

CHAPTER IV

RESULTS AND DISCUSSION

4.1. Preliminary study

4.1.1. Type of chromoionophore

Chromoionophore is one of important composition of optode membrane. Concentration of silver ion is observed by the optical response of chromoionophore. Therefore, a suitable type of chromoionophore was investigated.

Both chromoionophores were protonated at nitrogen atoms when immersed in $1 \text{ mol L}^{-1} \text{ HNO}_3$ [56]. The spectra of fully protonated form and fully deprotonated form of chromoionophore I and XIV were recorded in the wavelength range of 400-800 nm as illustrated in Figure 4.1. The protonated forms of both chromoionophores provided more stable structure because of the resonance forms resulting in the decrease of energy gap. The maximum absorption band was then shifted to longer wavelength.

For chromoionophore XIV, the optode membrane responded to H^+ by a color change from yellow to blue. The characteristic maximum absorption band of deprotonated form was found at 450 nm and that of protonated form was found at 650 nm (Figure 4.1 (A)).

For chromoionophore I, the optode membrane responded to H^+ by a color change from pink to blue. The characteristic maximum absorption band of deprotonated form was found at 545 nm and that of protonated form was found at 628 and 668 nm (Figure 4.1 (B)).



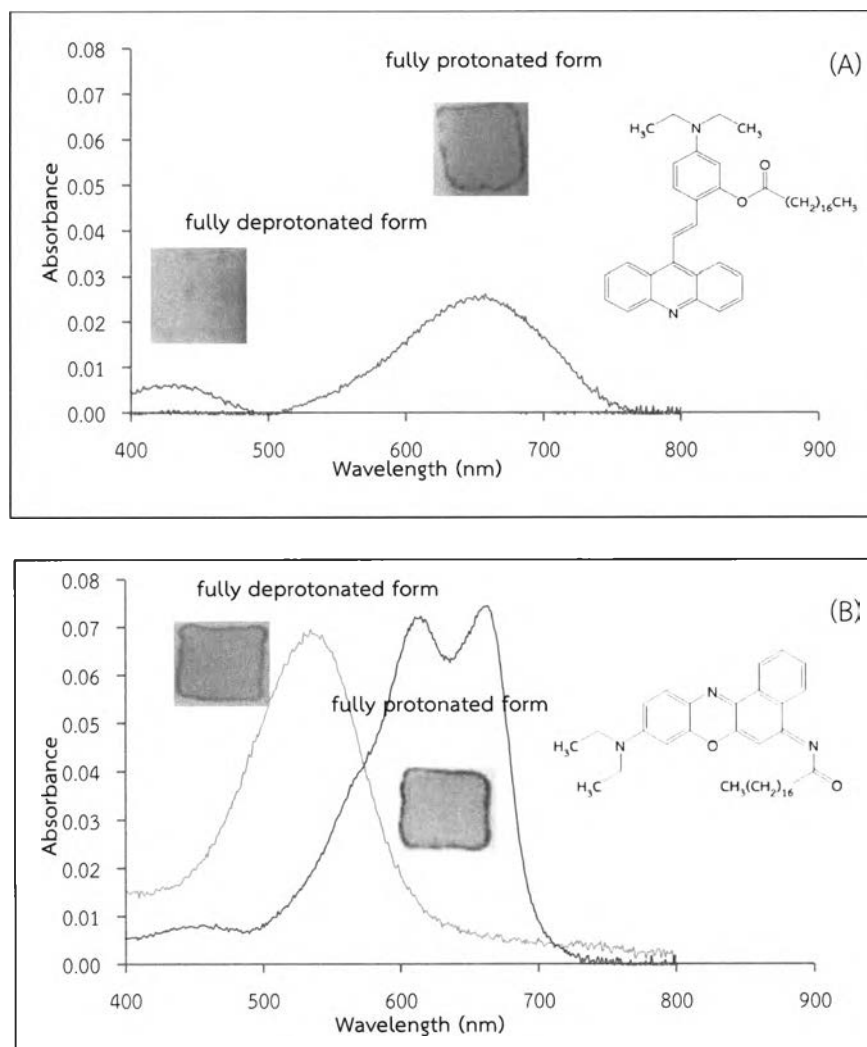


Figure 4.1 Absorption spectra of (A) chromoionophore XIV and (B) chromoionophore I

From the result, the color change of chromoionophore I was clearly and the absorbance was higher than that of chromoionophore XIV. Therefore, chromoionophore I was chosen for further experiment.

4.1.2. Effect of pH

In this study, the effect of pH was investigated in the pH range of 7.0-8.5. The optode membrane response were studied in the concentration range of

10^{-6} - 10^{-3} mol L⁻¹ silver ion at pH 7.0, 8.0 and 8.5 buffering by Tris solution. Example of the absorption spectra of the optode membranes at pH 8.0 was shown in Figure 4.2. When the concentration of silver ion increased, optode membranes changed from blue to pink. Therefore, a reduction in the absorption band of protonated form and an increase in absorption band of deprotonated form were obtained.

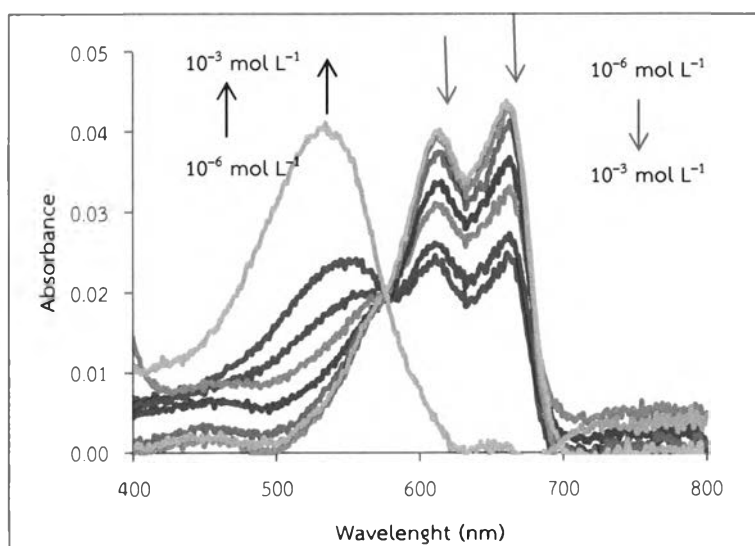


Figure 4.2 Absorption spectra of the optode membranes at pH 8.0

The responses of the optode membranes at pH 7.0, 8.0 and 8.5 are shown in Figure 4.3. The response of the optode membrane showed a sigmoidal shape at pH 8.0-8.5 and the working range was wider than at pH 7.0. Therefore, pH 8.0-8.5 was selected as pH range for further experiment.

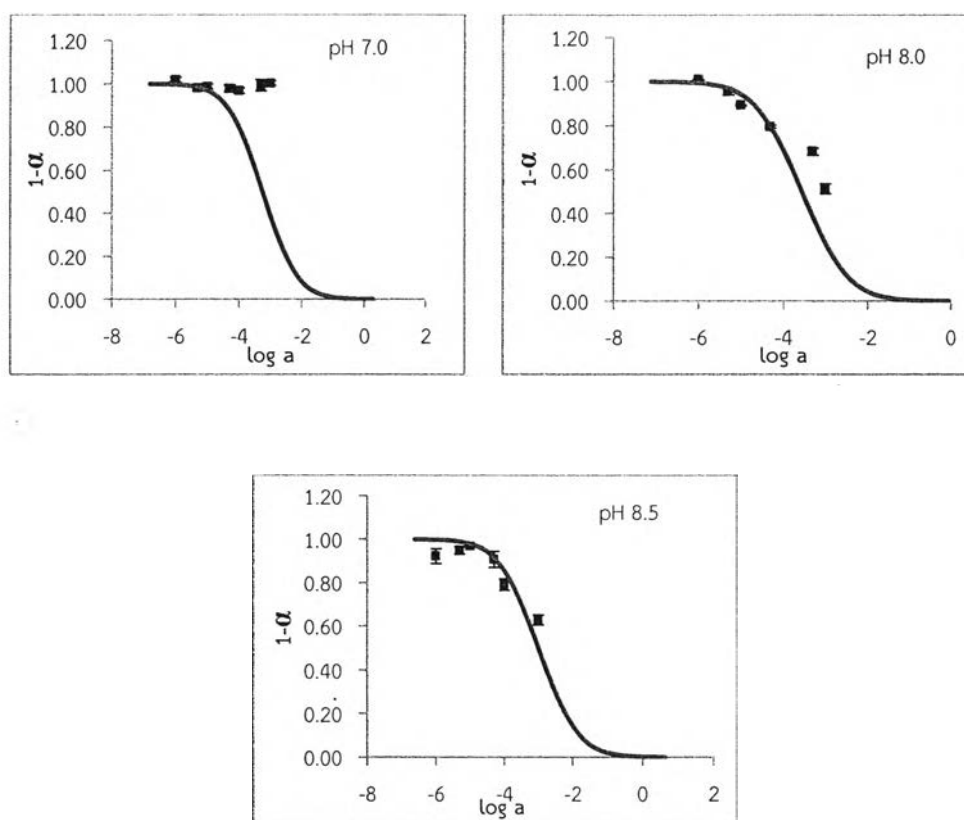


Figure 4.3 The response of the optode membranes at pH 7.0, 8.0 and 8.5 in various concentration of silver ion

From the results of the preliminary study, chromoionophore I was chosen for preparing optode membrane and the pH range of 8.0-8.5 was used for further experiment.

4.2. Ratio of composition of membrane

The major components of membrane are CU1, KTpClPB and chromoionophore I. The response behavior of optode membrane is dependent on type and percentage composition of all components. The amount of three components is always kept in the order of $L > R > C$ [38].

The ratio of CU1 and KTpClPB was used as described in our previous report [16] and chromoionophore I was varied from 2.50 to 5.98 mmol kg⁻¹ in PVC plasticized by DOS (1:2 w/w).

The plots between the degree of deprotonation ($1 - \alpha$) and $\log a_{\text{Ag}}$ of the optode membranes A, B and C are illustrated in Figure 4.4. From the results, the characteristic function of only optode membrane C showed a sigmoidal shape as predicted by theory and it also responded in a wide concentration range of 10^{-5} - 10^{-2} mol L⁻¹ AgNO₃. Therefore, the optode membrane prepared from composition C was chosen for further experiment.

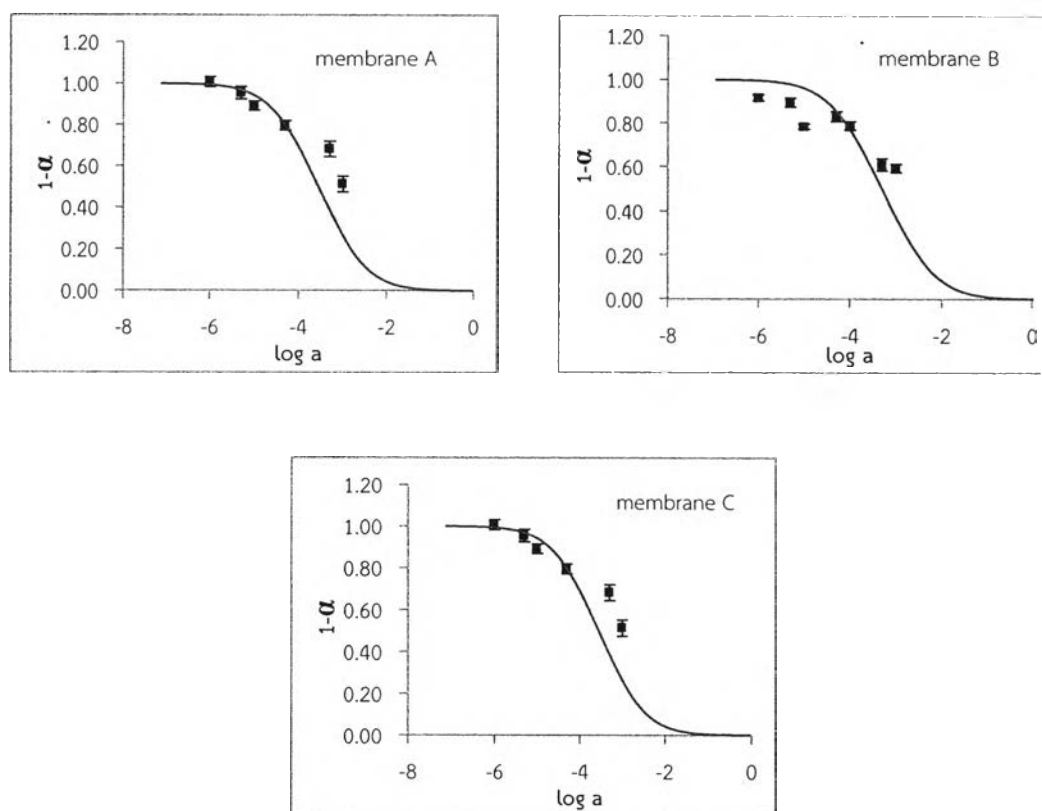


Figure 4.4 Responses of silver ionoptode membrane for membrane A, membrane B and membrane C at pH 8.0

4.3. Effect of pH

In this study, the effect of pH was investigated in the pH range of 5.0-9.0. After immersing the optode membranes in AgNO_3 solutions at various pHs, the color of the optode membranes changed from blue to pink at only pH 7.0-9.0 which can be detected by naked-eye. While at pH 5.0 and 6.0 no color change was observed.

From the result in Figure 4.5, the absorbance at 545 nm, corresponding to the deprotonated form of chromoionophore I, increased with increasing the pH until pH 8.0. At low pH, the chromoionophore was not deprotonated therefore the extraction of silver ion into the membrane phase was not good as the ion-exchange reaction shown in Equation (2.1). At pH 9.0, the absorbance decreased due to the formation of silver oxide. Therefore, pH 7.0-8.5 was the pH range for further experiment.

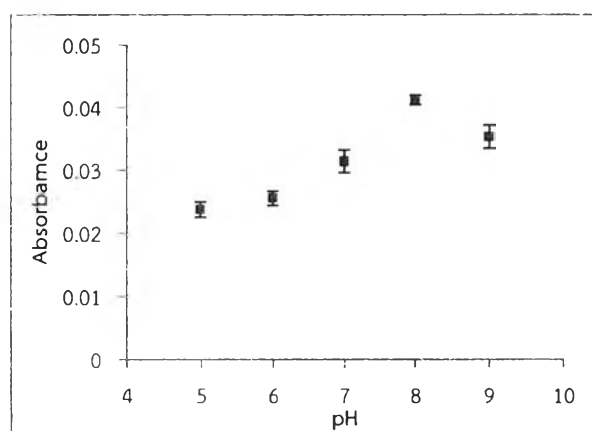


Figure 4.5 pH effect on the response of the optode membranes at 545 nm after immersing in $10^{-3} \text{ mol L}^{-1}$ silver ion solution

The optode membrane response were also studied in the concentration range of 10^{-5} - $10^{-2} \text{ mol L}^{-1}$ silver ion solution at pH 7.0, 8.0 and 8.5 buffering by Tris solution. The absorption spectra of the optode membranes at pH 8.5 and the color of calibration chart are shown in Figure 4.6. The optode membrane responded to silver ion by changing color from blue to pink. The color of calibration chart of

optode membrane related with the concentration of silver ion. The concentration range of silver ion can be detected by naked-eye. This is one of advantage of bulk optode technique that can be applied for the fieldwork application.

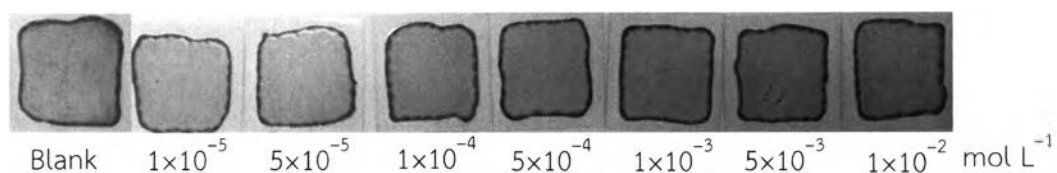
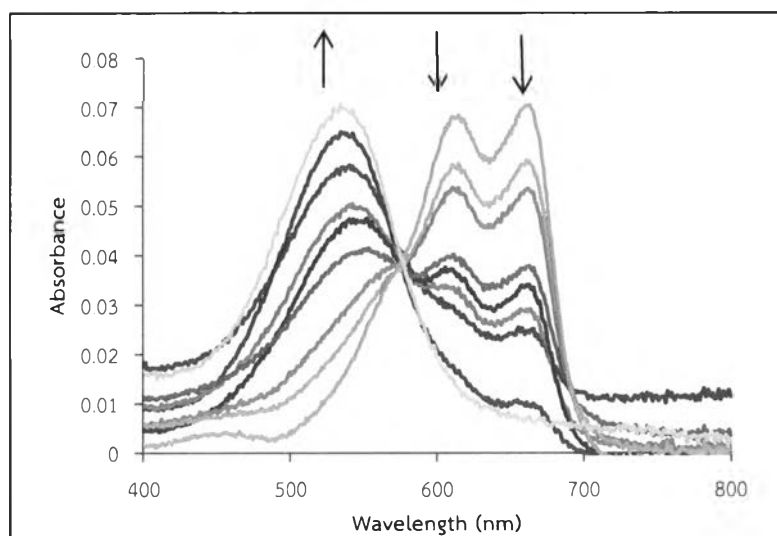


Figure 4.6 Absorption spectra of the optode membranes in 10^{-5} - 10^{-2} mol L⁻¹ silver ion solution at pH 8.5 and the color of calibration chart

The relation between $1-\alpha$ and $\log a$ at pH 7.0-8.5 are shown in Figure 4.7. The response of the optode membrane showed a sigmoidal shape at pH 8.0-8.5 and the working range was wider than at pH 7.0. Therefore, pH 8.0-8.5 was the optimum pH range for further experiment.

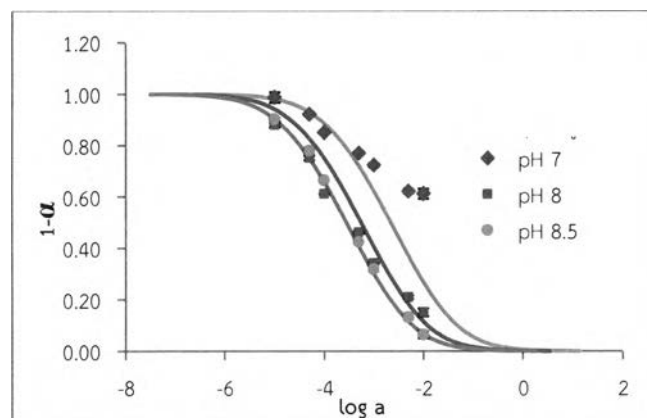


Figure 4.7 The response of the optode membranes at pH 7.0, 8.0, 8.5 in various concentration of silver ion

4.4. Response time

The response time is the time required for target ion transfer from bulk solution into the membrane. This process is depended on the membrane thickness, membrane composition, activity of the measuring ion and pH of the measurement [21, 57]. It is an important characteristic for any optode membrane.

Figure 4.8 shows the absorbance change of the optode membrane in the presence of 10^{-2} and 10^{-5} mol L⁻¹ silver ion. From the results, the response times were found at 15 min and 20 min for 10^{-2} and 10^{-5} mol L⁻¹ AgNO₃ solution, respectively. At higher concentration, silver ion was extracted into the membrane faster than at lower concentration [47, 53]. However, to ensure that the extraction of silver ion into the optode membrane reached the equilibrium, immersing time of 20 min was chosen for further experiment.



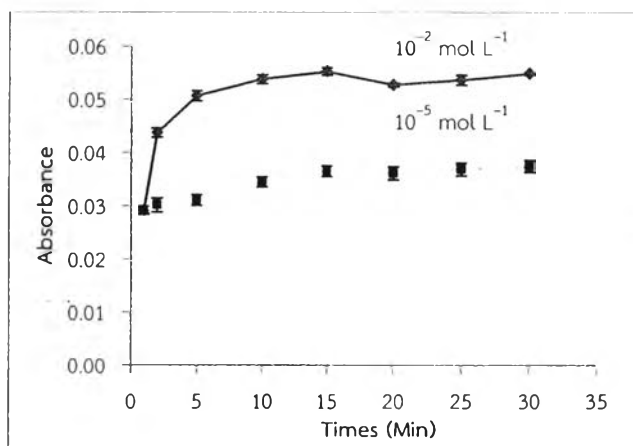


Figure 4.8 Response time of the optode membrane in the presence of 10^{-2} and 10^{-5} mol L⁻¹ AgNO₃

4.5. Selectivity

The selectivity behavior is one of important characteristics of bulk optode technique. The selectivity coefficients ($K_{Ag,M}^{opt}$) is a value to show selectivity behavior between ionophore and target ion. The sensor selectivity of the proposed optode membrane over different metal ions (M^{n+}) was studied by separate solution method (SSM) [21, 47].

Na⁺, K⁺, Ca²⁺ and Hg²⁺ were chosen for studying the selectivity behavior of the proposed optode membrane. Because Na⁺, K⁺ and Ca²⁺ are metal ions which can be found in commercial silver nanoparticles product whereas Hg²⁺ is soft metal like Ag⁺.

The activity of Ag⁺ in the mix solution is calculated by Equation (4.1) as follows:

$$a_{Ag^+} = (K_{Exch}^{Ag})^{-1} \left(\frac{\alpha}{1-\alpha} a_H \right)^{n_{Ag}} \times \frac{R_{tot} - (1-\alpha)C_{tot}}{n_{Ag} \left\{ L_{tot} - (R_{tot} - (1-\alpha)C_{tot}) \left(\frac{Z_{Ag}}{n_{Ag}} \right)^{Z_{Ag}} \right\}} \quad (4.1)$$

The activity of another cation in the mix solution is calculated by Equation (4.2) as follows:

$$a_M = (K_{\text{Exch}}^M)^{-1} \left(\frac{\alpha}{1-\alpha} a_H \right)^{n_M} \times \frac{R_{\text{tot}} - (1-\alpha)C_{\text{tot}}}{n_M \left\{ L_{\text{tot}} - (R_{\text{tot}} - (1-\alpha)C_{\text{tot}}) \left(\frac{z_M}{n_M} \right) \right\}^{z_M}} \quad (4.2)$$

From the Equations 4.1 and 4.2, a curve of each cation was plotted between $1-\alpha$ versus the logarithm of activity as shown in Figure 4.9.

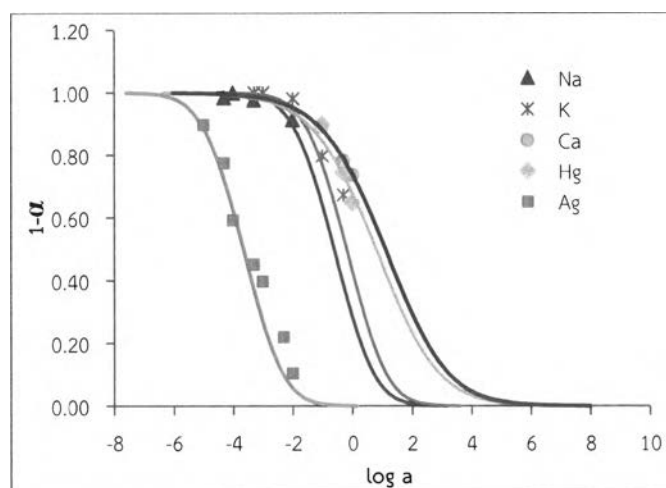


Figure 4.9 Curves between $1-\alpha$ versus the logarithm of the activity of Na^+ , K^+ , Ca^{2+} and Hg^{2+} at pH 8.5

The selectivity coefficients ($K_{\text{Ag},M}^{\text{opt}}$) was given by the ratio of two activities calculated at the highest sensitivity ($\alpha = 0.5$) followed by Equation (4.3) [58, 59].

$$K_{\text{Ag},M}^{\text{opt}} = \frac{a_{\text{Ag}}}{a_M} = \frac{n_M K_{\text{Exch}}^M}{n_{\text{Ag}} K_{\text{Exch}}^{\text{Ag}}} \left(\frac{\alpha}{1-\alpha} a_H \right)^{n_{\text{Ag}} - n_M} \frac{\left(L_{\text{tot}} - \frac{z_M}{n_M} \left\{ R_{\text{tot}} - (1-\alpha) C_{\text{tot}} \right\} \right)^{z_M}}{\left(L_{\text{tot}} - \frac{z_{\text{Ag}}}{n_{\text{Ag}}} \left\{ R_{\text{tot}} - (1-\alpha) C_{\text{tot}} \right\} \right)^{z_{\text{Ag}}}} \quad (4.3)$$

Table 4.1 shows the selectivity coefficients ($K_{Ag,M}^{opt}$) of each cation. From the result, silver ion showed high selectivity over other cations in the order of $Ag^+ > Na^+ > K^+ > Hg^{2+} > Ca^{2+}$.

Table 4.1 Selectivity coefficients ($K_{Ag,M}^{opt}$) of the optode membranes in Tris buffer solution at pH 8.5

Interfering ions	$\log K_{Ag,M}^{opt} (\log a_{Ag}/a_M)$
Ag^+	0
Na^+	-3.07
K^+	-3.47
Hg^{2+}	-4.47
Ca^{2+}	-4.77

4.6. Limit of detection

The estimation of lower detection limit can be approximated by two different methods:

1) the loss of sensitivity due to the sigmodal shape of the response function at low analyte concentrations. Using this method, it has been defined through:

- a) the standard deviation of background signal
- b) the variation of given fraction of the maximum slope of the response function
- c) the intersection point of two linear functions of maximum and minimum slope which is in the zone of lower activity of sigmoidal function

The upper limit can be obtained in analogy with previous definition b) and c) [21, 60, 61].

- 2) the interference from other ions present



In this work, the estimation of lower limit was approximated based on the loss of sensitivity due to the sigmoidal response curve which was determined in c). This approximation offered two advantages; a simple calculation and an interpolation with less error. The dynamic linear range in sigmoidal response curve is shown in Figure 4.10.

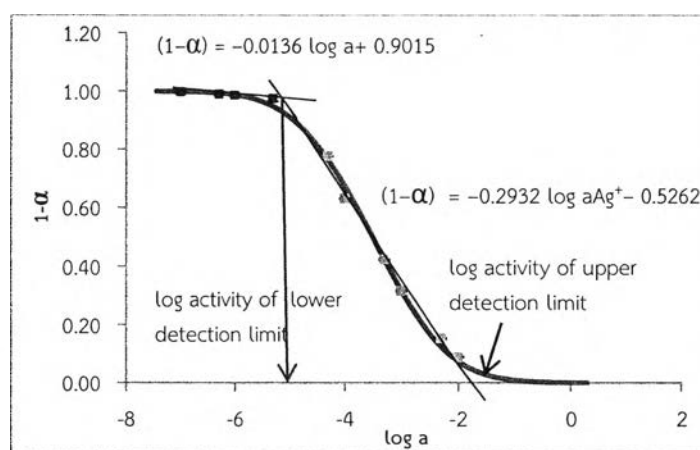


Figure 4.10 Dynamic linear range in sigmoidal response curve

From the Figure 4.10, linear functions of two series of standard were found to be

$$(1-\alpha) = -0.0136 \log a + 0.9015$$

$$\text{and } (1-\alpha) = -0.2932 \log a - 0.5262$$

The interception of both functions gave a lower detection limit (DL) of $7.83 \times 10^{-6} \text{ mol L}^{-1}$ and the detection limit was $1.58 \times 10^{-2} \text{ mol L}^{-1}$. A dynamic range of $1 \times 10^{-5} - 1 \times 10^{-2} \text{ mol L}^{-1}$ was obtained.

4.7. Reproducibility and repeatability

The reproducibility and repeatability are two important characteristics of the optical sensor. The reproducibility of the optode membranes was studied by

measuring the absorbance of 10 optode membranes made from the same cocktail solution.

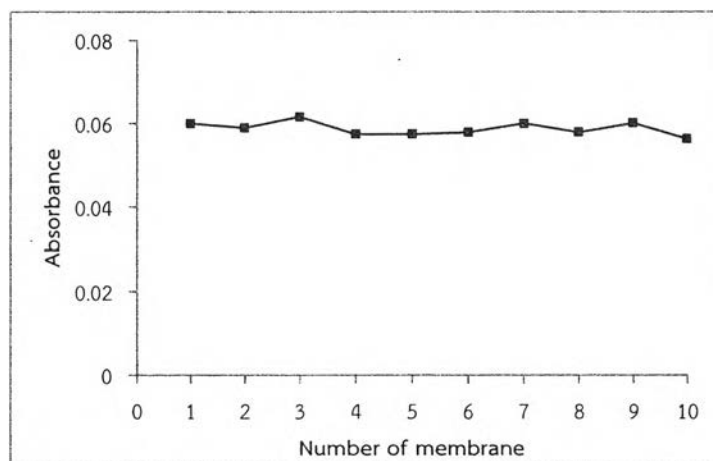


Figure 4.11 Absorbance at 545 nm of 10 optode membranes made from the same cocktail solution in Tris buffer solution at pH 8.5 containing 10^{-3} mol L^{-1} $AgNO_3$

From the result in Figure 4.11, the relative standard deviation (R.S.D.) of the absorbance was 2.80% ($n=10$). This result indicated the acceptable reproducibility compared with the other works [21, 45, 48, 62].

One of advantages of bulk optode technique is repeatability. The used optode membrane was regenerated by immersing in $1 \text{ mol } L^{-1}$ HNO_3 solution. Therefore, silver ion was released from the optode membrane to solution phase.

The repeatability of optode membrane was studied by measuring the absorbance of single optode membrane immersed in Tris buffer solution at pH 8.5 containing 5×10^{-5} mol L^{-1} $AgNO_3$ and another experiment, containing 10^{-3} and 5×10^{-5} mol L^{-1} $AgNO_3$. The results are shown in Figure 4.12 and Figure 4.13.

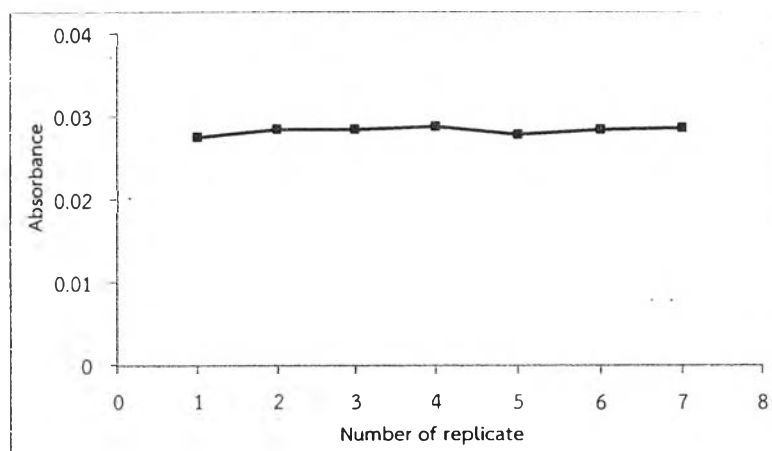


Figure 4.12 Absorbance at 545 nm of single optode membrane in Tris buffer solution at pH 8.5 containing $5 \times 10^{-5} \text{ mol L}^{-1} \text{ AgNO}_3$

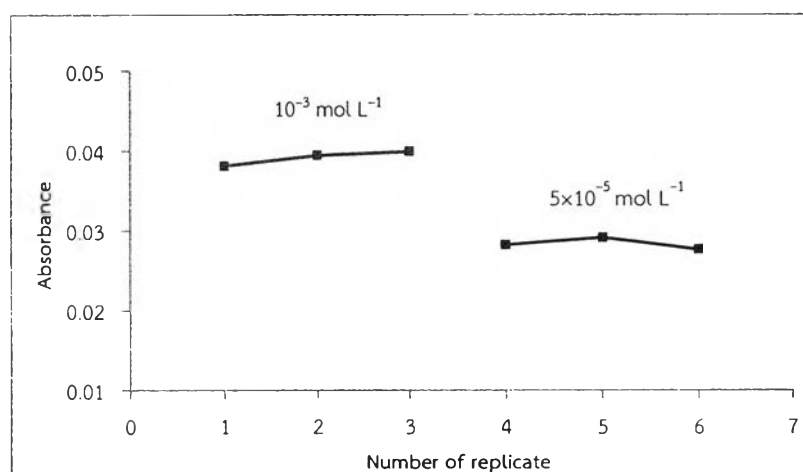


Figure 4.13 Absorbance at 545 nm of single optode membrane in Tris buffer solution at pH 8.5 containing $1 \times 10^{-3} \text{ mol L}^{-1}$ and $5 \times 10^{-5} \text{ mol L}^{-1} \text{ AgNO}_3$

From the result in Figure 4.12, the relative standard deviation (R.S.D.) of the absorbance was 1.47% ($n=7$). This result indicated the acceptable reversibility compared with the other works [21, 45, 48, 62].

From the result in Figure 4.13, the relative standard deviation (R.S.D.) of the absorbance was 2.39% ($n=3$) and 2.65% ($n=3$) for $1 \times 10^{-3} \text{ mol L}^{-1}$ and $5 \times 10^{-5} \text{ mol L}^{-1}$

AgNO₃ solution, respectively. This result indicated the acceptable reversibility compared with the other works [21, 45, 48, 62]. Moreover, it was also found that the regeneration of optode membrane was independent on the concentration of silver ion.

4.8. Lifetime

From the result in Figure 4.14, The difference of absorbance between the day of fabrication and 14 days after fabrication was 3.0% while the difference of absorbance between the day of fabrication and 16-18 days after fabrication was 7%. Therefore, the precision of measurement was good if the optode membrane kept in the box within 2 weeks.

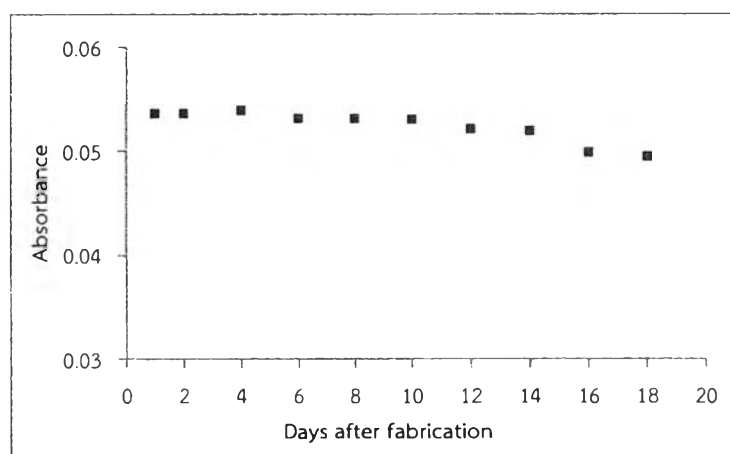


Figure 4.14 The absorbance of the optode membrane in function of days after fabrication



4.9. Summarization of the optimum conditions

Parameters of the optimized optode membrane used for determination of silver ion by batch system are summarized in Table 4.2.

Table 4.2 Parameters of the optimized optode membrane used for determination of silver ion

Parameters	Conditions/Results
pH and type of buffer	Tris buffer, pH 8.0-8.5
response time	20 min
regeneration	1 mol L ⁻¹ HNO ₃ for 5 min
dynamic range	1×10 ⁻⁵ to 1.0×10 ⁻² mol L ⁻¹
detection limit (DL)	lower detection limit = 7.83×10 ⁻⁶ mol L ⁻¹ upper detection limit = 1.58×10 ⁻² mol L ⁻¹
life time	14 days

4.10. Real sample analysis

The proposed optode membrane was used to determine silver ion in real sample as sample A and sample B by spiked method.

Real sample was diluted 50 folds because pH of real sample was not suitable for this method. From the results, calibration curves between $1 - \alpha$ versus the logarithm of activity at pH 8.5 prepared by Milli-Q water were used to determine silver ion (Figure 4.15 (A) and Figure 4.16 (A)) and by real sample (Figure 4.15 (B) and Figure 4.16 (B)) in order to investigate the matrix effect in real sample solution. From the results, it was found that the slopes of the calibration curves prepared by real sample solution and milli-Q were not different indicating that the matrix interference in real sample solution did not affect to the experiment. In addition, the percentage recoveries of silver ion in sample A and sample B were more than 90% indicating the acceptable accuracy [63] (Table 4.3).

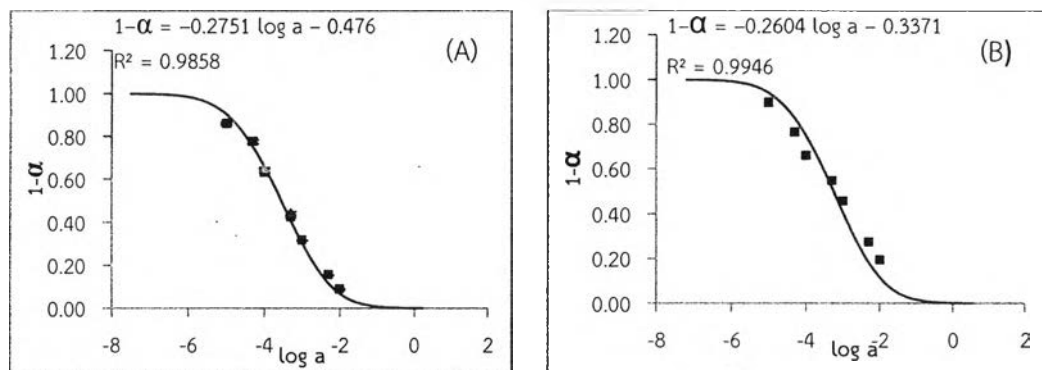


Figure 4.15 Calibration curves between $1-\alpha$ versus the logarithm of activity at pH 8.5 prepared by (A) Milli-Q water and (B) by sample A

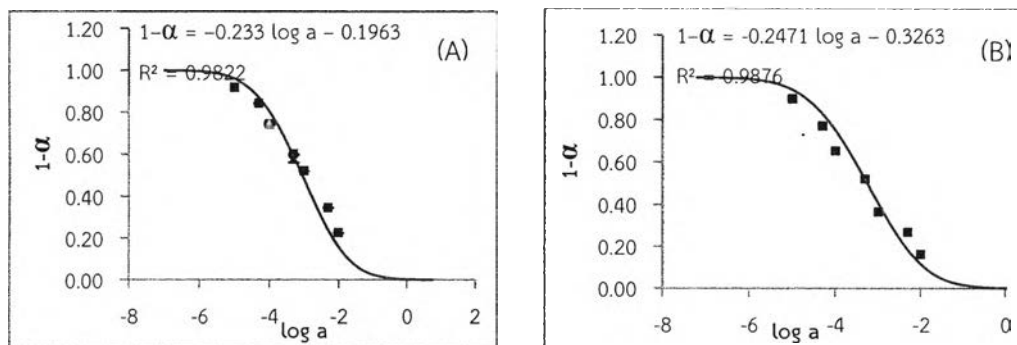


Figure 4.16 Calibration curves between $1-\alpha$ versus the logarithm of activity at pH 8.5 prepared by (A) Milli-Q water and (B) sample B

Table 4.3 The determination of silver ion in sample A and sample B

Sample	Added (mol L ⁻¹)	Founded (mol L ⁻¹)	%Recovery
Sample A	1×10^{-4}	9.43×10^{-5}	92.4
	5×10^{-4}	4.67×10^{-4}	93.3
Sample B	1×10^{-4}	9.25×10^{-5}	92.5
	5×10^{-4}	4.56×10^{-4}	91.2

4.11. Silver nanoparticles solution

The proposed optode membrane was used to determine silver nanoparticles in form of silver ion. Silver nanoparticles were synthesized from Environmental Analysis Research Unit. Silver nanoparticles (yellow solution) were changed to silver ion form (colorless solution) via oxidation process by hydrogen peroxide. The spiked silver nanoparticle solution with silver nitrate is the method for checking the accuracy of the experiment.

Figure 4.17 shows a calibration curve between $1-\alpha$ versus the logarithm of activity at pH 8.5 used to determine silver nanoparticle in form of silver ion diluted by Milli-Q and sample. From the results in Table 4.4 and 4.5, the percentage recovery of the spiked method was in the range of 90-110% indicating the acceptable accuracy [63].

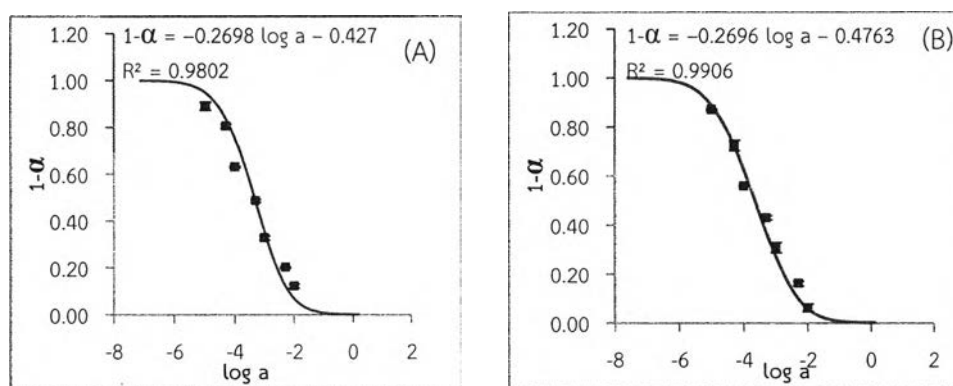


Figure 4.17 Calibration curves between $1-\alpha$ versus the logarithm of activity at pH 8.5 used to determined silver ion in silver nanoparticle solution in (A) Milli-Q (B) sample A and sample B

Table 4.4 The determination of silver nanoparticles in form of silver ion diluted by Milli-Q

	Added	Total silver (mol L ⁻¹)	%Recovery
10 folds dilute Ag nano solution	H ₂ O ₂	1.19×10 ⁻⁵	-
10 folds dilute Ag nano solution	1×10 ⁻⁵ mol L ⁻¹ Ag ⁺ + H ₂ O ₂	2.27×10 ⁻⁵	108.6
2 folds dilute Ag nano solution	H ₂ O ₂	2.07×10 ⁻⁵	-
2 folds dilute Ag nano solution	1×10 ⁻⁵ mol L ⁻¹ Ag ⁺ + H ₂ O ₂	3.10×10 ⁻⁵	104.0

Table 4.5 The determination of silver nanoparticles in form of silver ion diluted by sample A and sample B

	Added	Total silver (mol L ⁻¹)	%Recovery
Sample A 100 folds dilute Ag nano solution	-	9.19×10 ⁻⁶	-
100 folds dilute Ag nano solution	H ₂ O ₂	1.94×10 ⁻⁵	-
100 folds dilute Ag nano solution	10 ⁻⁴ mol L ⁻¹ Ag ⁺ + H ₂ O ₂	1.25×10 ⁻⁴	105.9
Sample B 100 folds dilute Ag nano solution	-	9.78×10 ⁻⁶	-
100 folds dilute Ag nano solution	H ₂ O ₂	1.30×10 ⁻⁵	-
100 folds dilute Ag nano solution	10 ⁻⁴ mol L ⁻¹ Ag ⁺ + H ₂ O ₂	1.05×10 ⁻⁴	91.9



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