CHAPTER 3

RESULTS AND DISCUSSION

The FI and SI spectrophotometric methods for speciation of cadmium(II) using rhodamine B in wastewater samples were developed. The experimental conditions considered were evaluated by univariate optimization method. The proposed FIA and SIA methods were applied to wastewater samples collected in Chiang Mai Province.

3.1 Preliminary Study of Absorption Spectra

Preliminary study showed that cadmium reacted with iodide in acidic medium and followed by rhodamine B resulting in a violet complex. The absorbances of the complex and reagent solutions were scanned between 400-700 nm using UV-Vis Lambda 25 spectrophotometer as shown in Figure. 3.1.

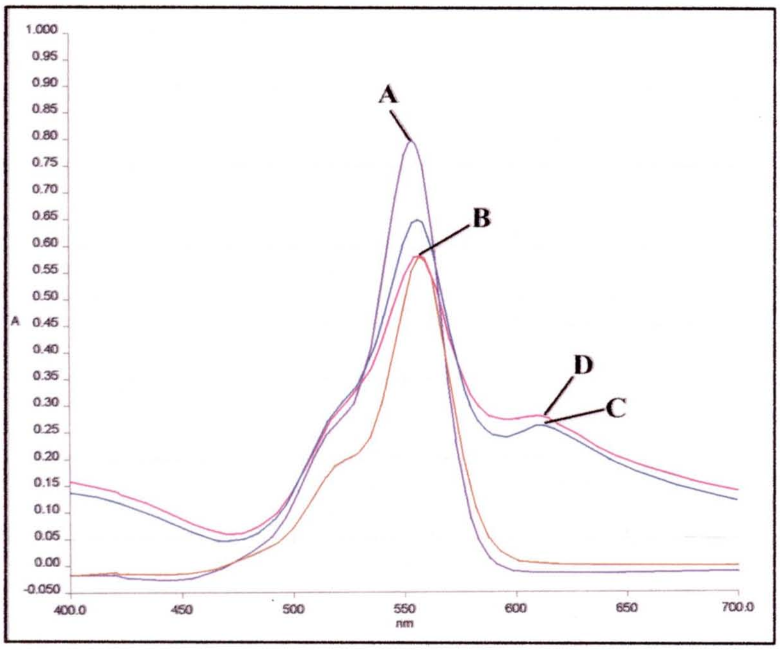

Figure 3.1 The absorption spectra of reagent and complex solutions:

(A) Rhodamine B, (B) Rhodamine B-KI, (C) Cd(II)-Rhodamine B, and

(D) Cd(II)-Rhodamine B-KI complexes against water at pH 3.0

The solution of ion-association complex presented maximum absorption at 612 nm while that reagent solution showed maximum absorption at 557 nm and low absorption at about 612 nm under the same experimental conditions which means that positive peak at 612 nm can be measured. In this study, a spectrophotometric procedure was proposed for determining cadmium(II) using rhodamine B as complexing agent.

3.2 Stoichiometry of the Complexes using Mole-Ratio Method

The mole ratio method was used to study the composition of Cd(II)-iodide-rhodamine B ternary complex. In this method, a series of solution were prepared containing a fixed amount of metal ion, with varying concentrations of ligand.

The absorbances of these solutions were then measured. A resulting plot of absorbance vs. ligand-to-metal ratio initially increased then became constant one and the ligand to metal ratio has been achieved. The point at which the slope of the line changed corresponded to the ligand: metal ratio of the complex [85]. The mole-ratio method of Cd(II)-Rhodamine B-Iodide complex was defined as 2 series of solution were prepared in which cadmium(II) and KI concentrations were fixed while the rhodamine B concentration was varied. Another one was prepared in which cadmium(II) and rhodamine B concentrations were fixed while the KI concentration was varied.

Series I; the various concentrations of rhodamine B were added to the solution containing 1×10^{-4} mol L^{-1} of Cd(II), 1×10^{-4} mol L^{-1} KI, 5 mL of 0.10 mol L^{-1} of phosphate buffer pH 3.0 and diluted with deionized water in 25 mL volumetric flasks. Absorbance of each solution was measured at 612 nm. The results were shown in Table 3.1 and Figure 3.2. It was found that the absorbance as peak height increased to maximum at concentration of rhodamine B was 3×10^{-4} mol L^{-1} and then it became constant. Therefore, 3×10^{-4} mol L^{-1} of rhodamine B was chosen for studying effect of KI concentration in series II.

Table 3.1 Effect of rhodamine B concentrations for mole-ratio of Cd(II)-Rhodamine B-Iodide complex

[Rhodamine B] (x 10 $^{-4}$ mol L^{-1})	**Mole ratio (Cd(II):Rhodamine B)**	**Absorbance (AU)***
0	1:0	0.060
1	1:1	0.063
2	1:2	0.066
3	1:3	0.070
4	1:4	0.071
5	1:5	0.071
6	1:6	0.071

*average of triplicate results

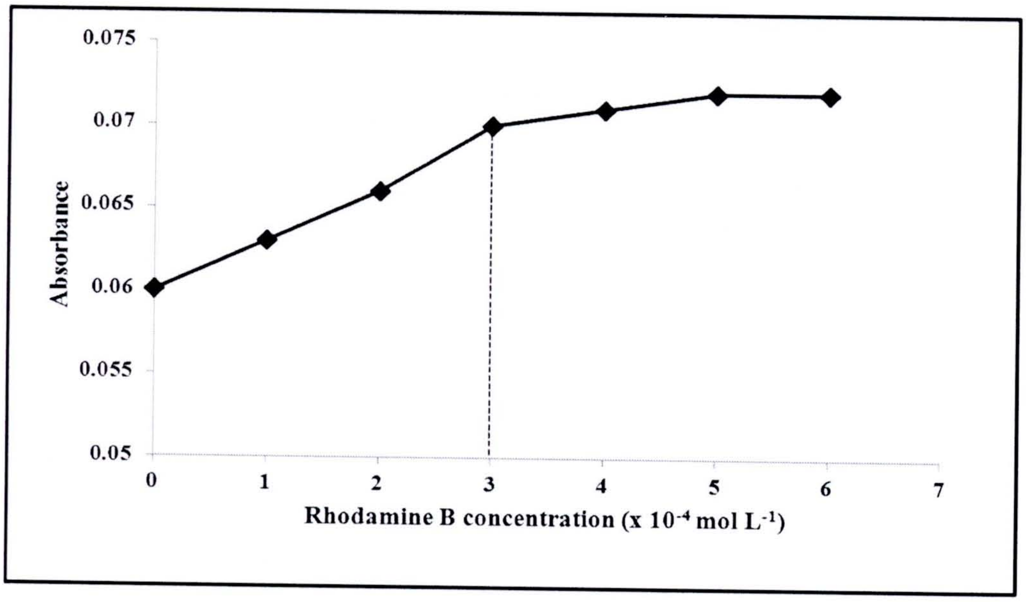

Figure 3.2 Mole-ratio study of Cd(II)-Rhodamine B-Iodide system; effect of rhodamine B concentration, Cd(II) 1x10^{-4} mol L^{-1}, KI 1x10^{-4} mol L^{-1}, pH 3.0, wavelength 612

Series II ; the various concentrations of KI were added to solution containing 1×10^{-4} mol L^{-1} of Cd(II), 3×10^{-4} mol L^{-1} of rhodamine B, 5 mL of 0.10 mol L^{-1} of phosphate buffer pH 3.0 and diluted with deionized water in 25 mL volumetric flasks. Absorbance of each solution was measured at 612 nm. The results are shown in Table 3.2 and Figure 3.3. It was found that the absorbance as peak height increased to maximum up to the KI concentration of 3×10^{-4} mol L^{-1} then it became constant and so did the absorbance in AU. Therefore, 3×10^{-4} mol L^{-1} of KI concentration was chosen.

Table 3.2 Effect of KI concentrations for mole-ratio of Cd(II)-Rhodamine B-Iodide complex

[KI] (x 10 $^{-4}$ mol L^{-1})	**Mole ratio (Cd(II):Rhodamine B:KI)**	**Absorbance (AU)***
0	1:3:0	0.059
1	1:3:1	0.062
2	1:3:2	0.066
3	1:3:3	0.071
4	1:3:4	0.072
5	1:3:5	0.073
6	1:3:6	0.073
7	1:3:7	0.074

*average of triplicate results

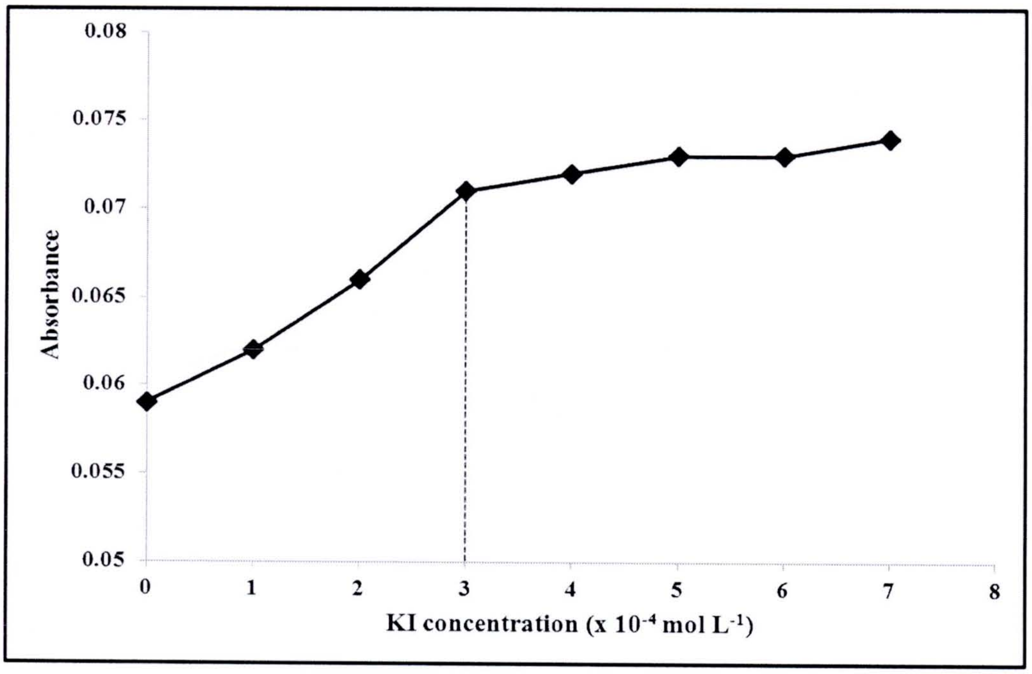

Figure 3.3 Mole-ratio study of Cd(II)-Rhodamine B-Iodide system; effect of KI concentration, Cd(II) $1x10^{-4}$ mol L^{-1},Rhodamine B $3x10^{-5}$ mol L^{-1}, pH 3.0, wavelength 612 nm

From experimental results in Tables 3.1-3.2 and Figures 3.2-3.3 gave a mole-ratio of Cd(II):Rhodamine B:KI at 1:3:3. So, the complexation of Cd(II)-Rhodamine B-KI was shown in Figure 3.4.

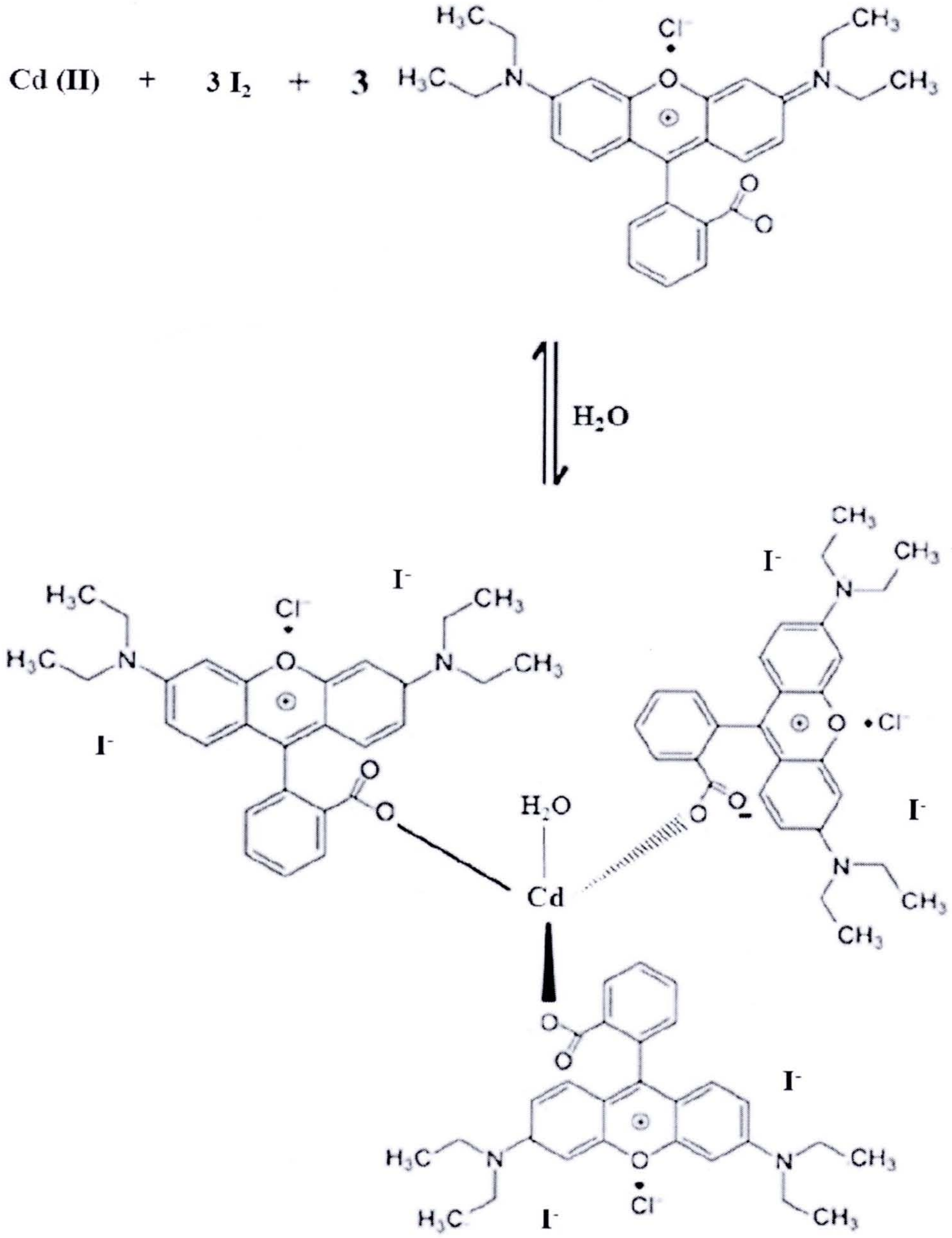

Figure 3.4 The complexation of Cd(II)-Rhodamine B-Iodide

3.3 FIA Spectrophotometric Determination of Cadmium(II) using Rhodamine B as A Complexing Agent

3.3.1 Optimization of the FIA system by univariate method

The conditions for the determination of cadmium(II) were optimized by studying the influences of the various parameters, such as wavelength, pH, concentration of rhodamine B, KI and hydroxylamine HCl, flow rate, reaction coil inner diameter, reaction coil length and sample volume, 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. The range of variables studied and the optimal values were chosen as shown in Table 2.1.

3.3.1.1 Optimum wavelength

Optimization of the experimental conditions was started with optimum wavelength for cadmium(II) determination. A comparative study of the absorption spectrum of the ion-association complex was carried out by FIA method with spectrophotometric detection using the experimental conditions (fixed by random) as shown in Table 2.2. The optimum wavelength was investigated over the range 605 to 620 nm by introducing 0.1, 0.2 and 0.3 ppm of cadmium standard solutions into the proposed FI system (Figure 2.1). The FI signals in the form of Δpeak height (ΔP.H.) and the highest sensitivity of the method (defined as slope of calibration curve) obtained at various operating wavelengths. Δpeak height (ΔP.H.) was the difference between peak height of blank and peak height of analyte. The results shown in Table 3.3 and Figure 3.5 indicated that the highest sensitivity of the method was obtained when the absorbance was measured at 612 nm. Therefore, this wavelength was chosen as optimum condition for further FIA measurements in order to assess the best sensitivity.

Table 3.3 Effect of wavelengths on the sensitivity

Wavelength (nm)	**ΔPeak height (mV)* obtained for standard Cd(II) (mg L^{-1})**			**Sensitivity (mV/ppm)**	**Correlation coefficient (r^2)**
	0.1	**0.2**	**0.3**		
605	0.099	0.286	0.481	0.191	0.9999
606	0.104	0.289	0.492	0.194	0.9993
607	0.102	0.287	0.491	0.195	0.9992
608	0.104	0.290	0.499	0.198	0.9989
609	0.105	0.295	0.504	0.200	0.9992
610	0.115	0.302	0.517	0.201	0.9984
611	0.115	0.305	0.520	0.203	0.9987
612	0.117	0.325	0.524	0.204	0.9998
613	0.114	0.313	0.506	0.196	0.9999
614	0.087	0.279	0.462	0.18	0.9998
615	0.085	0.269	0.444	0.180	0.9998
616	0.078	0.257	0.429	0.176	0.9999
617	0.076	0.225	0.416	0.170	0.9991
618	0.075	0.232	0.396	0.161	0.9998
619	0.072	0.218	0.380	0.154	0.9991
620	0.067	0.218	0.353	0.143	0.9990

*average of triplicate results

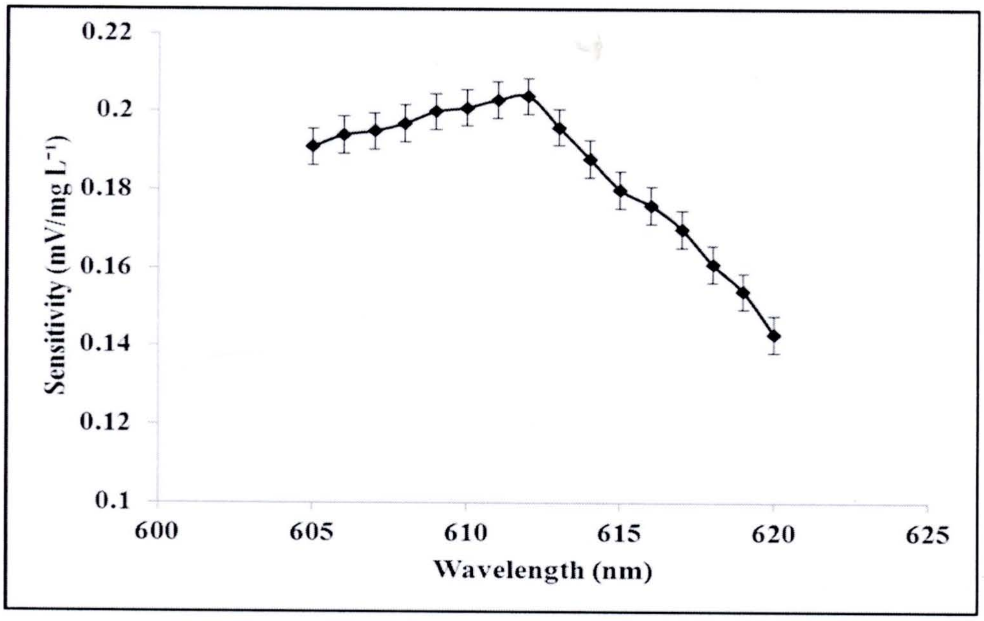

Figure 3.5 Relationship between various wavelengths and sensitivity of the calibration curve

3.3.1.2 Effect of pH

The effect of pH on the formation of Cd(II)-Rhodamine B was studied by varying of the pH of phosphate buffer from difference in the range of 1.5-4.0. The pH values of the solution were adjusted with phosphoric acid (H_3PO_4) and sodium dihydrogen phosphate (NaH_2PO_4). The results obtained are shown in Table 3.4 and Figure 3.6. The optimum pH for a maximum absorbance at 612 nm was found to be pH 3.0. The sensitivity (slope of the calibration curve) decreased significantly when the pH above 3.0. Therefore, pH 3.0 was selected because it provided the greatest sensitivity.

Table 3.4 Effect of pH on the sensitivity

pH	ΔPeak height* (mV) obtained from the standard Cd(II) (mg L^{-1})					Sensitivity (mV/mg L^{-1})	Correlation coefficient (r^2)
	0.1	0.2	0.3	0.4	0.5		
1.5	0.066	0.232	0.426	0.585	0.717	1.655	0.9959
2.0	0.061	0.215	0.433	0.603	0.748	1.762	0.9956
2.5	0.014	0.180	0.376	0.557	0.751	1.851	0.9993
3.0	0.066	0.247	0.439	0.623	0.812	1.868	0.9999
3.5	0.108	0.311	0.475	0.635	0.801	1.710	0.9978
4.0	0.103	0.162	0.238	0.311	0.367	0.677	0.9973

* average of triplicate results

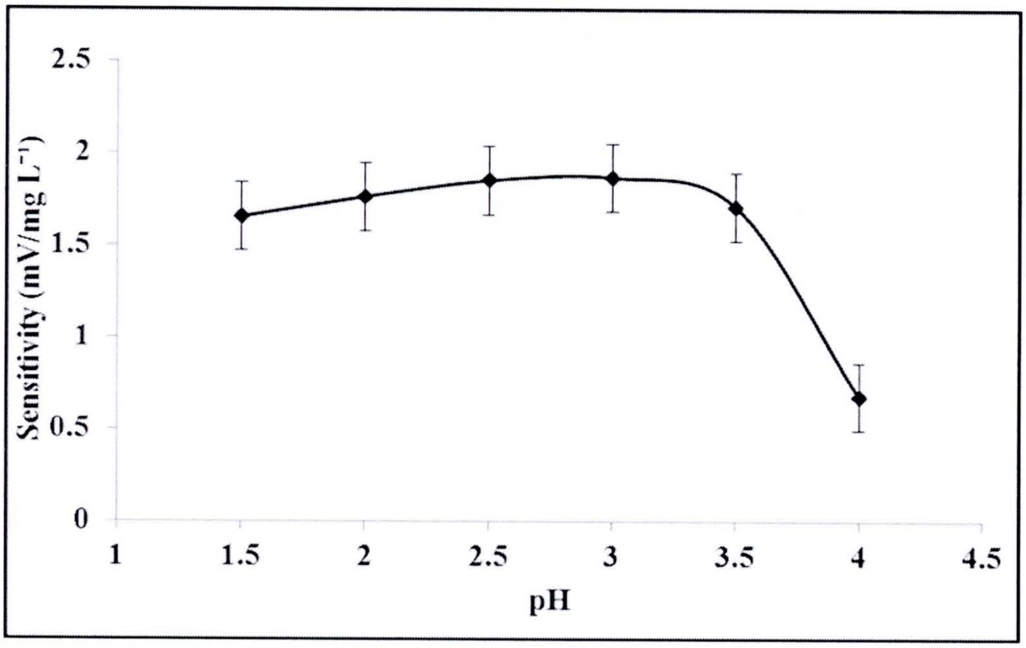

Figure 3.6 Relationship between pH and sensitivity of the calibration curve

3.3.1.3 Effect of KI concentration

Effect of KI concentrations on the determination of cadmium(II) was studied in the range 0.05-0.11 mol L^{-1}. The results are shown in Table 3.5 and Figure 3.7. The results indicated that the sensitivity increased rapidly with increasing KI concentration up to 0.07 mol L^{-1} and decreased rapidly when the concentration of KI was greater than 0.07 mol L^{-1}. Thus 0.07 mol L^{-1} of KI concentration was chosen as optimum concentration for subsequent experiment since it provided the highest sensitivity for this investigation.

Table 3.5 Effect of KI concentration on the sensitivity

KI concentration (mol L^{-1})	**ΔPeak height (mV)* obtained from the standard Cd(II) (mg L^{-1})**					**Sensitivity (mV/mg L^{-1})**	**Correlation coefficient (r^2)**
	0.1	**0.2**	**0.3**	**0.4**	**0.5**		
0.05	0.113	0.254	0.427	0.594	0.755	1.624	0.9991
0.06	0.071	0.259	0.437	0.594	0.778	1.749	0.9992
0.07	0.075	0.263	0.444	0.626	0.814	1.841	0.9999
0.08	0.060	0.236	0.447	0.627	0.766	1.803	0.9957
0.09	0.045	0.201	0.389	0.56	0.749	1.767	0.9991
0.10	0.039	0.202	0.382	0.56	0.709	1.698	0.9990
0.11	0.105	0.281	0.449	0.597	0.742	1.590	0.9980

* average of triplicate results

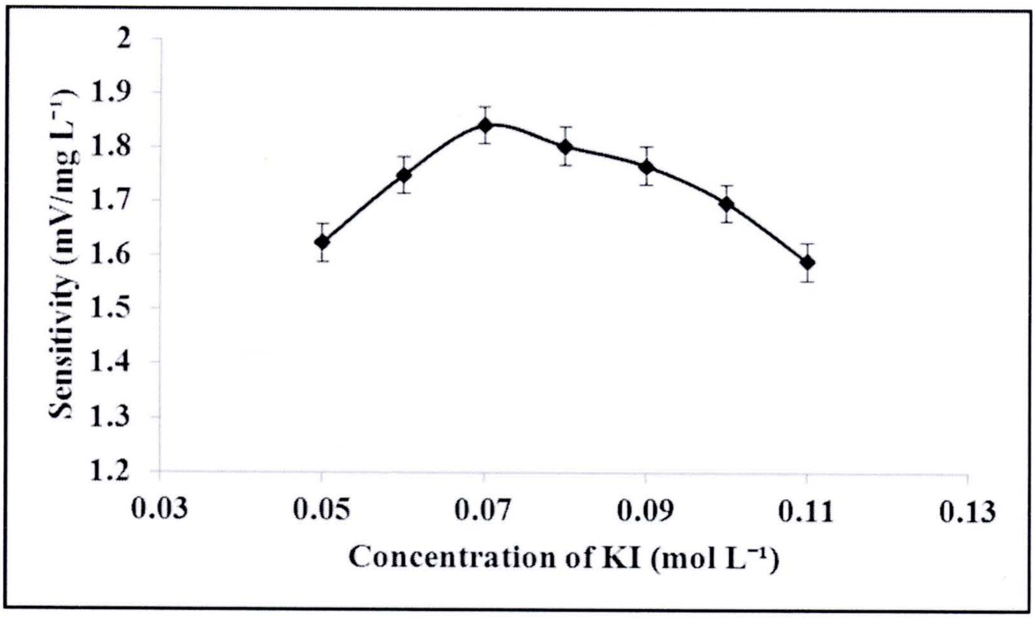

Figure 3.7 Relationship between KI concentration and sensitivity of the calibration curve

3.3.1.4 Effect of hydroxylamine HCl (H_3NOHCl) concentration

The hydroxylamine HCl (H_3NOHCl) solution was added as solubilizer to prevent precipitation in complexation reaction. Effects of various hydroxylamine HCl concentrations on peak height were investigated by varying the concentration of hydroxylamine HCl in the range of 0.5-3.0x10^{-4} mol L^{-1}. The results obtained were exhibited in Table 3.6 and Figure 3.8. From the experimental results, the sensitivity of calibration over the range of 0.1-0.5 mg L^{-1} cadmium(II) increased with hydroxylamine HCl concentration up to 1.5x10^{-4} mol L^{-1}, followed by a slight decrease in peak height at the concentration of 2.0x10^{-4} mol L^{-1}. Afterwards, the slope of calibration curve decreased slightly when the hydroxylamine HCl concentration reached 3.0x10^{-4} mol L^{-1}. The results indicated that the highest sensitivity was obtained when the concentration of hydroxylamine HCl was 1.5x10^{-4} mol L^{-1}.

Table 3.6 Effect of hydroxylamine HCl concentration on the sensitivity

H_3NOHCl concentration ($x10^{-4}$mol L^{-1})	ΔPeak height (mV)* obtained from the standard Cd(II) (mg L^{-1})					Sensitivity (mV/mg L^{-1})	Correlation coefficient (r^2)
	0.1	0.2	0.3	0.4	0.5		
0.5	0.060	0.187	0.347	0.493	0.601	1.388	0.9964
1.0	0.049	0.176	0.319	0.451	0.614	1.405	0.9982
1.5	0.051	0.190	0.359	0.522	0.703	1.636	0.9981
2.0	0.028	0.156	0.287	0.423	0.568	1.347	0.9994
2.5	0.026	0.150	0.276	0.419	0.556	1.329	1.9991
3.0	0.021	0.128	0.272	0.377	0.508	1.223	0.9980

* average of triplicate results

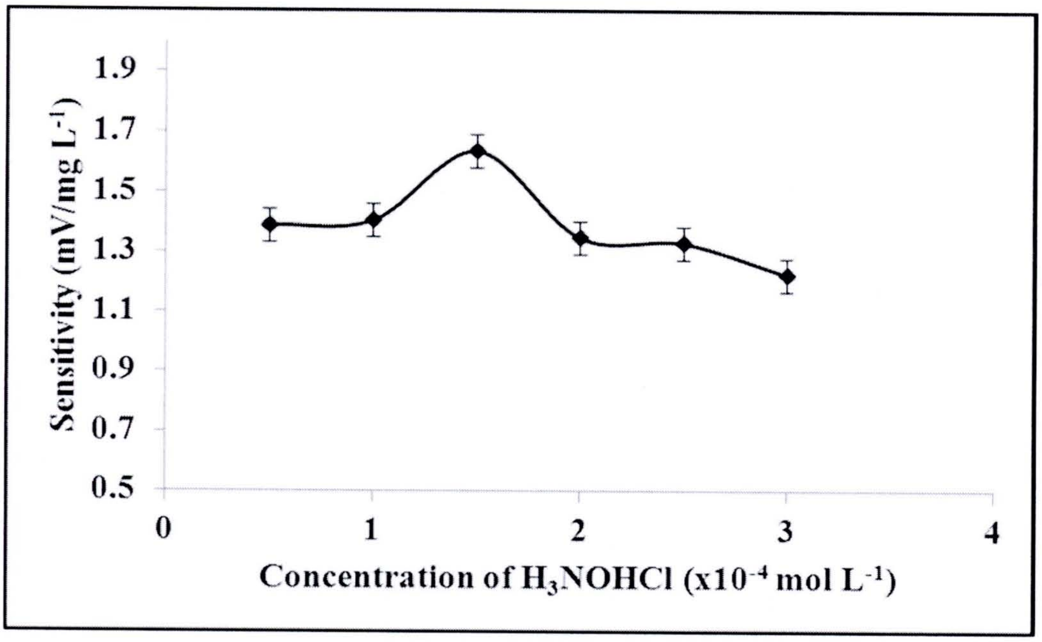

Figure 3.8 Relationship between hydroxylamine HCl concentration and sensitivity of the calibration curve

3.3.1.5 Effect of rhodamine B concentration

Effect of rhodamine B concentrations on the determination of cadmium(II) was studied in the range 1.0-5.0x10^{-4} mol L^{-1}. The results obtained are shown in Table 3.7 and Figure 3.9. The result was found that sensitivity increased very rapidly from the rhodamine B concentration of 1.0-2.5x10^{-4} mol L^{-1}. After that, the sensitivities were decreased. This is due to the fact the increasing the rhodamine B concentration leading to the increase in amounts of Cd(II)-Iodide-Rhodamine B complexation which results in a higher sensitivity. However, beyond the rhodamine B of 2.5x10^{-4} mol L^{-1}, the absorbance of Cd(II)-Iodide-Rhodamine B complex decreased. Consequently, a concentration of 2.5x10^{-4} mol L^{-1} of rhodamine B was selected as optimum concentration.

Table 3.7 Effect of rhodamine B concentration on the sensitivity

Rhodamine B concentration (x10^{-4}mol L^{-1})	**ΔPeak height (mV)* obtained from the standard Cd(II) (mg L^{-1})**					**Sensitivity (mV/mg L^{-1})**	**Correlation coefficient (r^2)**
	0.1	**0.2**	**0.3**	**0.4**	**0.5**		
1.0	0.011	0.068	0.134	0.200	0.272	0.654	0.9984
1.5	0.023	0.113	0.221	0.311	0.402	0.956	0.9990
2.0	0.032	0.119	0.231	0.336	0.464	1.081	0.9961
2.5	0.040	0.151	0.280	0.391	0.533	1.226	0.9983
3.0	0.040	0.118	0.250	0.338	0.468	1.076	0.9935
3.5	0.044	0.129	0.258	0.365	0.453	1.054	0.9957
4.0	0.041	0.117	0.219	0.337	0.452	1.042	0.9934
4.5	0.044	0.132	0.248	0.348	0.451	1.030	0.9987
5.0	0.039	0.124	0.225	0.335	0.438	1.009	0.9981

* average of triplicate results

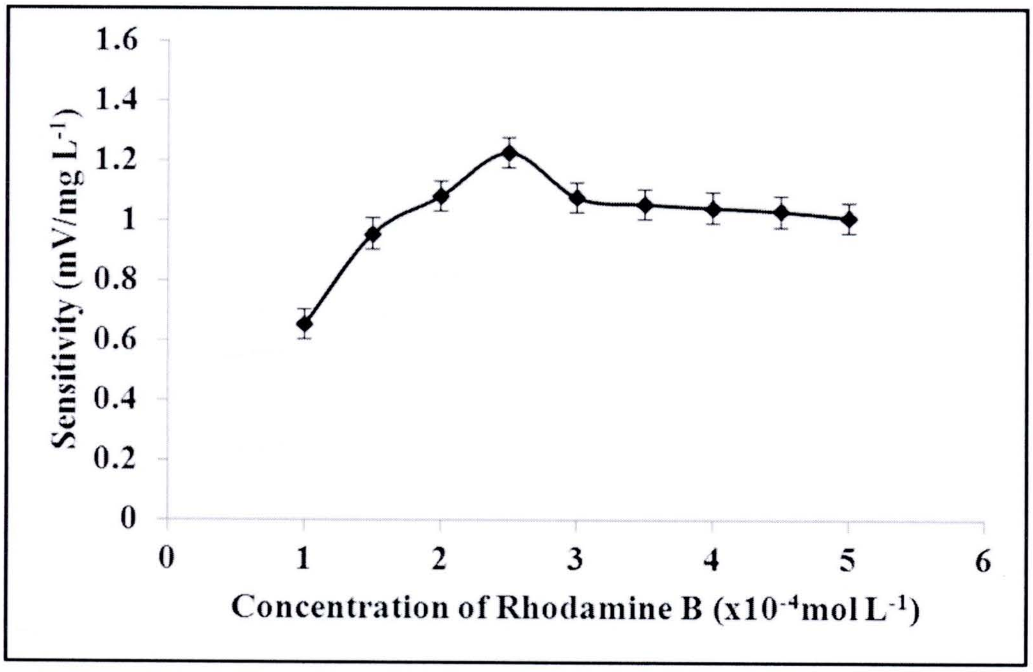

Figure 3.9 Relationship between rhodamine B concentration and sensitivity of the calibration curve

3.3.1.6 Effect of flow rate

In the FI system, the KI and rhodamine B reagent solutions were pumped into two-line manifold via peristaltic pump. The effect of flow rate was studied over the range 1.5-6.0 mL min^{-1} by injecting of various concentration of cadmium(II) standard solutions (0.1-0.5 mg L^{-1}) into the flow system as shown in Figure 2.1. The results obtained are shown in Table 3.8 and Figure 3.10. It was found that optimum flow rate for the carrier and reagent solutions were 3.5 mL min^{-1} because at the flow rate lower than 3.5 mL min^{-1}, it caused higher dispersion due to band broadening. Consequently, the sensitivity was lower, when the flow rate was higher than 3.5 mL min^{-1} and the reaction time was reduced. This resulted in less complex formation. Hence, the sensitivity was lower. Thus a flow rate 3.5 mL min^{-1} was chosen as optimum flow rate in order to obtain highest sensitivity.

Table 3.8 Effect of flow rate on the sensitivity

Flow rate (mL min^{-1})	ΔPeak height (mV)* obtained from the standard Cd(II) (mg L^{-1})					Sensitivity (mV/mg L^{-1})	Correlation coefficient (r^2)
	0.1	0.2	0.3	0.4	0.5		
1.5	0.046	0.15	0.246	0.346	0.434	0.972	0.9993
2.0	0.047	0.156	0.268	0.368	0.468	1.054	0.9993
2.5	0.047	0.156	0.268	0.371	0.487	1.095	0.9998
3.0	0.048	0.156	0.292	0.397	0.537	1.219	0.9979
3.5	0.048	0.169	0.305	0.433	0.575	1.318	0.9994
4.0	0.054	0.163	0.301	0.399	0.538	1.204	0.9975
4.5	0.039	0.143	0.262	0.365	0.479	1.102	0.9996
5.0	0.039	0.134	0.249	0.337	0.445	1.015	0.9988
5.5	0.036	0.126	0.237	0.325	0.428	0.983	0.9990
6.0	0.031	0.115	0.227	0.316	0.412	0.963	0.9985

* average of triplicate results

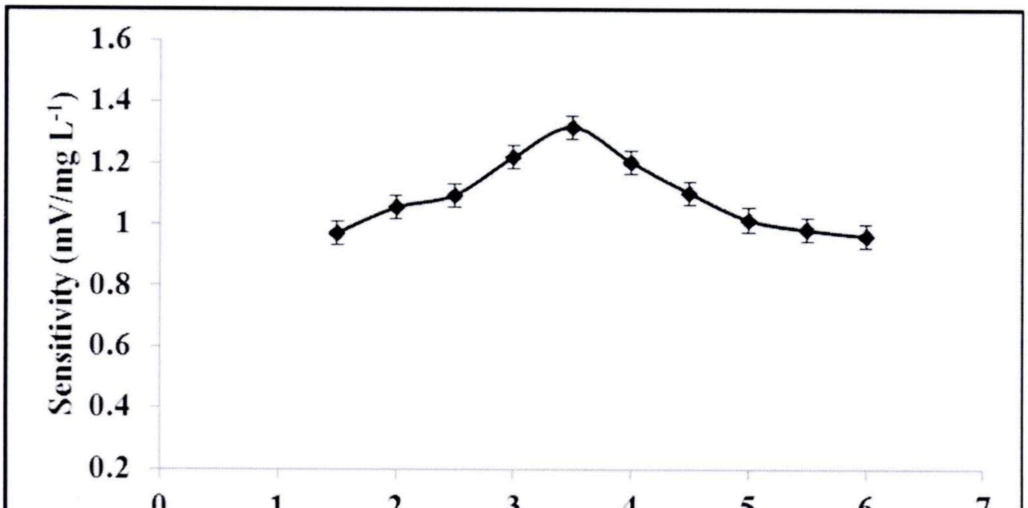

Figure 3.10 Relationship between flow rate and sensitivity of the calibration curve

3.3.1.7 Effect of reaction coil inner diameter (i.d.)

The tube diameter can affect the flow rate and hence affect the sensitivity. Therefore, it is important to find out an appropriate inner diameter of reaction coil. Optimization of inner diameter was studied over the range 0.051-0.132 cm. The results are shown in Table 3.9 and Figure 3.11 indicated that the sensitivity of calibration curve increased with the inner diameter (from 0.051-0.086 cm) when inner diameter was greater than 0.086 cm, the sensitivity decreased. Therefore, the reaction coil with the inner diameter of 0.086 cm was chosen as optimum with the highest sensitivity for this investigation.

Table 3.9 Effect of the reaction coil inner (i.d.) diameter on the sensitivity

reaction coil inner diameter (i.d. cm)	**ΔPeak height (mV)* obtained from the standard Cd(II) (mg L^{-1})**					**Sensitivity ($mV/mg\ L^{-1}$)**	**Correlation coefficient (r^2)**
	0.1	**0.2**	**0.3**	**0.4**	**0.5**		
0.051	0.056	0.198	0.335	0.510	0.667	1.534	0.9979
0.086	0.066	0.251	0.419	0.585	0.741	1.684	0.9991
0.107	0.078	0.231	0.384	0.547	0.665	1.490	0.9977
0.132	0.082	0.220	0.364	0.509	0.663	1.451	0.9996

* average of triplicate results

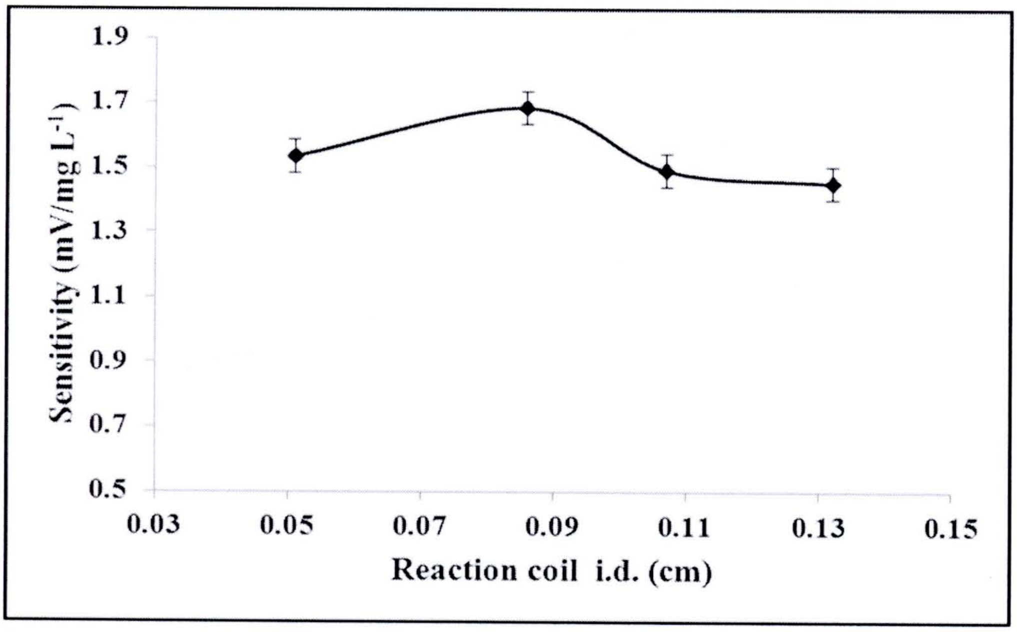

Figure 3.11 Relationship between the reaction coil inner diameter (i.d.) and sensitivity of the calibration curve

3.3.1.8 Effect of reaction coil length

The length of reaction coil is defined as the portion or part of the tubing, where the sample and reagent are mixed and reacted. The color develops and the absorbance of which is measured. The distance between the point of injection and the flow through cell should be kept as short as possible, yet should still be long to allow sufficient time for color development and/or effective reaction taking place. In this work, the effect of the reaction coil length was studied by varying the reaction coil length from 25-150 cm. The results are shown in Table 3.10 and Figure 3.12. It can be seen that the sensitivity increased with the length of reaction coil up to 75 cm. Afterwards, the sensitivity decreased when the longer reaction coil was used. Therefore the 75 cm was chosen as optimum length for reaction coil to obtain the highest sensitivity.

Table 3.10 Effect of the reaction coil length on the sensitivity

Reaction coil length (cm)	ΔPeak height (mV)* obtained from the standard Cd(II) (mg L^{-1})					Sensitivity (mV/mg L^{-1})	Correlation coefficient (r^2)
	0.1	0.2	0.3	0.4	0.5		
25	0.056	0.197	0.334	0.51	0.667	1.535	0.9978
50	0.065	0.205	0.384	0.564	0.698	1.625	0.9972
75	0.065	0.208	0.377	0.556	0.709	1.636	0.9987
100	0.056	0.198	0.335	0.510	0.667	1.534	0.9979
125	0.044	0.178	0.312	0.478	0.637	1.486	0.9975
150	0.07	0.226	0.369	0.512	0.665	1.476	0.9997

* average of triplicate results

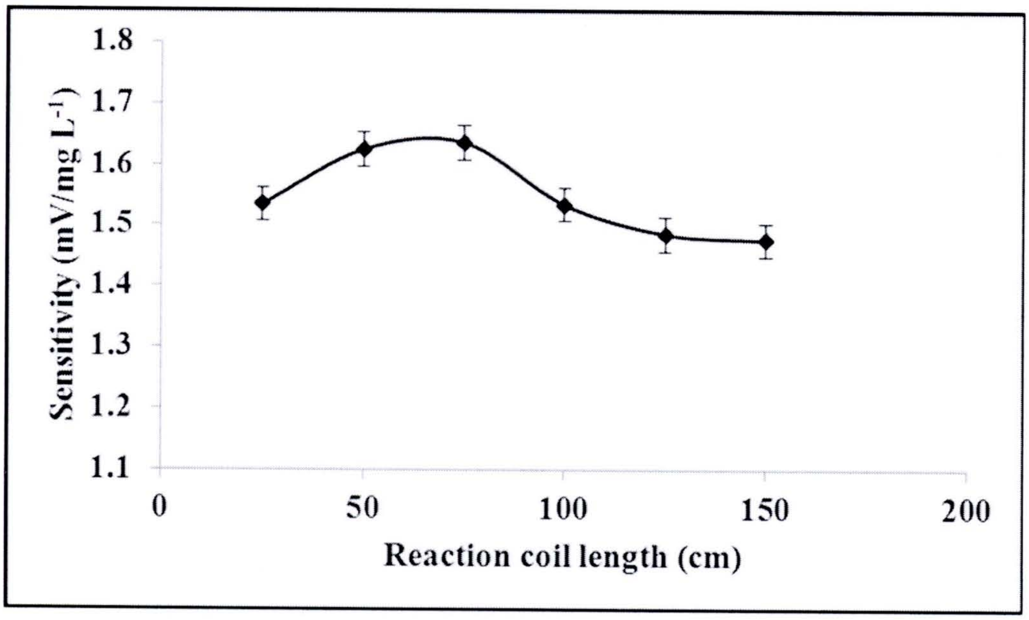

Figure 3.12 Relationship between the reaction coil length and sensitivity of the calibration curve

3.3.1.9 Effect of sample volume

The sample volume is one of the significant flow parameters. The dispersion in FIA system depends on sample volume. The sample volume was optimized in order to obtain adequate sensitivity by injected into the reagent stream has a significant effect on the peak height. This effect was studied by varying the loop length of injection valve to give a sample volume over the range 75-300 μl. The resulting experimental observations were shown in Tables 3.11 and Figures 3.13. It was found that the sensitivity increase with increasing sample volume up to 200 μL. Since, the increasing of sample volume leading to increases in the number of mole of cadmium(II) that caused higher absorbance. Therefore, a sample volume of 200 μl was found to be the optimum condition with low reagent consumption and greatest sensitivity.

Table 3.11 Effect of sample volume on the sensitivity

Sample volume (μL)	**ΔPeak height (mV)* obtained from the standard Cd(II) (mg L^{-1})**					**Sensitivity (mV/mg L^{-1})**	**Correlation coefficient (r^2)**
	0.1	**0.2**	**0.3**	**0.4**	**0.5**		
75	0.012	0.119	0.239	0.365	0.482	1.186	0.9993
100	0.013	0.138	0.276	0.435	0.57	1.411	0.9985
150	0.014	0.149	0.322	0.487	0.659	1.628	0.9983
200	0.023	0.197	0.380	0.579	0.751	1.838	0.9995
250	0.028	0.172	0.317	0.493	0.636	1.537	0.9987
300	0.022	0.140	0.292	0.399	0.536	1.287	0.9979

* average of triplicate results

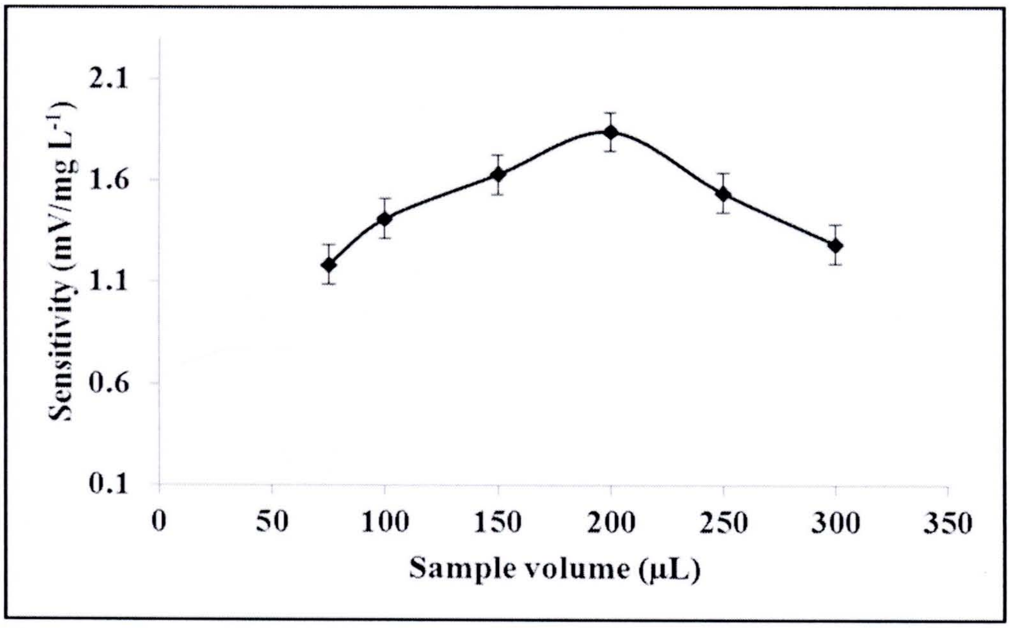

Figure 3.13 Relationship between sample volume and sensitivity of the calibration curve

3.3.1.10 Summary of the studied range and optimum conditions of FIA method for the determination of cadmium(II)

The optimization of the FIA system for cadmium(II) was studied with FIA manifold (Figure 2.1). The optimum experimental conditions for cadmium(II) speciation are summarized in Table 3.12. These optimum conditions were used for further investigation.

Table 3.12 Univariate optimum conditions for the FI determination of cadmium(II) using rhodamine B

Variable	Studied range	Optimum conditions
Wavelength (nm)	605-620	612
pH	1.5-4.0	3.0
Concentration of KI ((mol L^{-1})	0.05-0.11	0.07
Concentration of Hydroxylamine HCl (x10^{-4}mol L^{-1})	0.5-3.0	1.5
Concentration of Rhodamine B (mol L^{-1})	1.0-5.0	2.5
Flow rate (mL min^{-1})	1.5-6.0	3.5
Reaction coil inner diameter (i.d. cm)	0.051-0.132	75
Reaction coil length (cm)	25-150	0.086
Sample volume (μL)	75-300	200

3.3.2 Analytical characteristics of the FIA method for determination of cadmium(II) using rhodamine B

3.3.2.1 Linearity

The linear range of the proposed method was studied by injecting cadmium(II) standard solutions over the range 0.00-4.00 mg L^{-1} into FI system under the suitable conditions (Table 3.12). The results are presented in Table 3.13 and Figure 3.14.

The experimental results indicated that linear range was in the range of 0.00-1.00 mg L^{-1} for cadmium(II) with pentaplicate injections, that Beer's law was obeyed over a wide concentration range, which is useful for analysis of cadmium(II) in diverse matrices of sample.

Table 3.13 Study of linearity at Δpeak height of various cadmium(II) concentrations

Cadmium(II) (mg L^{-1})	**Peak height (mV)**						**ΔP.H.* (mV)**
	1	**2**	**3**	**4**	**5**	**$\bar{x}$**	
0.00	0.045	0.045	0.046	0.046	0.045	0.045	0
0.005	0.046	0.047	0.047	0.047	0.047	0.047	0.002
0.01	0.049	0.049	0.049	0.049	0.049	0.049	0.004
0.04	0.060	0.060	0.060	0.060	0.060	0.060	0.015
0.06	0.069	0.069	0.069	0.070	0.069	0.069	0.024
0.08	0.081	0.081	0.081	0.081	0.081	0.081	0.036
0.10	0.093	0.093	0.093	0.093	0.093	0.093	0.048
0.15	0.125	0.125	0.125	0.125	0.125	0.125	0.080
0.20	0.167	0.165	0.167	0.167	0.165	0.166	0.121
0.30	0.245	0.245	0.242	0.242	0.241	0.243	0.198
0.40	0.326	0.326	0.326	0.332	0.332	0.328	0.283
0.50	0.426	0.426	0.426	0.426	0.426	0.426	0.381
0.60	0.526	0.526	0.523	0.523	0.523	0.524	0.479
0.70	0.627	0.627	0.624	0.623	0.623	0.625	0.580
0.80	0.705	0.709	0.714	0.701	0.706	0.707	0.662
0.90	0.788	0.788	0.788	0.788	0.788	0.788	0.743
1.00	0.875	0.875	0.878	0.880	0.875	0.877	0.832
1.50	1.098	1.120	1.106	1.106	1.098	1.106	1.061
2.00	1.247	1.253	1.253	1.258	1.253	1.253	1.208
3.00	1.316	1.324	1.333	1.324	1.324	1.324	1.279
4.00	1.338	1.338	1.347	1.347	1.347	1.343	1.298

*average of pentaplicate results

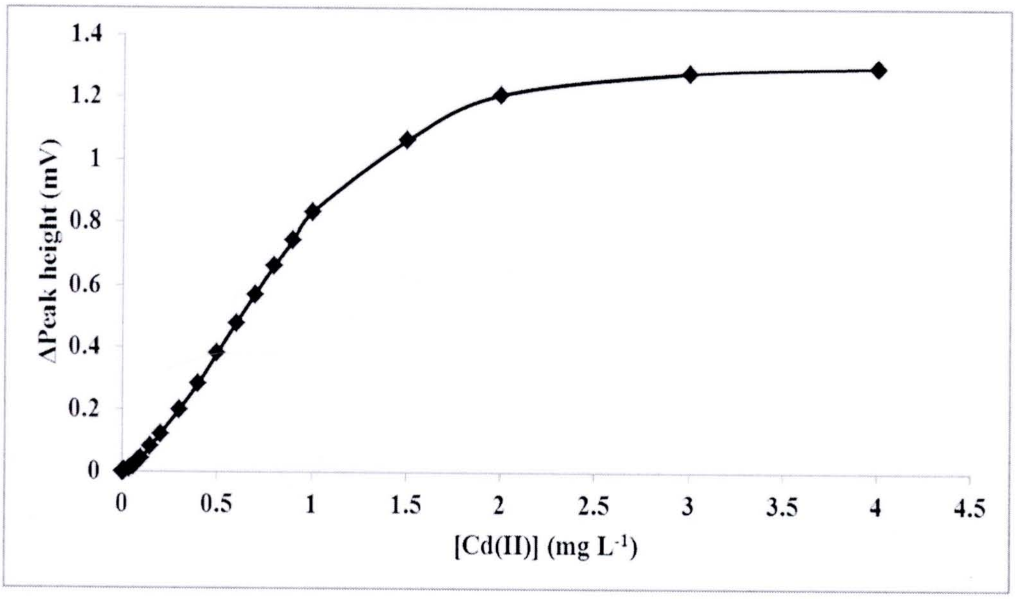

Figure 3.14 Linearity and relationship between net peak height and various concentrations of cadmium(II)

3.3.2.2 Calibration curve

A calibration curve was obtained by injecting cadmium(II) standard solutions containing 0.00-1.00 mg L^{-1} into the recommended FIA system (Figure 2.1) under the optimum conditions (Table 3.12). The resulting violet colored product was monitored at 612 nm. The results were shown in Table 3.14 and Figure 3.15. The calibration curve as shown in Figure 3.16 was established by plotting Δpeak heights versus the various cadmium(II) concentrations. It was found to be linear over the range of 0.00-1.00 mg L^{-1} with the following calibration equation:

$$y = 0.824x - 0.0137$$

When y is signal as peak height in mV

x is concentration of cadmium(II) solution in mg L^{-1} with a correlation coefficient (r^2) of 0.9996.

Table 3.14 ΔPeak height for calibration curve by FIA method

Cadmium(II) (mg L^{-1})	Peak height (mV)						ΔP.H.* (mV)
	1	2	3	4	5	$\bar{x}$	
0.00	0.045	0.045	0.045	0.046	0.045	0.045	0.000
0.06	0.070	0.070	0.069	0.073	0.069	0.070	0.025
0.10	0.110	0.112	0.110	0.110	0.10	0.110	0.065
0.20	0.187	0.187	0.190	0.190	0.190	0.189	0.144
0.40	0.358	0.358	0.358	0.355	0.355	0.357	0.312
0.60	0.529	0.529	0.521	0.521	0.521	0.524	0.479
0.80	0.696	0.691	0.691	0.701	0.691	0.694	0.649
1.00	0.856	0.857	0.858	0.857	0.857	0.857	0.812

*average of pentaplicate results

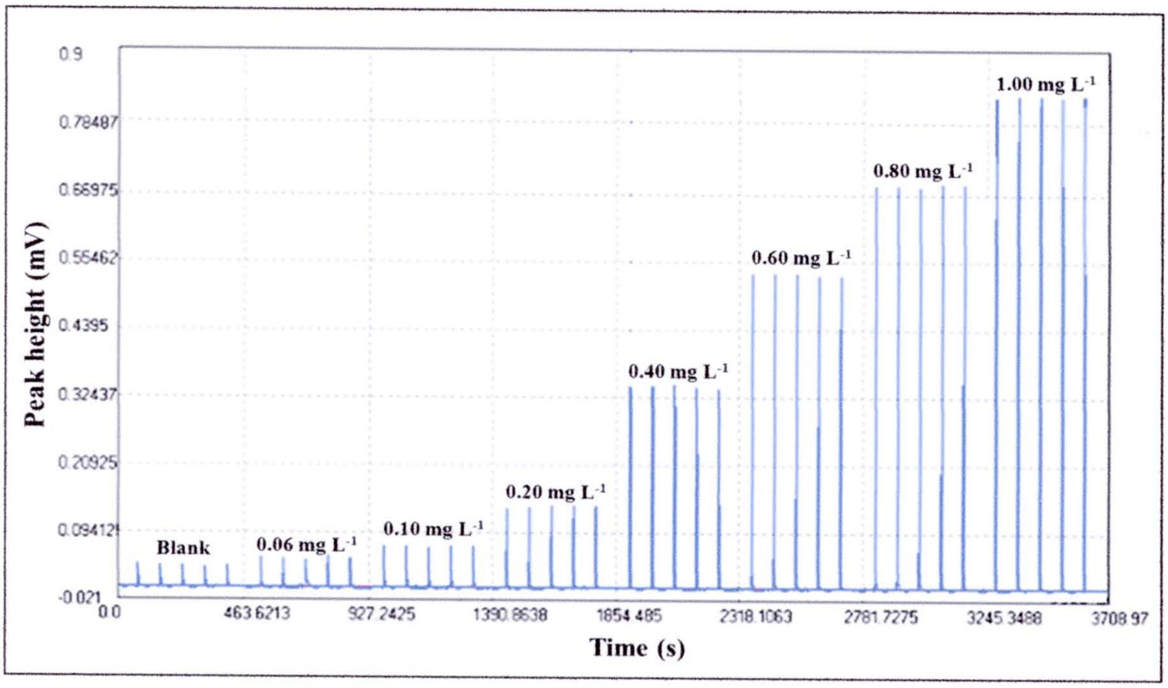

Figure 3.15 Calibration signal of FI spectrophotometric determination of cadmium(II) 0.00-1.00 mg L^{-1}

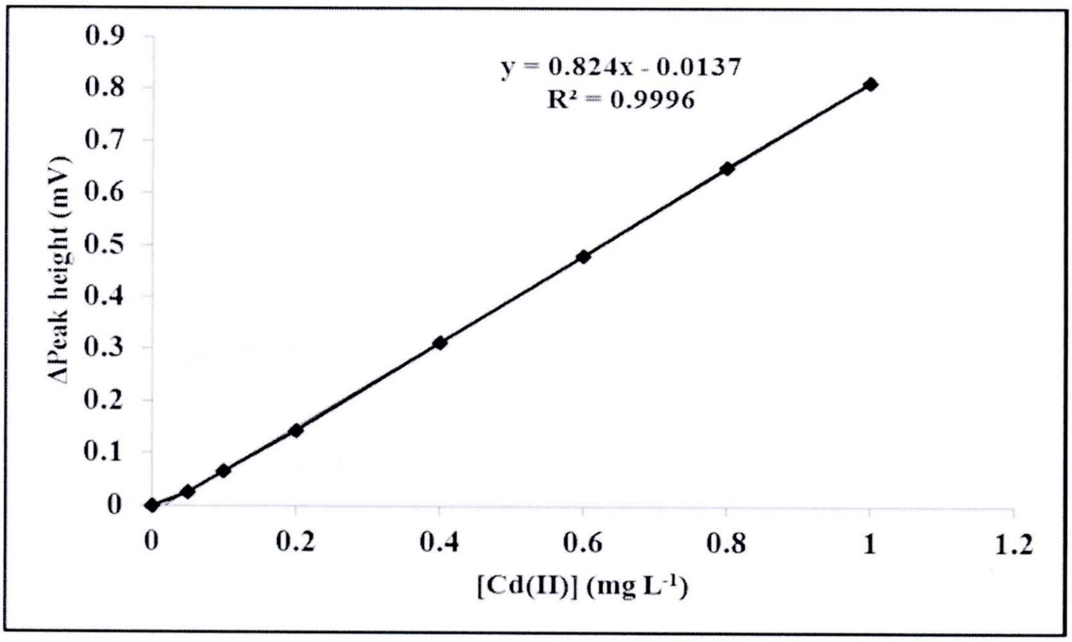

Figure 3.16 Calibration curve of FI spectrophotometric determination of cadmium(II) 0.00-1.00 mg L^{-1}

3.3.2.3 Precision

The precision of the proposed FIA method was verified by injecting 11 replicates of 0.2 mg L^{-1} cadmium(II) standard solution, using the optimum conditions in Table 3.12. The results were shown in Table 3.15. The relative standard deviation (RSD) was found to be 1.08%.

Table 3.15 Precision of verification using 0.2 mg L^{-1} cadmium(II) standard solution

Experimental number	Peak height (mV)*
1	0.111
2	0.109
3	0.110
4	0.110
5	0.111
6	0.109
7	0.112
8	0.112
9	0.111
10	0.112
11	0.110
$\bar{x}$	0.111
S.D.	0.0012
% R.S.D.	1.08

*average of triplicate results

3.3.2.4 Detection limit

The detection limit is defined as that concentration of the analyte producing a signal (peak height) which is the experimental blank signal plus three times of the standard deviation of the blank signal [82]. Using the FIA manifold (Figure 2.1) and the optimum conditions in Table 3.12. The results were presented in Table 3.16. The detection limit of the proposed method was found to be 0.007 mg L^{-1}.

Table 3.16 The detection limit of FIA method from blank signal resulting of 12 injections

Experimental number	**Peak height (mV)***
1	0.004
2	0.004
3	0.005
4	0.005
5	0.005
6	0.004
7	0.006
8	0.006
9	0.005
10	0.005
11	0.004
12	0.004
$\bar{x}$	0.005
S.D.	0.0008
LOD (mg L^{-1})	0.007
LOQ (mg L^{-1})	0.013

*average of triplicate results

3.3.2.5 Accuracy

The accuracy (defined as percentage recovery of the added analyte) of the proposed FIA method was verified by standard addition method. The method was examined by determining the recoveries of the added cadmium(II) with varying concentrations in sample solution. The results are presented in Tables 3.17. The average recovery was found to be 101.31% for cadmium(II). The proposed FIA method was found to be accurate for the determination of trace amounts of cadmium(II) in sample solutions.

Table 3.17 The %recovery by using standard addition method for cadmium(II) by FIA method

Cadmium(II) (mg L^{-1})	**Peak height (mV)***		**[Cd(II)] found (mg L^{-1})**	**% recovery**
	Std.	**Std.+sample**		
0.10	0.165	0.169	0.102	102.00
0.20	0.444	0.459	0.207	103.50
0.30	0.872	0.883	0.304	101.33
0.40	1.205	1.211	0.402	100.50
0.50	1.686	1.673	0.496	99.20
Mean			101.31	
S.D.			1.611	
% RSD			1.606	

*average of triplicate results

3.3.3 Interference studies

The interference effects of some possible foreign ions on the determination of cadmium(II) were studied by the proposed FIA procedure under the optimum conditions (Table 3.12). A systematic study to check for the effects of some possible foreign ions on the determination of cadmium(II) was undertaken for the maximum w/w ratio of cadmium(II) to foreign ions up to 1:5000 The solutions of a 0.1 mg L^{-1} cadmium(II) standard containing varying concentrations of diverse ions were determined using the FIA system. The tolerance is defined as the largest foreign-ion concentration causing % relative error ±10% for determining the analyte of interest. The tolerance values for the ions studied are given in Table 3.18.

Table 3.18 Interference studies for 0.1 mg L^{-1} standard cadmium(II) by FIA method

Interferences	**Cadmium(II) : Interference**	**Peak height (mV)**	**% Relative error**
Zn^{2+}	1 : 0	0.077	-
	1 : 100	0.076	-1.30
	1 : 1000	0.072	-6.49
	1 : 2500	0.068	-11.69
Cr^{3+}	1 : 0	0.077	-
	1 : 500	0.075	-2.60
	1 : 2500	0.071	-7.79
	1 : 5000	0.070	-9.09
Cu^{2+}	1 : 0	0.077	-
	1 : 5	0.079	+2.60
	1 : 10	0.081	+6.49
	1 : 20	0.086	+11.69

* average of triplicate results.

Table 3.18 (Continued)

Interference	Cadmium(II) : Interference	Peak height* (AU)	% Relative error
Ni^{2+}	1 : 0	0.077	-
	1 : 1000	0.077	-
	1 : 2500	0.078	+1.30
	1 : 5000	0.079	+2.60
Ba^{2+}	1 : 0	0.077	-
	1 : 1000	0.076	-1.30
	1 : 3000	0.073	-5.19
	1 : 5000	0.068	-11.69
Co^{2+}	1 : 0	0.077	-
	1 : 100	0.079	+2.60
	1 : 200	0. 082	+6.46
	1 : 500	0.085	+10.39
Al^{3+}	1 : 0	0.081	-
	1 : 100	0.080	-1.23
	1 : 1000	0.077	-4.94
	1 : 3000	0.072	-11.11
Fe^{2+}	1 : 0	0.081	-
	1 : 50	0.84	+3.70
	1 : 100	0.086	+6.17
	1 : 150	0.090	+11.11
Fe^{3+}	1 : 0	0.081	-
	1 : 100	0.082	+1.23
	1 : 300	0.085	+4.94
	1 : 400	0.091	+12.35
Mn^{2+}	1 : 0	0.081	-
	1 : 10	0.084	+3.70
	1 : 50	0.087	+7.41
	1 : 100	0.090	+11.11

* average of triplicate results.

Table 3.18 (Continued)

Interference	**Cadmium(II) : Interference**	**Peak height* (AU)**	**% Relative error**
Cl^-	1 : 0	0.081	-
	1 : 50	0.084	+3.70
	1 : 100	0.088	+8.64
	1 : 200	0.091	+12.35
I^-	1 : 0	0.077	-
	1 : 1000	0.078	+1.30
	1 : 2500	0.079	+2.59
	1 : 5000	0.081	+5.19
Br^-	1 : 0	0.077	-
	1 : 100	0.080	+3.90
	1 : 500	0.082	+6.49
	1 : 1000	0.085	+10.39
SO_4^{2-}	1 : 0	0.077	-
	1 : 500	0.081	+5.19
	1 : 1000	0.082	+6.49
	1 : 2500	0.086	+11.69
PO_4^{3-}	1 : 0	0.077	-
	1 : 100	0.079	+2.60
	1 : 200	0.081	+5.19
	1 : 300	0.085	+10.89
K^+	1 : 0	0.077	-
	1 : 500	0.077	-
	1 : 1000	0.078	+1.30
	1 : 5000	0.078	+1.30
NO_2^-	1 : 0	0.080	-
	1 : 10	0.081	+1.25
	1 : 50	0.084	+5.00
	1 : 100	0.089	+11.25

* average of triplicate results.

Table 3.18 (Continued)

Interference	**Cadmium(II) : Interference**	**Peak height*** **(AU)**	**% Relative error**
NO_3^-	1 : 0	0.080	-
	1 : 500	0.083	+3.75
	1 : 1000	0.086	+7.50
	1 : 2000	0.089	+11.25
Mg^{2+}	1 : 0	0.080	-
	1 : 1000	0.079	-1.25
	1 : 2500	0.079	-1.25
	1 : 5000	0.077	-3.75
Na^+	1 : 0	0.081	-
	1 : 500	0.082	+1.23
	1 : 1000	0.083	+2.47
	1 : 5000	0.083	+2.47
F^-	1 : 0	0.081	-
	1 : 500	0.081	-
	1 : 1000	0.082	+1.23
	1 : 5000	0.085	+4.94
CN^-	1 : 0	0.081	-
	1 : 500	0.081	-
	1 : 1000	0.081	-
	1 : 5000	0.083	+2.47

* average of triplicate results.

The experimental results obtained by investigation of some interfering species which may occur in water samples indicated that the presence of some interfering ions namely Cr^{3+}, Ni^{2+}, Ba^{2+}, I^-, K^+, Mg^{2+}, Na^+, F^-, CN^- at concentration ≥5000 folds that of cadmium(II) caused relative error ±10%. Furthermore, interfering ions of Al^{3+}, Zn^{2+} and SO_4^{2-}, NO_3^-, Br^-, Co^{2+}, Fe^{3+}, PO_4^{3-}, Cl^-, Fe^{2+}, Mn^{2+} and NO_2^- at concentration 3000, 2500, 2000, 1000, 500, 400, 300, 200, 150 and 100 folds, respectively that of cadmium(II) caused % relative error ±10%. While the presence of Cu^{2+} concentration up to 20 folds that of cadmium(II) caused relative error ±10%. The presence of interfering ions was found to interfere by increasing and decreasing the FIA signals. It was found that the serious positive interference for cadmium(II) determination was Cu^{2+}. Most interferences from various cations might be due to the formation of coloured complex and competitive complexation with the rhodamine dye and reagent structure contain numerous sites at which chelation with metal ions might occur that affect absorption of the complex. Ions which may oxidize iodide to triiodide under the experimental conditions, such as Cu^{2+} which may form large anionic ion-complexes which can form ion-associates with rhodamine B. Since interferences are many in which few interferences are significant under the optimum working conditions. Thus the method is selective and matrix effects are not severe to the determination cadmium(II) in wastewater, which referred standard quality of wastewater defined by Pollution Control Department (PCD) Ministry of Natural Resources and Environment (Appendix A), which can be evaluated by method of standard additions. In addition, such high concentrations of transition-metal interferences are normally not to be expected in wastewater samples. The interference effects of some possible foreign ions on cadmium(II) determination using FIA system were summarized in Table 3.19.

Table 3.19 Summary of interference effects of some ions on the response obtained from 0.1 mg L^{-1} of cadmium(II) by FIA method

Interference ions	**Tolerable concentration ratio*(mg L^{-1}) of ion/Cd(II)**
Cr^{3+}, Ni^{2+}, Ba^{2+}, I^-, K^+, Mg^{2+}, Na^+, F^-, CN^-	≥5000
Al^{3+}	3000
Zn^{2+}, SO_4^{2-}	2500
NO_3^-	2000
Br^-	1000
Co^{2+}	500
Fe^{3+}	400
PO_4^{3-}	300
Cl^-	200
Fe^{2+}	150
Mn^{2+}, NO_2^-	100
Cu^{2+}	20

*The concentration of an ion is considered to be interfering when causing a relative error of more than ±10% with respect to the signal of cadmium(II) alone.

3.3.4 Determination of cadmium(II) in wastewater samples using standard addition method by FIA method

The proposed FIA spectrophotometric method was applied to the simultaneous determination of cadmium(II) in wastewater samples which were collected from several districts in Chiang Mai. The results were given in Table 3.20. The cadmium(II) contents in the wastewater samples were in the range of 0.015-0.069 mg L^{-1}.

Table 3.20 Determination of cadmium(II) in wastewater samples using standard addition method by FIA method

Wastewater samples	**Cadmium(II) found (mg L^{-1})**				**SD**	**Cadmium(II) concentration* (mg L^{-1})**
	1	**2**	**3**	$\bar{x}$		
Wastewater 1	0.025	0.026	0.025	0.025	0.0007	0.025 ± 0.0007
Wastewater 2	ND**	ND**	ND**	ND**	-	-
Wastewater 3	0.029	0.028	0.027	0.028	0.0010	0.028 ± 0.0010
Wastewater 4	0.015	0.019	0.018	0.017	0.0020	0.017 ± 0.0020
Wastewater 5	0.031	0.028	0.028	0.029	0.0017	0.029 ± 0.0017
Wastewater 6	0.031	0.032	0.031	0.031	0.0007	0.031 ± 0.0007
Wastewater 7	0.013	0.016	0.017	0.015	0.0020	0.015 ± 0.0020
Wastewater 8	0.019	0.015	0.019	0.018	0.0020	0.017 ± 0.0020
Wastewater 9	0.041	0.040	0.039	0.040	0.0010	0.040 ± 0.0010
Wastewater 10	0.067	0.069	0.070	0.069	0.0016	0.069 ± 0.0016

*average of triplicate results
**not detected

3.3.5 Comparative determination of cadmium(II) in wastewater samples of the proposed FIA method with ICP-MS method and validation method

In order to validate the proposed FIA method for cadmium(II) determination, a comparative determination of cadmium(II) by ICP-MS method was carried out. The experimental results were shown in Table 3.21. There was good agreement between the proposed FIA method and the ICP-MS method. The cadmium(II) contents in the wastewater samples were in the range of 0.015-0.069 mg L^{-1} and 0.013-0.073 mg L^{-1} using the proposed FIA method and ICP-MS respectively. The results obtained by the proposed FI spectrophotometric method compared favorably with those obtained by ICP-MS using the student *t*-test (Table 3.21 and Appendix B in Table B.1). It was seen that experimental *t*-value for cadmium(II) assay, which was smaller than the theoretical *t*-value at a confidence interval of 95% indicating that results obtained by both methods were in excellent agreement.

Table 3.21 Comparative determination of cadmium(II) in wastewater samples by the proposed FIA method and ICP-MS method

Wastewater samples	**Concentrations of cadmium (mg L^{-1})**		$t_{\text{calculated}}$
	FIA*	**ICP-MS***	
Wastewater 1	0.025	0.027	-4.206
Wastewater 2	ND**	ND**	-
Wastewater 3	0.028	0.026	3.464
Wastewater 4	0.017	0.013	3.724
Wastewater 5	0.029	0.027	2.038
Wastewater 6	0.031	0.029	5.691
Wastewater 7	0.015	0.017	-1.472
Wastewater 8	0.018	0.019	-1.126
Wastewater 9	0.040	0.037	5.196
Wastewater 10	0.069	0.073	-4.655

*average of triplicate results
**not detected

3.4 SIA Spectrophotometric Determination of Cadmium(II) using Rhodamine B as A Complexing Agent

The conditions for the determination of cadmium(II) were optimized by studying the influences of various parameters, such as sample and reagent volumes, reagent/carrier flow rates, 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 Figure 2.2 and 2.3 and preliminary experimental conditions in Table 2.5 were used. The range of variables studied and the optimal values were chosen as shown in Table 2.4.

3.4.1 Study the aspiration order

The complexation of Cd(II)-Iodide-Rhodamine B was studied at different aspiration orders. The sensitivities obtained are shown in Table 3.22. It was found that the aspiration order of first series provided the highest sensitivity. So, aspiration order of the second series was chosen for further optimization of SIA method.

Table 3.22 Aspiration order on the sensitivity

Series	Aspiration order	Sensitivity (mV/mg L^{-1})
1	A-B-C	0.386
2	A-C-B	0.434
3	B-A-C	0.260
4	B-C-A	0.300
5	C-A-B	0.327
6	C-B-A	0.348

A was 0.2 mg L-1 Cd(II) standard solution

B was 0.07 mol L^{-1} KI reagent solution (mixed in 1.5 mol L^{-1} Hydroxylamine HCl)

C was 2.5×10^{-4} mol L^{-1} Rhodamine B reagent solution

3.4.2 Optimization of the SIA system by univariate method

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

3.4.2.1 Effect of pH

The effect of pH on the formation of Cd(II)-Rhodamine B was studied by varying the pH of phosphate buffer in the range of pH 2.0-4.0. The pH value of the solution was adjusted with phosphoric acid (H_3PO_4) and sodium dihydrogen phosphate (NaH_2PO_4). The results obtained are shown in Table 3.23 and Figure 3.17. The optimum pH for a maximum absorbance at 612 nm was found to be pH 3.0. The sensitivity (slope of the calibration curve) decreased significantly when the pH value more than 3.0. So, pH 3.0 was selected as optimum pH because it provided the greatest sensitivity.

Table 3.23 Effect of pH on the sensitivity

pH	ΔPeak height* (mV) obtained from the standard Cd(II) (mg L^{-1})					Sensitivity (mV/mg L^{-1})	Correlation coefficient (r^2)
	0.1	**0.2**	**0.3**	**0.4**	**0.5**		
2.0	0.385	0.432	0.485	0.542	0.595	0.530	0.9990
2.5	0.383	0.432	0.485	0.547	0.597	0.543	0.9985
3.0	0.373	0.433	0.491	0.56	0.615	0.611	0.9991
3.5	0.372	0.433	0.481	0.531	0.588	0.530	0.9984
4.0	0.319	0.359	0.386	0.419	0.457	0.336	0.9966

*average of pentaplicate results

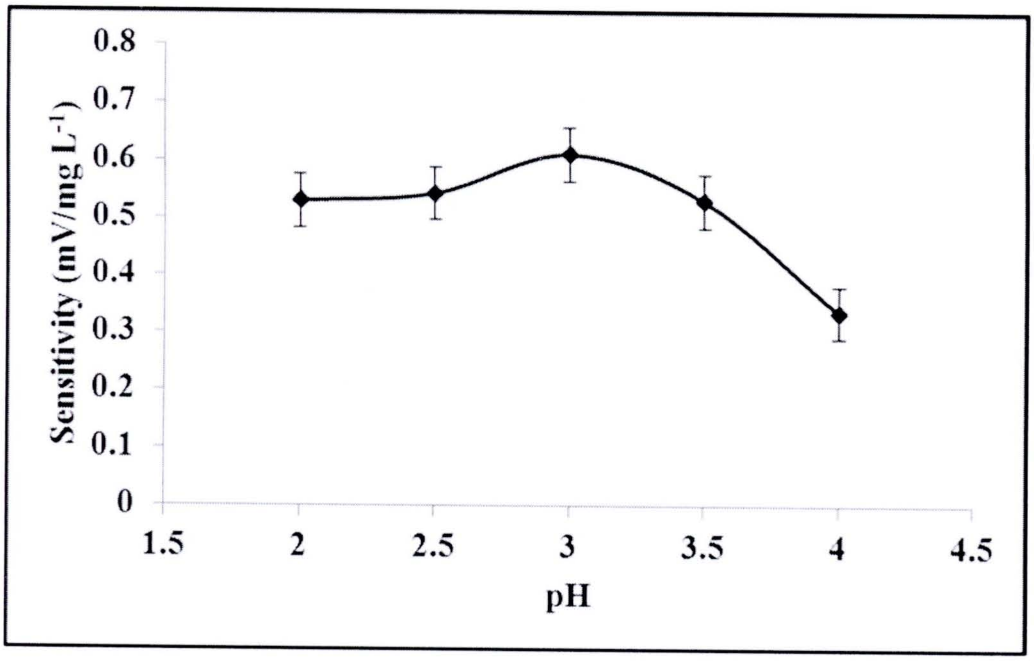

Figure 3.17 Relationship between various pH and sensitivity of the calibration curve

3.4.2.2 Effect of concentration of phosphate buffer pH 3.0

The effect of the concentration of phosphate buffer solution was studied in the range 0.05-0.25 mol L^{-1}. The results are shown in Table 3.24 and Figure 3.18. It was found that the sensitivity of the SIA method increased from the phosphate buffer concentration of 0.05-0.15 mol L^{-1}. After that, the sensitivity was decreased. Therefore, 0.15 mol L^{-1} of phosphate buffer solution was chosen for subsequent experiments since it gave the highest sensitivity.

Table 3.24 Effect of concentration of phosphate buffer pH 3.0 on the sensitivity

Concentration of phosphate buffer pH 3.0 (mol L^{-1})	**ΔPeak height* (mV) obtained from the standard Cd(II) (mg L^{-1})**					**Sensitivity (mV/mg L^{-1})**	**Correlation coefficient (r^2)**
	0.1	**0.2**	**0.3**	**0.4**	**0.5**		
0.05	0.377	0.420	0.446	0.483	0.520	0.349	0.9959
0.10	0.382	0.425	0.462	0.511	0.549	0.420	0.9985
0.15	0.385	0.427	0.474	0.527	0.569	0.468	0.9985
0.20	0.385	0.422	0.472	0.514	0.551	0.424	0.9975
0.25	0.367	0.414	0.448	0.494	0.536	0.418	0.9981

*average of pentaplicate results

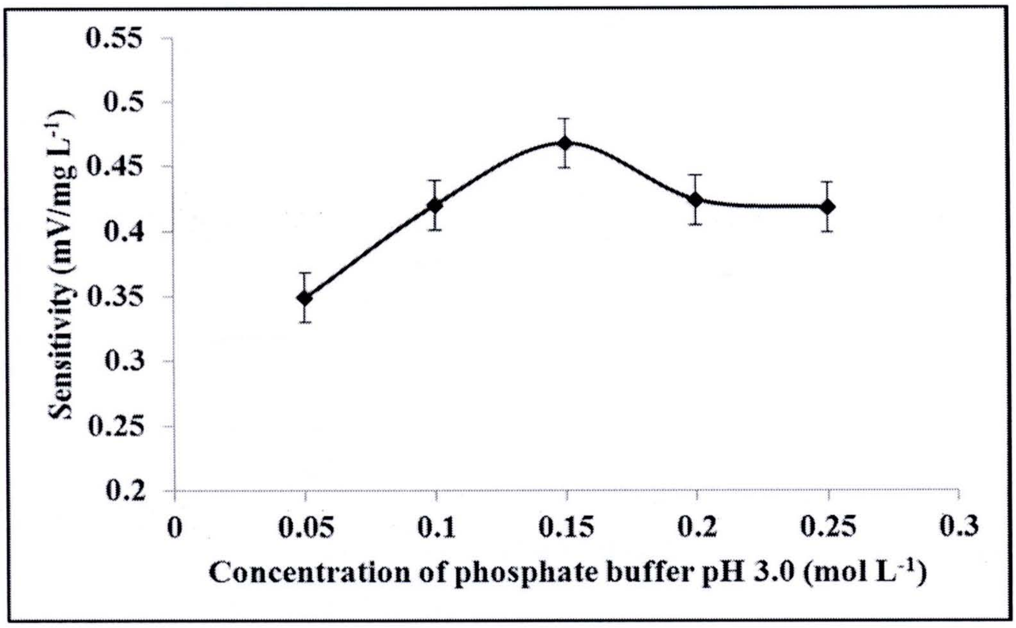

Figure 3.18 Relationship between concentration of phosphate buffer pH 3.0 and sensitivity of the calibration curve

3.4.2.3 Effect of KI concentration

Effect of KI concentrations on the determination of cadmium(II) was studied in the range 0.05-0.15 mol L^{-1}. The results are shown in Table 3.25 and Figure 3.19. The results indicated that the sensitivity increased rapidly with increasing KI concentration up to 0.11 mol L^{-1} and decreased rapidly when the concentration of KI was greater than 0.11 mol L^{-1}. Thus 0.11 mol L^{-1} of KI concentration was chosen as optimum concentration for subsequent experiment since it provided the highest sensitivity for this investigation.

Table 3.25 Effect of KI concentration on the sensitivity

KI concentration (mol L^{-1})	**ΔPeak height (mV)* obtained from the standard Cd(II) (mg L^{-1})**					**Sensitivity (mV/mg L^{-1})**	**Correlation coefficient (r^2)**
	0.1	**0.2**	**0.3**	**0.4**	**0.5**		
0.05	0.285	0.335	0.397	0.444	0.505	0.549	0.9984
0.07	0.408	0.478	0.548	0.615	0.681	0.683	0.9998
0.09	0.520	0.601	0.676	0.753	0.821	0.754	0.9992
0.11	0.632	0.701	0.779	0.860	0.939	0.773	0.9992
0.13	0.631	0.698	0.766	0.840	0.904	0.688	0.9996
0.15	0.609	0.677	0.741	0.807	0.866	0.664	0.9995

*average of pentaplicate results

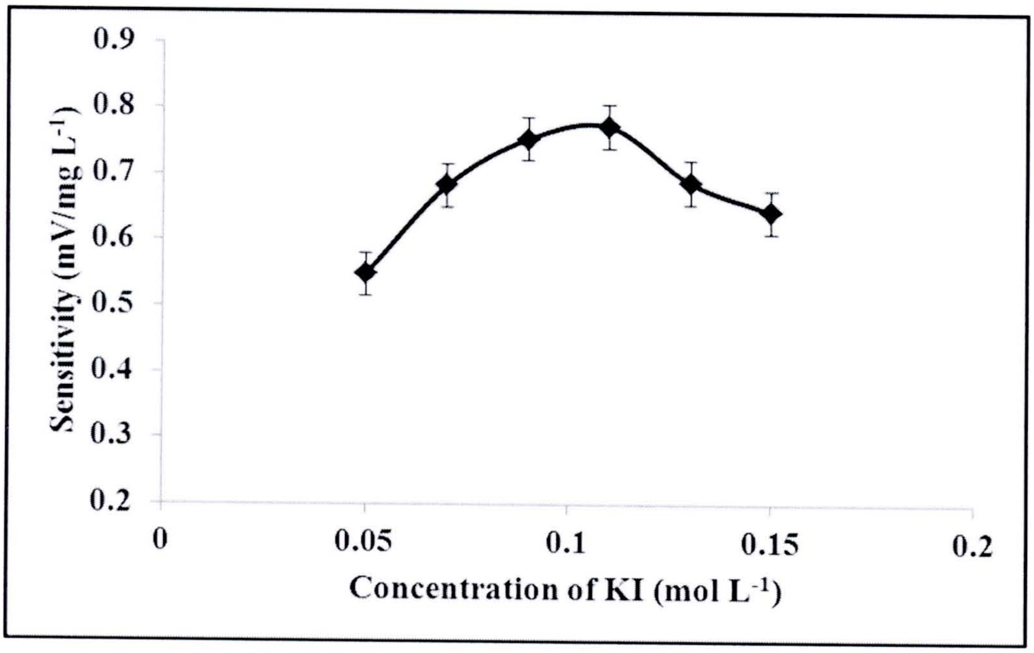

Figure 3.19 Relationship between KI concentrations and sensitivity of the calibration curve

3.4.2.4 Effect of hydroxylamine HCl concentration

The hydroxylamine HCl (H_3NOHCl) solution was added as solubilizer to prevent precipitation in complexation reaction. Effects of various hydroxylamine HCl concentrations on peak height were investigated by varying the concentration of hydroxylamine HCl in the range of 0.5-2.5x10^{-4} mol L^{-1}. The results obtained were exhibited in Table 3.26 and Figure 3.20. From the experimental results, the sensitivity of calibration over the range 0.1-0.5 mg L^{-1} cadmium(II) increased with hydroxylamine HCl concentration up to 1.5x10^{-4} mol L^{-1}, followed by a slight decrease in peak height at the concentration of 2.0x10^{-4} mol L^{-1}. Afterwards, the slope of calibration curve decreased slightly when the hydroxylamine HCl concentration reached 2.5x10^{-4} mol L^{-1}. The results indicated that the highest sensitivity was obtained when the concentration of hydroxylamine HCl was 1.5x10^{-4} mol L^{-1}.

Table 3.26 Effect of hydroxylamine HCl concentration on the sensitivity

H_3NOHCl concentration (x10^{-4}mol L^{-1})	**ΔPeak height (mV)* obtained from the standard Cd(II) (mg L^{-1})**					**Sensitivity (mV/mg L^{-1})**	**Correlation coefficient (r^2)**
	0.1	**0.2**	**0.3**	**0.4**	**0.5**		
0.5	0.260	0.387	0.547	0.693	0.801	1.388	0.9964
1.0	0.249	0.376	0.519	0.651	0.814	1.405	0.9982
1.5	0.251	0.390	0.559	0.722	0.903	1.636	0.9981
2.0	0.028	0.356	0.487	0.623	0.768	1.347	0.9994
2.5	0.226	0.350	0.476	0.619	0.756	1.329	1.9991

*average of pentaplicate results

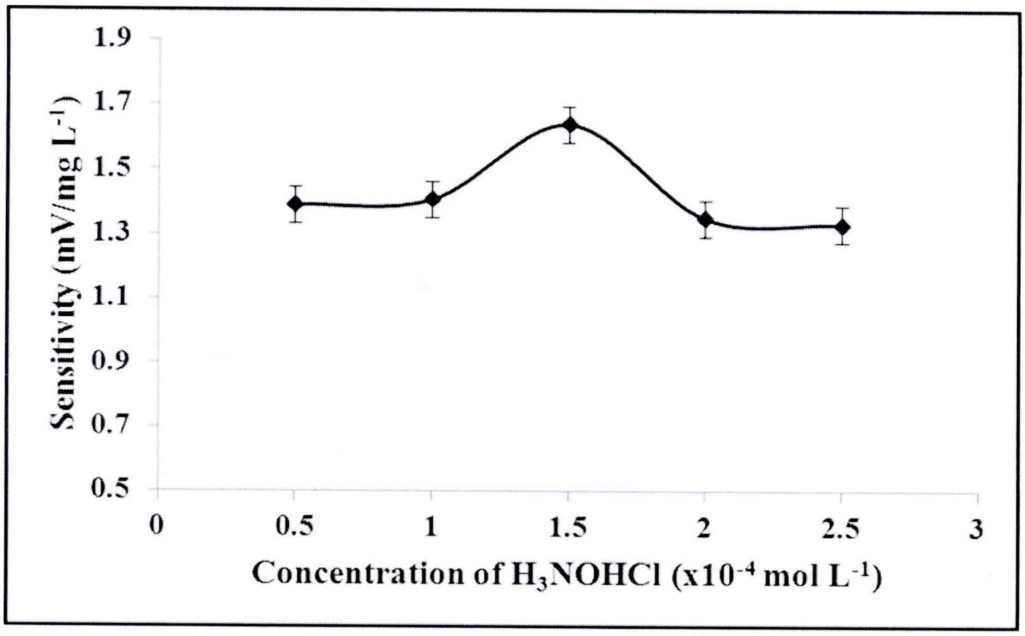

Figure 3.20 Relationship between hydroxylamine HCl concentrations and sensitivity of the calibration curve

3.4.2.5 Effect of rhodamine B concentration

Effect of rhodamine B concentrations on the determination of cadmium(II) was studied in the range 1.5-4.0x10^{-4} mol L^{-1}. The results obtained are shown in Table 3.27 and Figure 3.21. The result was found that sensitivity increased very rapidly from the rhodamine B concentration of 1.5-3.0x10^{-4} mol L^{-1}. After that, the sensitivities were decreased. This is due to the fact that the increasing the rhodamine B concentration leading to the increase in amounts of Cd(II)-Iodide-Rhodamine B complexation which results in a higher sensitivity. However, beyond the rhodamine B of 3.0x10^{-4} mol L^{-1}, the absorbance of Cd(II)-Iodide-Rhodamine B complex decreased. Consequently, a concentration of 3.0x10^{-4} mol L^{-1} of rhodamine B was selected as optimum concentration.

Table 3.27 Effect of rhodamine B concentration on the sensitivity

Rhodamine B concentration ($\times10^{-4}$mol L^{-1})	**ΔPeak height (mV)* obtained from the standard Cd(II) (mg L^{-1})**					**Sensitivity (mV/mg L^{-1})**	**Correlation coefficient (r^2)**
	0.1	**0.2**	**0.3**	**0.4**	**0.5**		
1.5	0.219	0.265	0.301	0.343	0.382	0.404	0.9989
2.0	0.291	0.344	0.399	0.453	0.504	0.535	0.9998
2.5	0.295	0.375	0.465	0.547	0.631	0.844	0.9998
3.0	0.299	0.423	0.545	0.673	0.805	1.262	0.9998
3.5	0.287	0.392	0.503	0.628	0.731	1.124	0.9991
4.0	0.218	0.316	0.419	0.526	0.631	1.036	0.9997

*average of pentaplicate results

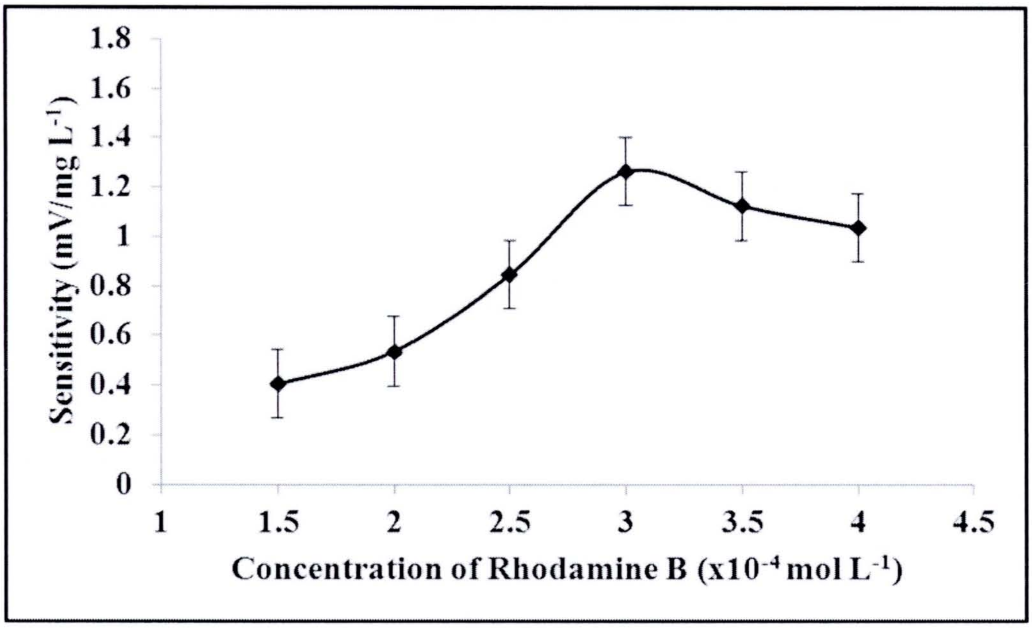

Figure 3.21 Relationship between rhodamine B concentrations and sensitivity of the calibration curve

3.4.2.6 Effect of the aspiration volumes of phosphate buffer pH 3.0

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 0.15 mol L^{-1} of phosphate buffer pH 3.0 was studied over the range 50–175 μL at every 25 μL interval (Table 3.28 and Figure 3.22).

Table 3.28 Effect of the aspiration volumes of 0.15 mol L^{-1} of phosphate buffer pH 3.0 on the sensitivity

Aspiration volume of buffer (μL)	**ΔPeak height (mV)* obtained from the standard Cd(II) (mg L^{-1})**					**Sensitivity (mV/mg L^{-1})**	**Correlation coefficient (r^2)**
	0.1	**0.2**	**0.3**	**0.4**	**0.5**		
50	0.317	0.417	0.517	0.634	0.726	1.035	0.9990
75	0.317	0.419	0.521	0.629	0.740	1.056	0.9996
100	0.322	0.421	0.536	0.641	0.754	1.084	0.9995
125	0.325	0.427	0.537	0.660	0.764	1.111	0.9991
150	0.295	0.396	0.506	0.622	0.731	1.098	0.9995
175	0.228	0.326	0.429	0.539	0.655	1.067	0.9988

*average of pentaplicate results

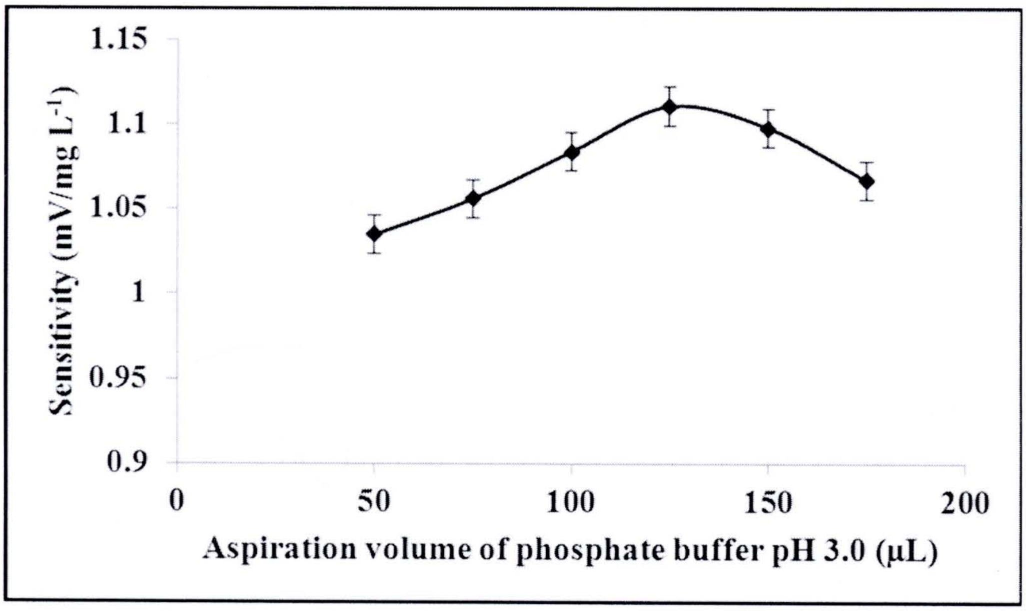

Figure 3.22 Relationship between various aspiration volumes of 0.15 mol L^{-1} of phosphate buffer pH 3.0 and sensitivity of the calibration curve

The result was found that the sensitivity increased when the aspiration volume of phosphate buffer 0.15 mol L^{-1} at pH 3.0 was increased and reached a maximum sensitivity at 125 μL, above which the sensitivity started to decline. So, a volume of 125 μL was chosen as an optimum for subsequent measurements.

3.4.2.7 Effect of the aspiration volumes of KI

The Effect of the aspiration volumes of KI (0.11 mol L^{-1}) on the determination of cadmium(II) (0.1-0.5 mg L^{-1}) was studied at different concentrations in the range of 75-200 μL. The results are shown in Table 3.29 and Figure 3.23. It was found that the sensitivity decreased, when the aspiration volume of KI lower and higher than 150 μL. Thus, the optimum aspiration volume of KI was 150 μL because it gave highest sensitivity.

Table 3.29 Effect of the aspiration volumes of 0.11 mol L^{-1} of KI on the sensitivity

Aspiration volume of KI (μL)	**ΔPeak height (mV)* obtained from the standard Cd(II) (mg L^{-1})**					**Sensitivity (mV/mg L^{-1})**	**Correlation coefficient (r^2)**
	0.1	**0.2**	**0.3**	**0.4**	**0.5**		
75	0.219	0.307	0.401	0.506	0.608	0.977	0.9987
100	0.281	0.368	0.48	0.582	0.675	1.002	0.9987
125	0.301	0.399	0.513	0.628	0.732	1.091	0.9993
150	0.350	0.465	0.580	0.687	0.800	1.122	0.9998
175	0.294	0.388	0.492	0.609	0.720	1.073	0.9983
200	0.342	0.435	0.525	0.631	0.728	0.968	0.9992

*average of pentaplicate results

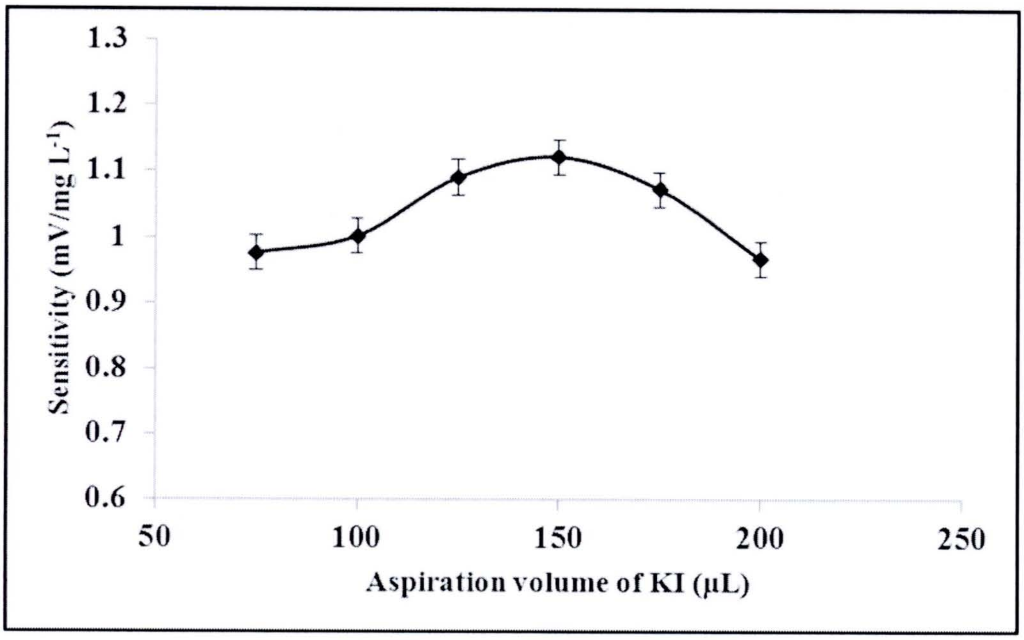

Figure 3.23 Relationship between various aspiration volumes of 0.11 mol L^{-1} of KI and sensitivity of the calibration curve

3.4.2.8 Effect of the aspiration volumes of rhodamine B

Effect of the aspiration volumes of rhodamine B ($3.0x10^{-4}$ mol L^{-1}) on the determination of cadmium(II) was studied in the range 75-200 μL. The results obtained are shown in Table 3.30 and Figure 3.24. The result was found that sensitivity increased very rapidly from the aspiration volumes of rhodamine B in range the 75-150 μL. After that, the sensitivities were decreased. This is due to the fact that the increasing the aspiration volume of $3.0x10^{-4}$ mol L^{-1} rhodamine B leading to the increase in amounts of Cd(II)-Iodide-Rhodamine B complexation which results in a higher sensitivity. However, beyond 150 μL, the absorbance of Cd(II)-Iodide-Rhodamine B complex decreased. Thus, the optimum aspiration volume of rhodamine B was 150 μL because it gave the highest sensitivity.

Table 3.30 Effect of the aspiration volumes of $3.0x10^{-4}$ mol L^{-1} rhodamine B on the sensitivity

Aspiration volume of rhodamine B (μL)	**ΔPeak height (mV)* obtained from the standard Cd(II) (mg L^{-1})**					**Sensitivity (mV/mg L^{-1})**	**Correlation coefficient (r^2)**
	0.1	**0.2**	**0.3**	**0.4**	**0.5**		
75	0.350	0.433	0.528	0.599	0.676	0.818	0.9976
100	0.371	0.461	0.566	0.651	0.737	0.922	0.9986
125	0.381	0.484	0.600	0.717	0.817	1.105	0.9992
150	0.397	0.500	0.621	0.735	0.859	1.159	0.9991
175	0.385	0.493	0.614	0.714	0.823	1.097	0.9993
200	0.304	0.405	0.512	0.627	0.72	1.054	0.9991

*average of pentaplicate results

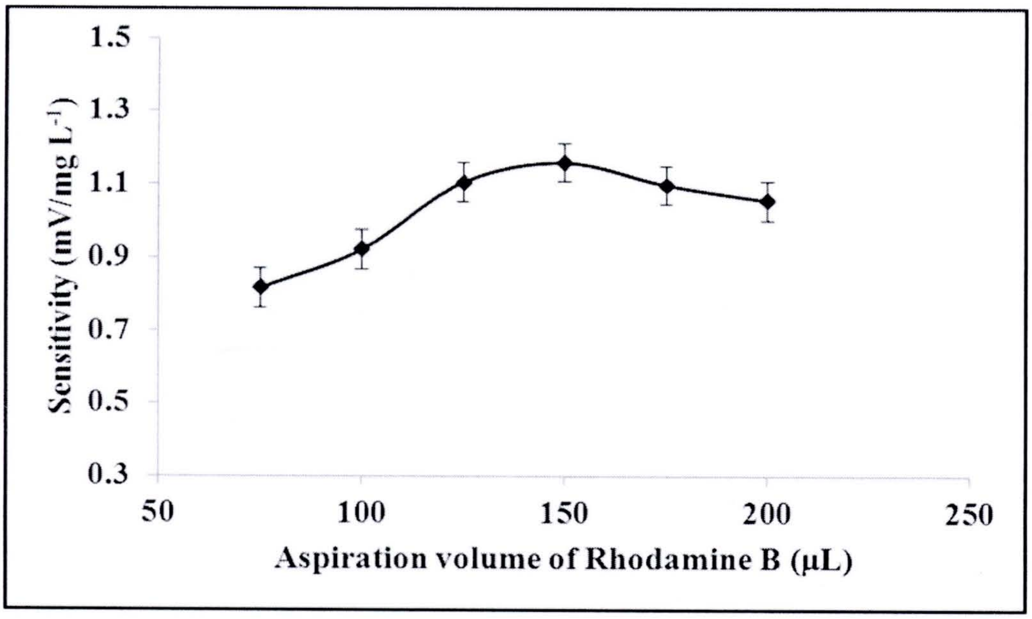

Figure 3.24 Relationship between various aspiration volumes of 3.0x10^{-4} mol L^{-1} rhodamine B and sensitivity of the calibration curve

3.4.2.9 Effect of the aspiration volumes of sample

The influence of the sample volumes were examined in the range of 100-250 µL at every 25 µL interval and it was found that the sensitivity increased markedly up to 200 µL, above which the sensitivity started to decline (Table 3.31 and Figure 3.25). So, an aspiration volume of 200 µL was chosen as an appropriate sample volume for further investigations.

Table 3.31 Effect of the aspiration volumes of sample on the sensitivity

Aspiration volume of sample (μL)	**ΔPeak height (mV)* obtained from the standard Cd(II) (mg L^{-1})**					**Sensitivity (mV/mg L^{-1})**	**Correlation coefficient (r^2)**
	0.1	**0.2**	**0.3**	**0.4**	**0.5**		
100	0.397	0.484	0.582	0.674	0.764	0.924	0.9997
125	0.420	0.518	0.633	0.746	0.840	1.068	0.9988
150	0.421	0.539	0.666	0.786	0.892	1.189	0.9991
175	0.439	0.586	0.713	0.843	0.982	1.343	0.9994
200	0.447	0.601	0.755	0.905	1.040	1.490	0.9993
225	0.435	0.564	0.709	0.837	0.955	1.313	0.9988
250	0.365	0.495	0.629	0.755	0.870	1.270	0.9992

*average of pentaplicate results

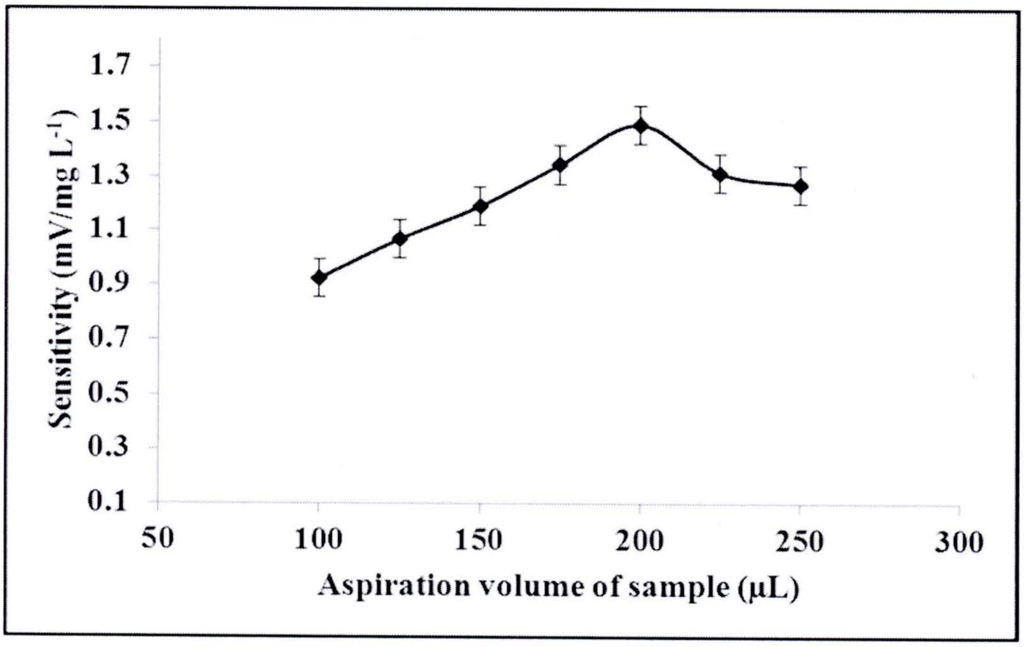

Figure 3.25 Relationship between various aspiration volumes of sample and sensitivity of the calibration curve

3.4.2.10 Effect of flow rate

In the SIA system, the sample and reagent solutions were pumped into SIA manifold via syringe pump. The effect of flow rate was studied over the range 100-225 μL s^{-1} by aspirating of various concentration of cadmium(II) standard solutions (0.1-0.5 mg L^{-1}) into the SIA system as shown in Figure 2.2 and 2.3. The results obtained are shown in Table 3.32 and Figure 3.26. It was found that maximum sensitivity was obtained at a flow rate of 175 μL s^{-1}. Increasing the flow rates above 175 μL s^{-1} did not significantly enhance the sensitivity, but they increased the pressure in the tubing, resulting in the more consumption of the reagents. Therefore, a flow rate of 175 μL s^{-1} was chosen for further investigations.

Table 3.32 Effect of flow rate on the sensitivity

Flow rate (μL s^{-1})	**ΔPeak height (mV)* obtained from the standard Cd(II) (mg L^{-1})**					**Sensitivity (mV/mg L^{-1})**	**Correlation coefficient (r^2)**
	0.1	**0.2**	**0.3**	**0.4**	**0.5**		
100	0.370	0.495	0.623	0.746	0.858	1.227	0.9994
125	0.378	0.504	0.641	0.788	0.902	1.332	0.9987
150	0.372	0.507	0.655	0.781	0.937	1.404	0.9992
175	0.392	0.526	0.669	0.825	0.969	1.453	0.9994
200	0.371	0.492	0.636	0.778	0.906	1.356	0.9992
225	0.338	0.452	0.584	0.688	0.815	1.190	0.9990

*average of pentaplicate results

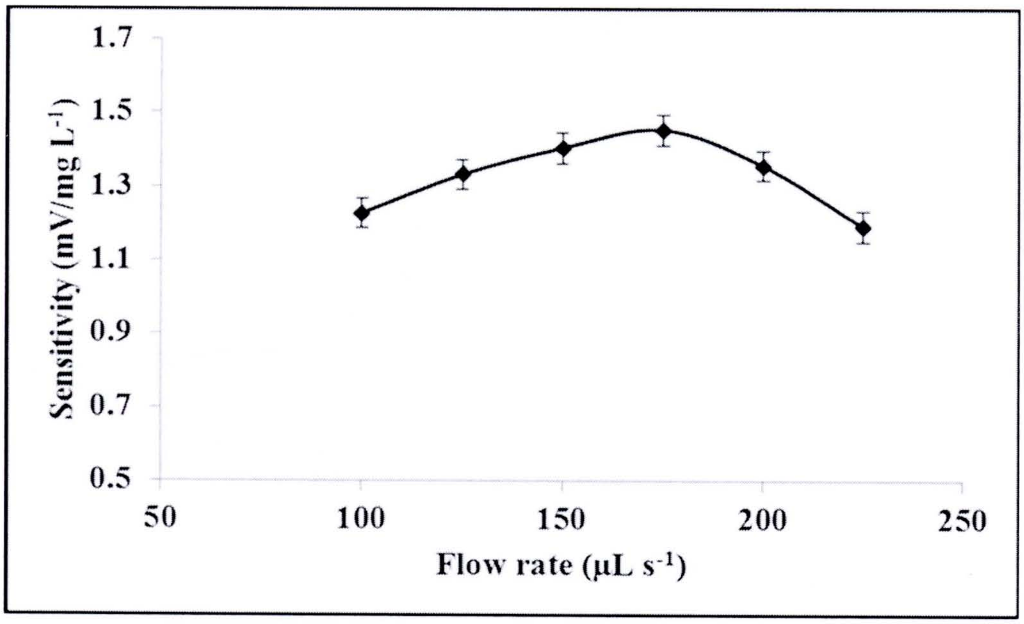

Figure 3.26 Relationship between various flow rate and sensitivity of the calibration curve

3.4.2.11 Effect of holding time

The effect of holding time on the determination of cadmium(II) was studied in the range of 0-50 s .The results are shown in Table 3.33 and Figure 3.27. The sensitivity increased to a maximum at holding time 20 s. It can be explained that increasing the holding time results in an increase in Cd(II)-Iodide-Rhodamine B complex because its mixing is better than that occurred at the holding time below 20 s. The sensitivity decreased when holding time greater than 20 s because of dispersion. Thus, a holding time of 20 s was chosen as optimum since it provided the greatest sensitivity.

Table 3.33 Effect of holding time on the sensitivity

Holding time (s)	ΔPeak height (mV)* obtained from the standard Cd(II) ($mg\ L^{-1}$)					Sensitivity ($mV/mg\ L^{-1}$)	Correlation coefficient (r^2)
	0.1	0.2	0.3	0.4	0.5		
0	0.441	0.585	0.738	0.879	0.996	1.404	0.9979
10	0.438	0.574	0.706	0.863	1.006	1.425	0.9991
20	0.447	0.586	0.746	0.882	1.031	1.464	0.9995
30	0.436	0.576	0.730	0.869	1.001	1.423	0.9993
40	0.429	0.575	0.716	0.865	0.977	1.386	0.9979
50	0.427	0.575	0.704	0.843	0.958	1.330	0.9984

*average of pentaplicate results

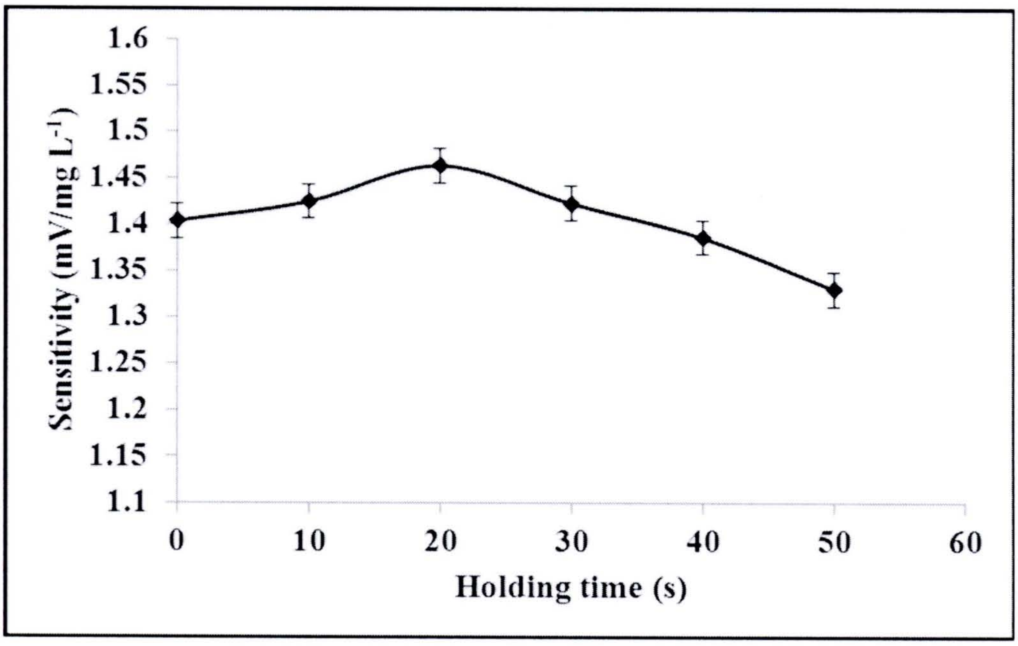

Figure 3.27 Relationship between various holding time and sensitivity of the calibration curve

3.4.2.12 Summary of the studied range and optimum conditions of SIA method for the determination of cadmium(II)

The optimization of the SIA system for cadmium(II) was studied with SIA manifold (Figure 2.2 and 2.3). The optimum experimental conditions for cadmium(II) speciation are summarized in Table 3.34. These optimum conditions were used for further investigation.

Table 3.34 Univariate optimum conditions for SI determination of cadmium(II) using Rhodamine B

Variable	**Studied range**	**Optimum conditions**
pH	2.0-4.0	3.0
Concentration of phosphate buffer (mol L^{-1})	0.05-0.25	0.15
Concentration of KI (mol L^{-1})	0.05-0.15	0.11
Concentration of hydroxylamine HCl (x 10^{-4} mol L^{-1})	0.5-2.5	1.5
Concentration of rhodamine B (x 10^{-4} mol L^{-1})	1.5-4.0	3.0
Aspiration volume of phosphate buffer (μL)	50-175	125
Aspiration volume of KI (μL)	75-200	150
Aspiration volume of rhodamine B (μL)	75-200	150
Aspiration volume of sample (μL)	100-250	200
Flow rate (μL s^{-1})	100-225	175
Holding time (s)	0-50	20

3.4.3 Analytical characteristics of SIA method for determination of cadmium(II) using rhodamine B

3.4.3.1 Linearity

The linear range of the proposed SIA method was studied by injecting cadmium(II) standard solutions over the range 0.00-5.00 mg L^{-1} into SI system under the suitable conditions (Table 3.34). The results are presented in Table 3.35 and Figure 3.28. The experimental results indicated that linear range was in the range 0.00-1.00 mg L^{-1} for cadmium(II) with pentaplicate injections, that Beer's law was obeyed over a wide concentration range, which is useful for analysis of cadmium(II) in diverse matrices of sample.

Table 3.35 Study of Linearity at Δpeak height of various cadmium(II) concentrations

Cadmium(II)	**Peak height (mV)**						**ΔP.H.***
(mg L^{-1})	**1**	**2**	**3**	**4**	**5**	**$\bar{x}$**	**(mV)**
0.00	0.366	0.349	0.349	0.345	0.345	0.347	0.000
0.005	0.355	0.358	0.355	0.352	0.352	0.354	0.007
0.01	0.363	0.363	0.363	0.363	0.362	0.363	0.016
0.02	0.378	0.378	0.378	0.379	0.379	0.378	0.031
0.04	0.401	0.401	0.401	0.407	0.401	0.402	0.055
0.06	0.433	0.435	0.435	0.435	0.430	0.434	0.087
0.08	0.453	0.456	0.456	0.456	0.453	0.455	0.108
0.10	0.484	0.485	0.488	0.481	0.488	0.485	0.138
0.15	0.541	0.544	0.544	0.544	0.537	0.542	0.195
0.20	0.612	0.608	0.604	0.617	0.617	0.612	0.265
0.30	0.757	0.742	0.757	0.737	0.752	0.749	0.402
0.40	0.884	0.862	0.878	0.884	0.878	0.877	0.530
0.50	1.007	1.010	1.010	1.010	1.010	1.009	0.662
0.60	1.115	1.117	1.117	1.117	1.113	1.116	0.769
0.70	1.204	1.198	1.206	1. 206	1.198	1.202	0.855
0.80	1.300	1.308	1.300	1.300	1.300	1.302	0.955
0.90	1.378	1.376	1.376	1.376	1.378	1.377	1.030
1.00	1.458	1.458	1.458	1.458	1.458	1.458	1.111
1.50	1.640	1.642	1.642	1.642	1.642	1.642	1.295
2.00	1.716	1.712	1.712	1.712	1.712	1.713	1.366
2.50	1.780	1.766	1.767	1.767	1.767	1.769	1.422
3.00	1.843	1.847	1.843	1.844	1.834	1.842	1.495
4.00	1.854	1.865	1.867	1.867	1.891	1.869	1.522
5.00	1.902	1.903	1.907	1.909	1.908	1.906	1.559

*average of pentaplicate results

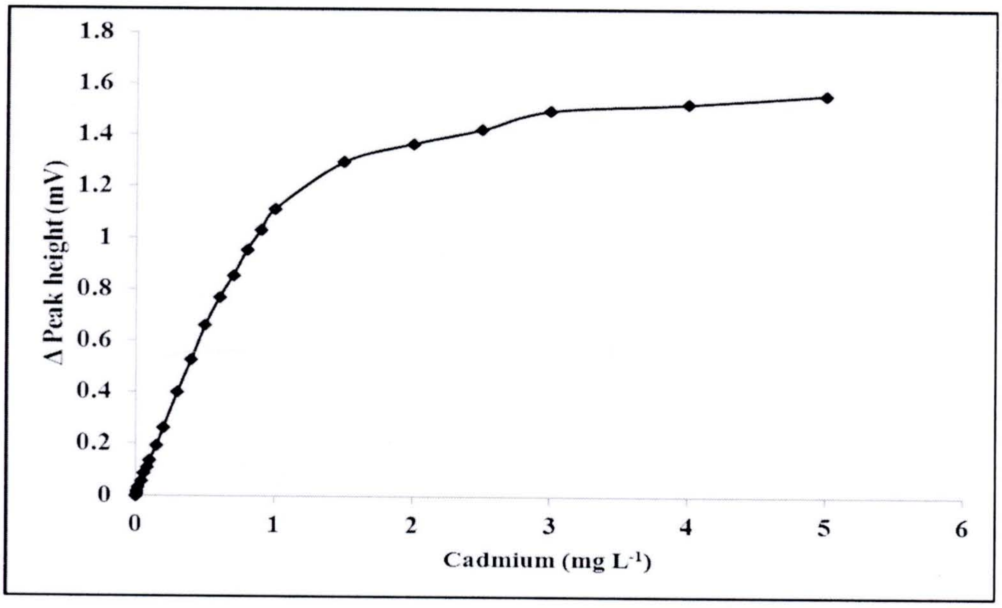

Figure 3.28 Linearity and relationship between net peak height and various concentrations of cadmium(II)

3.4.3.2 Calibration curve

A calibration curve was obtained by injecting cadmium(II) standard solutions containing 0.00-1.00 mg L^{-1} into the recommended SIA system (Figure 2.1) under the optimum conditions (Table 3.34). The resulting violet colored product was monitored at 612 nm. The results were shown in Table 3.36 and Figure 3.29. The calibration curve as shown in Figure 3.30 was established by plotting Δpeak heights versus the various cadmium(II) concentrations. It was found to be linear over the range 0.00-1.00 mg L^{-1} with the following calibration equation:

$$y = 1.3968x + 0.0146$$

When y is signal as peak height in mV

x is concentration of cadmium(II) solution in mg L^{-1} with a correlation coefficient (r^2) of 0.9997

Table 3.36 ΔPeak height for calibration curve

Cadmium(II) (mg L^{-1})	Peak height (mV)						ΔP.H.* (mV)
	1	2	3	4	5	$\bar{x}$	
0.00	0.646	0.64	0.346	0.646	0.646	0.645	0.000
0.10	0.809	0.811	0.811	0.811	0.811	0.811	0.116
0.20	0.941	0.944	0.944	0.944	0.944	0.944	0.298
0.30	1.079	1.084	1.076	1.084	1.076	1.080	0.435
0.40	1.225	1.225	1.227	1.227	1.227	1.226	0.581
0.60	1.485	1.485	1.494	1.494	1.494	1.490	0.845
0.80	1.729	1.729	1.729	1.729	1.728	1.729	1.084
1.00	2.059	2.059	2.064	2.064	2.064	2.062	1.417

*average of pentaplicate results

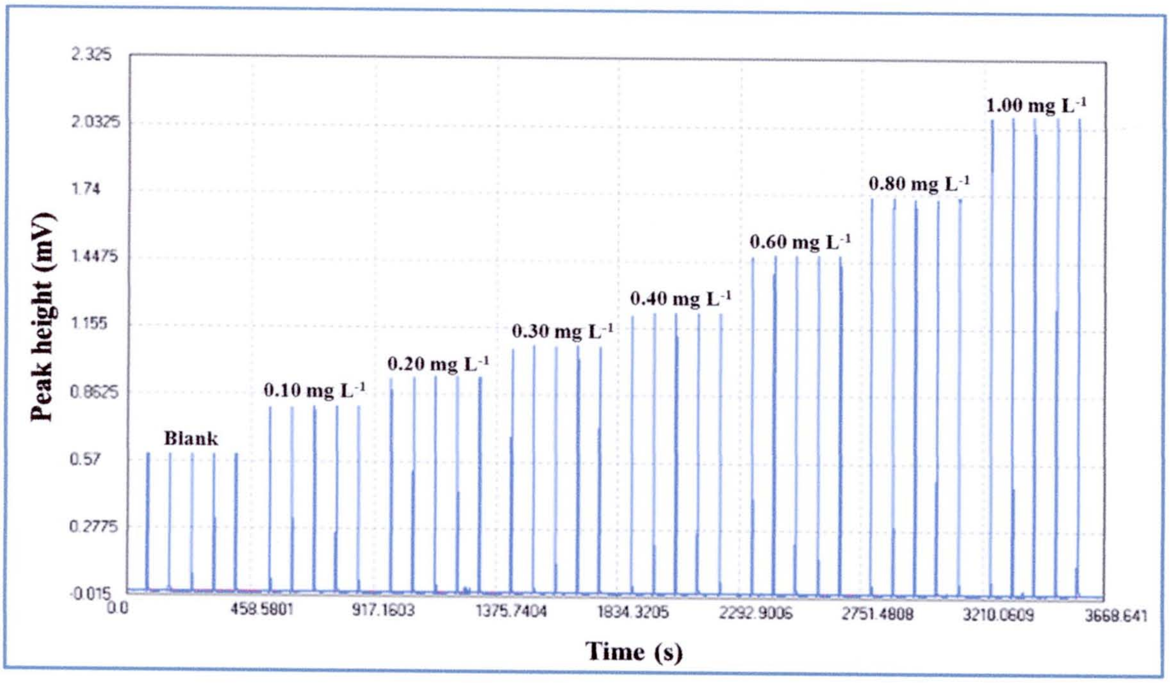

Figure 3.29 Calibration signal of SI spectrophotometric determination of cadmium(II) 0.00-1.00 mg L^{-1}

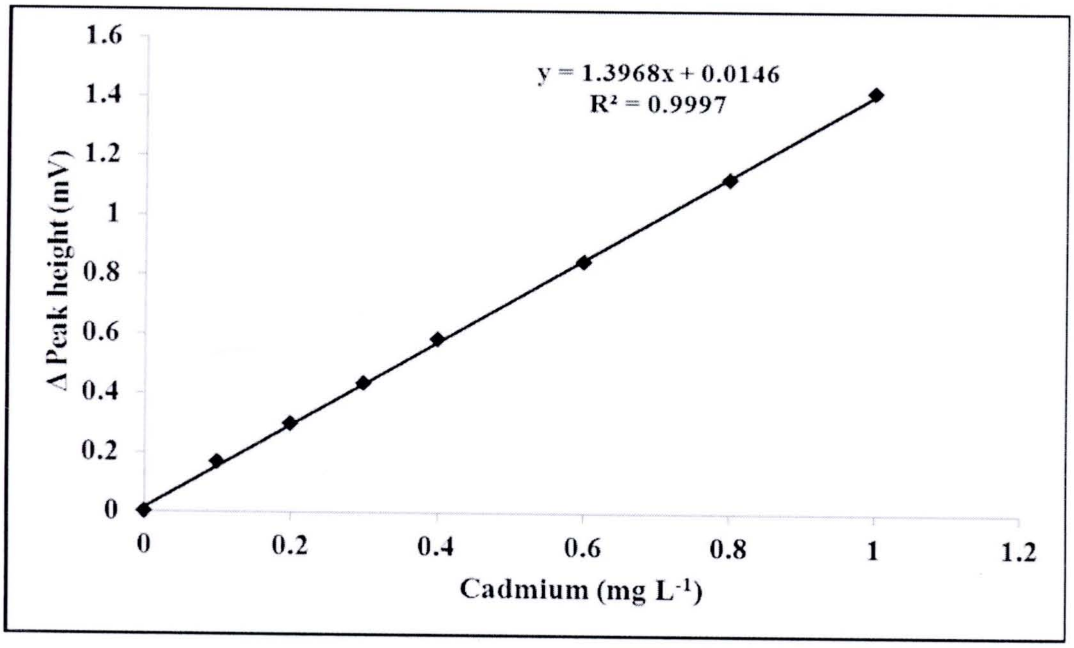

Figure 3.30 Calibration curve of SI spectrophotometric determination of cadmium(II) 0.00-1.00 mg L^{-1}

3.4.3.3 Precision

The precision of the proposed SIA method was verified by injecting 11 replicates of 0.2 mg L^{-1} cadmium(II) standard solution, using the optimum conditions in Table 3.34. The results were shown in Table 3.37. The relative standard deviation was found to be 0.16%.

Table 3.37 Precision of verification using 0.2 mg L^{-1} cadmium(II) standard solution of the SIA method

Experimental number	**Peak height (mV)***
1	0.618
2	0.618
3	0.617
4	0.619
5	0.617
6	0.618
7	0.616
8	0.616
9	0.617
10	0.618
11	0.617
$\bar{\mathbf{x}}$	0.617
S.D.	0.001
% R.S.D.	0.16

*average of pentaplicate results

3.4.3.4 Detection limit

The detection limit is defined as that concentration of the analyte producing a signal (peak height) which is the experimental blank signal plus three times of the standard deviation of the blank signal [82]. Using the SIA manifold (Figure 2.2 and 2.3) and the optimum conditions in Table 3.34. The results were presented in Table 3.38. The detection limit of the proposed method was found to be 0.005 mg L^{-1}.

Table 3.38 The detection limit of SIA method from blank signal resulting of 12 injections

Experimental number	Peak height (mV)*
1	0.003
2	0.003
3	0.003
4	0.002
5	0.002
6	0.002
7	0.002
8	0.003
9	0.003
10	0.003
11	0.003
12	0.004
$\bar{x}$	0.003
S.D.	0.0007
LOD (mg L^{-1})	0.005
LOQ (mg L^{-1})	0.010

*average of pentaplicate results

3.4.3.5 Accuracy

The accuracy (defined as percentage recovery of the added analyte) of the proposed SIA method was verified by standard addition method. The method was examined by determining the recoveries of the added cadmium(II) with varying concentrations in sample solution. The results are presented in Tables 3.39. The average recovery was found to be 100.54% for cadmium(II). The proposed SIA method for determination of cadmium(II) was found to be accurate for the determination of trace amounts of cadmium(II) in sample solutions.

Table 3.39 The %recovery by using standard addition method for cadmium(II) by SIA method

Cadmium(II) (mg L^{-1})	**Peak height (mV)***		**[Cd(II)] found (mg L^{-1})**	**% recovery**
	Std.	**Std.+sample**		
0.1	0.823	0.853	0.104	104.00
0.2	0.907	0.914	0.202	101.00
0.3	1.109	1.104	0.299	99.67
0.4	1.257	1.260	0.401	100.25
0.5	1.426	1.394	0.489	97.80
Mean			100.54	
S.D.			2.266	
% RSD			2.254	

*average of pentaplicate results

3.4.4 Interference studies

The interference effects of some possible foreign ions on the determination of cadmium(II) were studied by the proposed SIA procedure under the optimum conditions (Table 3.34). A systematic study to check for the effects of some possible foreign ions on the determination of cadmium(II) was undertaken for the maximum w/w ratio of cadmium(II) to foreign ions up to 1:5000 The solutions of a 0.1 mg L^{-1} cadmium(II) standard containing varying concentrations of diverse ions were determined using the SIA system. The tolerance is defined as the largest foreign-ion concentration causing % relative error ±10% for determining the analyte of interest. The tolerance values for the ions studied are given in Table 3.40.

Table 3.40 Interference studies for 0.1 mg L^{-1} standard cadmium(II) by SIA method

Interferences	Cadmium : Interference	Peak height (mV)	% Relative error
Zn^{2+}	1 : 0	0.497	-
	1 : 100	0.491	-1.21
	1 : 1000	0.472	-5.03
	1 : 2500	0.444	-10.66
Cr^{2+}	1 : 0	0.497	-
	1 : 500	0.489	-1.61
	1 : 2500	0.474	-4.63
	1 : 5000	0.453	-8.85
Cu^{2+}	1 : 0	0.487	-
	1 : 5	0.491	+0.82
	1 : 10	0.511	+4.93
	1 : 20	0.548	+12.53

* average of triplicate results.

Table 3.40 (Continued)

Interference	Cadmium(II) : Interference	Peak height* (AU)	% Relative error
Ni^{2+}	1 : 0	0.487	-
	1 : 1000	0.488	+0.21
	1 : 2500	0.490	+0.62
	1 : 5000	0.507	+4.11
Ba^{2+}	1 : 0	0.487	-
	1 : 1000	0.482	-1.03
	1 : 3000	0.462	-5.13
	1 : 5000	0.442	-9.24
Co^{2+}	1 : 0	0.487	-
	1 : 100	0.493	+1.23
	1 : 200	0. 509	+4.52
	1 : 500	0.538	+10.47
Al^{3+}	1 : 0	0.487	-
	1 : 100	0.483	-0.82
	1 : 1000	0.463	-4.93
	1 : 3000	0.434	-10.88
Fe^{2+}	1 : 0	0.487	-
	1 : 50	0.512	+5.13
	1 : 100	0.521	+6.98
	1 : 150	0.552	+13.35
Fe^{3+}	1 : 0	0.487	-
	1 : 100	0.499	+2.46
	1 : 300	0.512	+5.13
	1 : 400	0.536	+10.06
Mn^{2+}	1 : 0	0.427	
	1 : 10	0.441	+3.28
	1 : 50	0.454	+6.32
	1 : 100	0.475	+11.24

* average of triplicate results.

Table 3.40 (Continued)

Interference	**Cadmium(II) : Interference**	**Peak height* (AU)**	**% Relative error**
Cl^-	1 : 0	0.427	-
	1 : 50	0.445	+4.22
	1 : 100	0.459	+7.49
	1 : 200	0.478	+11.94
I^-	1 : 0	0.427	-
	1 : 500	0.439	+2.81
	1 : 1000	0.443	+3.75
	1 : 5000	0.443	+3.75
Br^-	1 : 0	0.427	-
	1 : 100	0.443	+3.75
	1 : 500	0.452	+5.85
	1 : 1000	0.471	+10.30
SO_4^{2-}	1 : 0	0.427	-
	1 : 500	0.448	+4.92
	1 : 1000	0.453	+6.09
	1 : 2500	0.473	+10.77
PO_4^{3-}	1 : 0	0.427	-
	1 : 100	0.445	+4.22
	1 : 200	0.449	+5.15
	1 : 400	0.475	+11.24
K^+	1 : 0	0.449	-
	1 : 500	0.472	+5.12
	1 : 1000	0.472	+5.12
	1 : 5000	0.479	+6.68
NO_2^-	1 : 0	0.449	-
	1 : 10	0.455	+1.34
	1 : 50	0.463	+3.12
	1 : 100	0.497	+10.69

* average of triplicate results.

Table 3.40 (Continued)

Interference	**Cadmium(II) : Interference**	**Peak height*** **(AU)**	**% Relative error**
NO_3^-	1 : 0	0.449	-
	1 : 500	0.475	+5.79
	1 : 1000	0.483	+7.57
	1 : 2000	0.499	+10.91
Mg^{2+}	1 : 0	0.449	-
	1 : 1000	0.437	-2.67
	1 : 2500	0.422	-6.01
	1 : 5000	0.419	-6.68
Na^+	1 : 0	0.449	-
	1 : 500	0.464	+3.34
	1 : 1000	0.472	+5.12
	1 : 5000	0.479	+6.68
F^-	1 : 0	0.449	-
	1 : 500	0.455	+1.34
	1 : 1000	0.467	+4.00
	1 : 5000	0.474	+5.57
CN^-	1 : 0	0.449	-
	1 : 500	0.449	-
	1 : 1000	0.452	+0.67
	1 : 5000	0.460	+2.45

* average of triplicate results.

The experimental results obtained by investigation of some interfering species which may occur in water samples indicated that the presence of some interfering ions namely Cr^{3+}, Ni^{2+}, Ba^{2+}, I^-, K^+, Mg^{2+}, Na^+, F^-, CN^- at concentration ≥5000 folds that of cadmium(II) caused relative error ±10%. Furthermore, interfering ions of Al^{3+}, Zn^{2+} and SO_4^{2-}, NO_3^-, Br^-, Co^{2+}, Fe^{3+} and PO_4^{3-}, Cl^-, Fe^{2+}, Mn^{2+} and NO_2^- at concentration 3000, 2500, 2000, 1000, 500, 400, 200, 150 and 100 folds, respectively that of cadmium(II) caused % relative error ±10%. While the presence of Cu^{2+} concentration up to 20 folds that of cadmium(II) cause relative error ±10%. The presence of interfering ions was found to interfere by increasing and decreasing SIA signals. It was found that the serious positive interferences for cadmium(II) determination was Cu^{2+}. Most interferences from various cations might be due to the formation of coloured complex and competitive complexation with the rhodamine dye and reagent structure contain numerous sites at which chelation with metal ions might occur that affect absorption of complex. Ions which may oxidize iodide to triiodide under the experimental conditions, such as Cu^{2+} which may form large anionic ion-complexes which can form ion-associates with rhodamine B. Since interferences are many in which few interferences are significant under the optimum working conditions. Thus the method is selective and matrix effects are not severe to the determination cadmium(II) in wastewater, which referred standard quality of wastewater defined by Pollution Control Department (PCD) Ministry of Natural Resources and Environment (Appendix A), which can be evaluated by method of standard additions. In addition, such high concentrations of transition-metal interferences are normally not to be expected in wastewater samples. The interference effects of some possible foreign ions on cadmium(II) determination using SIA system were summarized in Table 3.41.

Table 3.41 Summary of interference effects of some ions on the response obtained from 0.1 mg L^{-1} of cadmium(II) by SIA method

Interference ions	**Tolerable concentration ratio* (mg L^{-1}) of ion/Al (III)**
Cr^{3+}, Ni^{2+}, Ba^{2+}, I^-, K^+, Mg^{2+}, Na^+, F^-, CN^-	≥5000
Al^{3+}	3000
Zn^{2+}, SO_4^{2-}	2500
NO_3^-	2000
Br^-	1000
Co^{2+}	500
Fe^{3+}, PO_4^{3-}	400
Cl^-	200
Fe^{2+}	150
Mn^{2+}, NO_2^-	100
Cu^{2+}	20

*The concentration of an ion is considered to be interfering when causing a relative error of more than ±10% with respect to the signal of cadmium(II) alone.

3.4.5 Determination of cadmium(II) in wastewater samples using standard addition method by SIA method

The proposed SIA spectrophotometric method was applied to the simultaneous determination of cadmium(II) in wastewater samples which were collected from several districts in Chiang Mai. The results were given in Table 3.42. The cadmium(II) contents in the wastewater samples were in the range of 0.015-0.065 mg L^{-1}.

Table 3.42 Determination of cadmium(II) in wastewater samples using standard addition method by SIA method

Wastewater samples	**Cadmium concentration* (mg L^{-1})**				**SD**	**Cadmium(II) concentration* (mg L^{-1})**
	1	**2**	**3**	**$\bar{x}$**		
Wastewater 1	0.029	0.027	0.028	0.028	0.0010	0.028 ± 0.0010
Wastewater 2	ND**	ND**	ND**	ND**	-	-
Wastewater 3	0.023	0.022	0.024	0.023	0.0010	0.023 ± 0.0010
Wastewater 4	0.014	0.015	0.015	0.015	0.0007	0.015 ± 0.0007
Wastewater 5	0.026	0.028	0.025	0.026	0.0016	0.026 ± 0.0016
Wastewater 6	0.032	0.037	0.033	0.034	0.0030	0.034 ± 0.0030
Wastewater 7	0.018	0.017	0.018	0.018	0.0007	0.018 ± 0.0007
Wastewater 8	0.017	0.016	0.015	0.016	0.0010	0.016 ± 0.0010
Wastewater 9	0.034	0.031	0.032	0.032	0.0016	0.032 ± 0.0016
Wastewater 10	0.065	0.068	0.063	0.065	0.0030	0.065 ± 0.0030

*average of triplicate results

**not detected

3.4.6 Comparative determination of cadmium(II) in wastewater samples of the proposed SIA method with ICP-MS method and validation method

In order to validate the proposed SIA method for cadmium(II) determination, a comparative determination of cadmium(II) by ICP-MS method was carried out. The experimental results were shown in Table 3.43. There was good agreement between the proposed SIA method and the ICP-MS method. The cadmium(II) contents in the wastewater samples were in the range of 0.015-0.065 mg L^{-1} and 0.013-0.073 mg L^{-1} using the proposed SIA method and ICP-MS respectively. The results obtained by the proposed SI spectrophotometric method compared favorably with those obtained by ICP-MS using the student *t*-test (Table 3.43 and Appendix B in Table B.2). It was seen that experimental *t*-value for cadmium(II) assay, which was smaller than the theoretical *t*-value at a confidence interval of 95% indicating that results obtained by both methods were in excellent agreement.

Table 3.43 Comparative determination of cadmium(II) in wastewater samples by the proposed SIA method and ICP-MS method

Wastewater samples	**Concentrations of cadmium (mg L^{-1})**		$t_{\text{calculated}}$
	SIA[*]	**ICP-MS**[*]	
Wastewater 1	0.028	0.027	1.732
Wastewater 2	ND**	ND**	-
Wastewater 3	0.023	0.026	-5.196
Wastewater 4	0.015	0.013	4.206
Wastewater 5	0.026	0.027	-0.758
Wastewater 6	0.034	0.029	2.887
Wastewater 7	0.018	0.017	1.732
Wastewater 8	0.016	0.019	-5.196
Wastewater 9	0.032	0.037	-5.088
Wastewater 10	0.065	0.073	-4.446

[*] average of triplicate results
[**] not detected