity (AU/mg L ¹)	0.004 0.003 0.002 0.001	UN	VER		795.
ເສົາຣ໌	0 25	50 75 flo	100 125 w rate (uL s ⁻¹	150 175	5 200
Figure 3.25 the SIA method	Relationship	between var	rious flow r	ates on the	sensitivity

 Table 3.30 Effect of various flow rates on the sensitivity

3.3.2.11 Effect of holding time

The effect of holding time on the determination of Se(IV) $(0.10 - 0.90 \text{ mg L}^{-1})$ was studied in the range of 0 - 40 s. The results are shown in Table 3.31 and Figure 3.26. It was shown that the greater sensitivity was obtained when the holding time at 0 s. It can be explained that increasing the holding time the sensitivity decreased when holding time greater than 0 s because of dispersion. So, a holding time of 0 s was chosen as optimum since it provided the greatest sensitivity.

					27 M	
Holding time		Sensitivity ((AU/mg L ⁻¹)			
(\$)	1	2	3	x *	- SD	
0	0.0069	0.0072	0.0074	0.0071	0.000250	
10	0.0053	0.0046	0.0046	0.0048	0.000400	
20	0.0044	0.0044	0.0043	0.0043	0.000057	
30	0.0037	0.0038	0.0037	0.0037	0.000057	
40	0.0033	0.0032	0.0034	0.0033	0.000100	ļ

 Table 3.31 Effect of various holding times on the sensitivity

*average of triplicate results

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม Copyright[©] by Chiang Mai University All rights reserved



Figure 3.26 Relationship between various holding times on the sensitivity of the SIA method

A diagram in the SIA manifold is displayed in Figures 2.2 and 2.3. Table 3.32 shows the ranges over which the variables involved in the SIA system were studied and their optimum values.

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่ Copyright[©] by Chiang Mai University All rights reserved

Variable	Studied range	Optimum value
Concentration of CH ₃ COONa (mol L ⁻¹)	0.1 - 0.6	0.2
Concentration of KI (% w/v)	1.0 - 4.0	2.0
Concentration of HCl (mol L ⁻¹)	0.2 - 1.0	0.4
Concentration of ethanol in VB solution (% v/v)	15 - 40	30
Concentration of VB (mg L ⁻¹)	50 - 200	100
Aspiration volume of KI (µL)	75 – 200	125
Aspiration volume of HCl (µL)	75 – 175	-125
Aspiration volume of VB (µL)	75 - 200	100
Aspiration volume of sample (µL)	50 - 175	100
Flow rate (μ L s ⁻¹)	50 - 175	125
Holding time (s)	0-40	0

Table 3.32 Optimum conditions for selenium determination

3.3.3 Analytical Characteristics of the method

3.3.3.1 Linear range

The linear range of the proposed method was studied by aspiration of appropriate volume of selenium standard solution into the SIA system under the suitable conditions as shown in Table 3.32. Linear calibration graph was obtained for selenium standards solution over the concentration range of $0.01 - 2.50 \text{ mg L}^{-1}$. All measurements were made in five replicate injections. The results obtained are shown in Table 3.33 and Figure 3.27.

	selenium			Peak hei	ght (AU)			ΔΡ.Η. [*]
	(mg L ⁻¹)	1	2	3	4	5	x	(AU)
	0.00	0.0306	0.0306	0.0306	0.0306	0.0306	0.0306	0.0000
	0.01	0.0317	0.0317	0.0317	0.0318	0.0318	0.0317	0.00114
	0.02	0.0326	0.0326	0.0326	0.0326	0.0326	0.0326	0.00200
	0.03	0.0334	0.0334	0.0333	0.0335	0.0335	0.0334	0.00282
	0.04	0.0348	0.0348	0.0348	0.0349	0.0349	0.0348	0.00424
	0.05	0.0355	0.0361	0.0362	0.0360	0.0360	0.0359	0.00536
	0.06	0.0372	0.0372	0.0372	0.0372	0.0369	0.0371	0.00654
	0.07	0.0394	0.0394	0.0395	0.0395	0.0395	0.0394	0.00886
	0.08	0.0420	0.0418	0.0419	0.0418	0.0418	0.0418	0.01126
	0.10	0.0440	0.0440	0.0450	0.0450	0.0450	0.0446	0.01400
	0.20	0.0480	0.0480	0.0480	0.0480	0.0480	0.0480	0.01740
	0.40	0.0650	0.0650	0.0650	0.0650	0.0650	0.0650	0.03440
	0.60	0.0790	0.0790	0.0790	0.0790	0.0890	0.0810	0.05040
	0.80	0.0990	0.0990	0.0990	0.0990	0.0990	0.0990	0.06840
	1.00	0.1140	0.1150	0.1150	0.1150	0.1150	0.1140	0.08420
	C _{1.50}	0.1560	0.1560	0.1560	0.1560	0.1560	0.1560	0.12540
D	2.00	0.1950	0.1950	0.1950	0.1940	0.1940	0.1940	0.16400
	2.50	0.2320	0.2320	0.2320	0.2330	0.2310	0.2320	0.20140
	3.00	0.2620	0.2620	0.2620	0.2620	0.2620	0.2620	0.23140
	4.00	0.2970	0.2970	0.2970	0.2970	0.2970	0.2970	0.26640
	5.00	0.3390	0.3390	0.3390	0.3390	0.3390	0.3390	0.30840

 Table 3.33
 Linearity of selenium determination

*average of five replicate results



Figure 3.27 Relationship between Apeak height and concentration of selenium

3.3.3.2 Precision of the sequential injection system

The precision of the proposed method was verified by 11 replicated determination of selenium standard solution, using the optimum conditions (Table 3.32). Table 3.34 as shown the relative standard deviation was found to be 0.27, 0.40, 0.33 and 0.39 % of selenium 0.05, 0.10, 0.60 and 2.00 mg L^{-1} , respectively.

ลิขสิทธิ์มหาวิทยาลัยเชียงไหม Copyright[©] by Chiang Mai University All rights reserved

Experimental	Peak height (A	U) obtained from	m the standard S	Se(IV) (mg L ⁻¹)
number	0.05	0.10	0.60	2.00
1	0.00537	0.0141	0.0505	0.1632
2	0.00536	0.0140	0.0501	0.1632
3	0.00536	0.0140	0.0505	0.1640
4	0.00535	0.0140	0.0503	0.1640
5	0.00536	0.0141	0.0504	0.1646
6	0.00535	0.0140	0.0503	0.1640
573	0.00534	0.0141	0.0506	0.1646
8	0.00533	0.0140	0.0503	0.1632
9	0.00535	0.0140	0.0504	0.1646
10	0.00535	0.0141	0.0507	0.1646
11	0.00532	0.0140	0.0504	0.1632
x	0.00535	0.0140	0.0504	0.1640
SD	0.0000447	0.0000562	0.000166	0.000632
% R.S.D.	0.27	0.40	0.33	0.39

 Table 3.34
 Precision verification using various concentrations of selenium standard

3.3.3.3 Calibration curve

As depicted in Figures 2.2 and 2.3, the standard solutions containing 0.01 - 2.50 mg L⁻¹ were aspirated with appropriate volume into the SIA system under the established optimum conditions (Table 3.32). The results are shown in Table 3.35. The calibration curve as shown in Figure 3.29 was established by plotting Δ peak height versus the various selenium concentrations. A correlation coefficients (r²) and the regression equation are as follows:

 $y = 0.0807x + 0.0023 (r^2 = 0.9990) (Se 0.01-2.50 mg L^{-1})$

Where y is $\Delta peak$ height in AU

x is concentration of selenium in mg L^{-1}

Table 3.35 $\Delta Peak$ height for calibration curve	
---	--

Selenium			Peak hei	ght (AU)		Δ Ρ. Η.*	
(mg L ⁻¹)	1	2	3	4	5	x	(AU)
0.00	0.0306	0.0306	0.0306	0.0306	0.0306	0.0306	0.0000
0.01	0.0317	0.0317	0.0317	0.0318	0.0318	0.0317	0.00114
0.02	0.0326	0.0326	0.0326	0.0326	0.0326	0.0326	0.00200
0.03	0.0334	0.0334	0.0333	0.0335	0.0335	0.0334	0.00282
0.05	0.0355	0.0361	0.0362	0.0360	0.0360	0.0359	0.00536
0.08	0.0420	0.0418	0.0419	0.0418	0.0418	0.0418	0.01126
0.10	0.0440	0.0440	0.0450	0.0450	0.0450	0.0446	0.01400
0.20	0.0480	0.0480	0.0480	0.0480	0.0480	0.0480	0.01740
0.40	0.0650	0.0650	0.0650	0.0650	0.0650	0.0650	0.03440
0.80	0.0990	0.0990	0.0990	0.0990	0.0990	0.0990	0.06840
1.00	0.1140	0.1150	0.1150	0.1150	0.1150	0.1140	0.08420
1.50	0.1560	0.1560	0.1560	0.1560	0.1560	0.1560	0.12540
2.00	0.1950	0.1950	0.1950	0.1940	0.1940	0.1940	0.16400
2.50	0.2320	0.2320	0.2320	0.2330	0.2310	0.2320	0.20140

average of five replicate results

ht[©] by Chiang Mai University rights reserved



Figure 3.29 The Calibration curve of SIA spectrophotometric determination of selenium $0.01 - 2.50 \text{ mg L}^{-1}$

3.3.3.4 Detection limit [61]

For investigation of detection limit, the detection limit of the proposed method was investigated by preparing various standard solutions containing low concentration of selenium which were introduced into the proposed SIA system. The detection limit (defined as the concentration of the analyte which give the signal equivalent to three time of the standard deviation of the blank signal) was found to be 0.0005 mg L⁻¹. from selenium 0.01 – 2.50 mg L⁻¹. The limit of quantitation was 0.0015 mg L⁻¹. The concentration at limit of detection (*S_m*) can be calculated from equation 2.2.

$$S_m = S_{bl} + kSD_{bl}$$

Where

Sm is the minimum analytical signal

S_{bl} is the mean blank signal

SD_{bl} is the standard deviation of blank

k is the confidence level of detection : 3

The linear regression equation is y = 0.0807x + 0.0023

Sm, y = 0.0023 + 3(0.000012)= 0.00233y = 0.0807x + 0.0023x, LOD = $0.0005 \text{ mg L}^{-1} \text{Se(IV)}$ LOQ, Sm = 0.0023 + 10(0.000012)= 0.00242x, LOQ = $0.0015 \text{ mg L}^{-1} \text{Se(IV)}$

3.3.3.5 Interference studies

The interference effects of some possible foreign ions on the determination of selenium were studied by the proposed SIA procedure under the optimum conditions obtained (Table 3.32). A systematic study to check for the effects of some possible foreign ions on the determination of selenium was undertaken for the maximum w/w ratio of selenium to foreign ions up to 1 : 5000. The solutions of a 1.00 mg L⁻¹ selenium standard containing varying concentrations of diverse ions were determined using the SIA system. The tolerance is defined as the largest foreign-ion concentration causing % error between 90 – 110% for determining the analyte of interest. The tolerance values for the ions studied are given in Table 3.36.



ลิขสิทธิมหาวิทยาลัยเชียงไหม Copyright[©] by Chiang Mai University All rights reserved

Interference	Selenium : Interference	Peak height [*] (AU)	% Error
Cu ²⁺	1:091	0.0140	-
0	1: 0.2	0.0149	106
	1: 0.5	0.0155	110
	1: 0.7	0.0159	113
Fe ²⁺	1: 0	0.0140	63 -
	1: 0.1	0.0146	104
	1: 0.5	0.0155	110
26	1: 1.0	0.0162	115
Fe ³⁺	1:0	0.0140	5252
	1: 0.1	0.0151	107
9	1: 0.3	0.0155	110
	1: 0.5	0.0162	115
NO ₂ ⁻	1: 0	0.0140	
	1: 0.1	0.0149	106
	1: 0.3	0.0153	109
	1: 0.5	0.0166	118
Na ⁺	1: 0	0.0140	-
5	1: 500	0.0136	98
INS I	1: 2500	0.0140	100

5000

0

1000

2500

1: 500

1:

1:

1:

1:

0.0140

0.0140

0.0152

0.0155

0.0148

100

105

108

110

Table 3.36 Interference studies for 1.00 mg L^{-1} selenium standard solution by SIA method

average of five replicate results

 K^+

Table 3.36	(Continued)
-------------------	-------------

Interference	Selenium : Interference	Peak height [*] (AU)	% Error
Mg ²⁺	1:091	0.0140	-
	1: 300	0.0148	105
2	1: 1500	0.0140	100
	1: 4000	0.0137	98
Ca ²⁺	1:0	0.0140	6
	1: 500	0.0143	102
	1: 2500	0.0148	105
-SIZA	1: 5000	0.0152	108
Ba ²⁺		0.0140	295
0,000	1: 100	0.0149	106
G	1: 500	0.0152	108
E	1: 1000	0.0156	111
Al ³⁺	1: 0	0.0140	
	1: 10	0.0148	105
	1: 50	0.0151	107
	1 : 100	0.0156	111
Mn ²⁺	1: 0	0.0140	-
~	1: 10	0.0146	104
angi	1:50	0.0152	108
GIION	1: 80	0.0154	110
Co ²⁺	by 1: hiang	0.0140	niversit
	1: 10	0.0146	104
I r I	3 1: 40	e 0.0155 e	110 e
	1: 60	0.0158	112

*average of five replicate results

Table 3.36	(Continued)
-------------------	-------------

Interference	Selenium : Interference	Peak height [*] (AU)	% Error
Zn ²⁺	1:091	0.0140	-
0	1: 500	0.0148	105
	1: 1500	0.0152	108
	1: 2000	0.0155	110
Ni ²⁺	1:0	0.0140	3 -
	1: 10	0.0145	103
	1: 100	0.0153	109
24	1: 150	0.0157	112
Cr ³⁺	1:0	0.0140	5057
	1: 10	0.0142	101
9	1: 50	0.0149	106
	1: 100	0.0155	110
Cd ²⁺	1: 0	0.0140	
	1: 10	0.0145	103
	1: 50	0.0149	106
	1: 100	0.0153	109
F ⁻	1: 0	0.0140	-
ວ. ວັ.	1: 500	0.0143	102
ansi	1: 1500	0.0136	98
d	1: 2000	0.0128	92
PO4 ³⁻		0.0140	inversi
l ri	g 1: 10	0.0143	102
	1: 500	0.0151	107
	1: 1000	0.0153	109

*average of five replicate results

Table 3.36 (Continued)

Interference	Selenium : Interference	Peak height [*] (AU)	% Error
NO ₃ -	1:0	0.0140	-
0	1: 100	0.0143	102
	1: 2500	0.0143	102
	1: 5000	0.0143	102
SO4 ²⁻	1:0	0.0140	3 -
	1 : 500	0.0145	103
	1: 2500	0.0136	98
502	1: 5000	0.0135	97
CO3 ²⁻	1:0	0.0140	204
	1: 300	0.0140	100
E	1: 1000	0.0148	105
	1: 3000	0.0156	111
Br⁻	1:0	0.0140	· / -
	1: 500	0.0151	107
	1: 2500	0.0146	104
	1: 5000	0.0135	97
Cl	1: 0	0.0140	-2
สทรเ	1: 100	0.0140	100
	1:800	0.0147	105
yright ^e	1 : 2500	0.0156	111

The interference effects of some possible foreign ions in the SIA system for determination of selenium were summarized in Table 3.37. It was found that Fe^{2+} , Fe^{3+} , Cu^{2+} and NO_2^{-} interfered the determination a selenium in surface waters, which referred to standard quality of surface waters defined by Pollution Control Department Ministry of Natural Resources and Environment (Appendix A).

Table 3.37 Summary of interference effects of some ions on the response obtainedfrom selenium 1.00 mg L^{-1} by SIA method

Interference ion	Tolerable concentration ratio [*]
Na ⁺ , Ca ²⁺ , Cl ⁻ , NO ₃ , SO ₄ ²⁻ , Br ⁻	5000
Mg ²⁺	4000
CO ₃ ²⁻	3000
Κ+	2500
Zn ²⁺ , F	2000
PO ₄ ³⁻ , Ba ²⁺	1000
Ni ²⁺	200
$Cr^{3+}, Al^{3+}, Cd^{2+}$	100
Mn ²⁺	
co ²⁺ ight [©] by Chia	ng Mai Universi
Cu ²⁺ , Fe ²⁺	rese ^{0.5} rve
Fe^{3+} , NO_2^-	0.3

^{*}The concentration of interference ion is considered to be interfered when causing % error less than 90% and more than 110% with respect of the signal of selenium alone.

3.3.3.6 Effects of masking agents and interference

The effects of masking agents and interference were studied by the proposed SIA procedure under the optimum conditions. In order to overcome interference effects of Fe^{2+} , Fe^{3+} , NO_2 and Cu^{2+} on Se(IV) determination, various masking agents were investigated. The results are shown in Table 3.38. It was found that Fe^{2+} and Fe^{3+} were masked with 0.1% (w/v) of NaF and 0.5% (w/v) of NaF respectively [63]. Cu^{2+} was masked with 0.3% (w/v) of EDTA [64] and NO_2^- was masked with 0.005% (w/v) NH₃SO₃[64].

Table 3.38 Effect of masking agents for mask Fe^{2+} , Fe^{3+} , Cu^{2+} and NO_2^{-} on the response obtained from selenium 1.00 mg L⁻¹

Interference	Concentration of masking agent	Selenium :Interference	Peak height [*] (AU)	% Error
Fe ²⁺		1:0	0.0140	-
	0.05% (w/v) NaF	1:1	0.0145	103
		1:5	0.0147	105
		1: 10	0.0152	108
	2	1: 0	0.0140	-
alânŝ	0.1% (w/v) NaF		0.0146	104
		1: 10	0.0153	109
opyrigh		hiarig 15 Aai	0.0159	113
		1: 0	0.0140	
	0.5% (w/v) NaF		0.0143	102
		1: 10	0.0149	106
		1: 15	0.0157	112

^{*}average of five replicate results

NaF was sodium fluoride

Table 3.38 (continued)

Interference	Concentration of masking agent	Selenium :Interference	Peak height [*] (AU)	% Error
Fe ³⁺	-12	1:0	0.0140	-
	0.1% (w/v) NaF	1: 0.5	0.0145	103
			0.0156	111
6		1:-5	0.0206	147
6	0.3% (w/v) NaF	1:0	0.0140	-
6		<u> </u>	0.0140	100
		1: 5	0.0142	101
S S S		1: 10	0.0176	125
500	0.5% (w/v) NaF	1: 0	0.0140	
CH		1:1	0.0130	93
		1: 5	0.0145	103
		1: 10	0.0151	107
Cu ²⁺		1: 0	0.0140	-
	0.1% (w/v) EDTA	1: 5	0.0152	108
		1: 10	0.0162	115
		1: 20	0.0180	128
	0.3% (w/v) EDTA	1: 0	0.0140	-
		1: 10	0.0152	108
		1:20	0.0155	110
		1: 50	0.0159	113
	t = by t		0.0140	rsity
	0.5% (w/y) EDTA	S ¹ : r ⁵ e S	0.0152	108
	0.370 (w/V) ED1A	1: 10	0.0155	110
		1: 20	0.0162	115

*average of five replicate results

NaF was sodium fluoride

EDTA was ethylenediaminetetraacetic acid

Table 3.38 (continued)

Interference	Concentration of masking agent	Selenium :Interference	Peak height [*] (AU)	% Error
NO ₂ -	0.001% (w/v) NH ₃ SO ₃	0 1:0	0.0140	-
		1: 5	0.0145	103
		1: 10	0.0156	111
		1:-20	0.0162	115
	0.005% (w/v) NH ₃ SO ₃	1:0	0.0140	-
		1: 10	0.0146	104
		1: 20	0.0155	110
		1: 50	0.0160	114
	0.01% (w/v) NH ₃ SO ₃	1: 0	0.0140	2 -
		1: 10	0.0148	105
		1: 20	0.0156	111
		1: 50	0.0167	119

*average of five replicate results

NH₃SO₃ was sulfamic acid

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่ Copyright[©] by Chiang Mai University All rights reserved

3.3.3.7 Determination of selenium in waters

The proposed SIA spectrophotometric method was applied to the simultaneous determination of selenium in surface water samples which were collected from Chiang Mai canal and Chiang Mai University reservoir. The peak heights of each sample were compared with standard calibration curve. The results were given in Table 3.39.

224	Peak heights (AU)				Selenium	0/.	
Water samples	1 8	2	3	x	SD	concentration [*] (mg L ⁻¹)	⁷⁰ Recovery [*]
Suan Dok Gate	ND ^{**}	ND**	ND**	J	-	ND**	-
Hua Lin Corner	0.015	0.016	0.017	0.016	0.001	0.016 ± 0.001	100.16
Chang Phuak Gate	ND ^{**}	ND ^{**}	ND**		-	ND**	-
Sri Poom Corner	0.050	0.054	0.052	0.052	0.002	0.052 ± 0.002	101.24
Thaphae Gate	ND**	ND**	ND ^{**}	TT	ER	ND**	-
Khatum Corner	0.022	0.024	0.025	0.023	0.002	0.024 ± 0.002	99.58
Chiang mai Gate	0.030	0.028	0.026	0.028	0.002	0.028 ± 0.002	100.08
Khu Huang Corner	ND**	ND**	ND**	98	191	ND**	K 31
Ang-Keaw I	ND**	ND**	ND**	-	-	ND ^{**}	-
Ang-Keaw II	ND**	ND**	ND ^{**}	ng	Ma	ND** Ver	SITY
l ri	σ	h t	S		A	e r v	

 Table 3.39 Determination of selenium in water samples by SIA method

*average of five replicate results

**not detected
The selenium contents in the water samples were in the range of $0.016 - 0.052 \text{ mg L}^{-1}$ and $0.016 - 0.050 \text{ mg L}^{-1}$ using the proposed method and ICP-MS respectively. The results obtained by the proposed SIA spectrohotometric method were compared with those obtained by ICP-MS using the student t-test (Table 3.40 and Appendix B in Table B.2). It was evident that the t-value for Se(IV) contents in water samples determined by comparison the results obtained by SIA spectrohotometric method with those obtained by ICP-MS was 0.387. It was seen that the experimental t-value for Se(IV) assay(0.387) which was smaller than the theoretical t-value with a confidence interval of 95% (3.18) indicating that the results obtained by both methods were in excellent agreement.

Table 3.40 Comparative determination of selenium in water samples by proposed

 SIA method and ICP-MS

Water samples	Concentrations (mg L ⁻¹)		
	SIA [*]	ICP-MS*	^x d
Suan Dok Gate	ND***	ND**	-
Hua Lin Corner	0.016	0.016	0.000
Chang Phuak Gate	ND ^{**}	ND ^{**}	-
Sri Poom Corner	0.052	0.050	0.002
Thaphae Gate	ND ^{**}	ND**	- 1
Khatum Corner	0.023	0.024	-0.001
Chiang mai Gate	0.028	0.028	0.000
Khu Huang Corner	ND**	ND ^{**}	lnive
Ang-Keaw I	ND**	ND ^{**}	
Ang-Keaw II	ND**	ND**	r - 1/
Σ			0.001
Sd			0.00129
Т			0.387

^{*}average five replicate results

** not detected