CHAPTER III

EXPERIMENT

3.1. Chemicals and apparatus

All chemicals were used without further purification and listed in Table 3.1 as follows:

Table 3.1 Chemicals list

Chemicals	Supplier/Grade
25,27-Di(benzothiazolyl)-26,28-dihydroxy-calix[4]arene (CU1)	synthesized from Environmental Analysis Research Unit
Silver nanoparticles solution	synthesized from Environmental Analysis Research Unit
9-(Diethylamino)-5-(octadecanoylimino)-5H-benzo[*a*]phenoxazine, chromoionophore I	Fluka/ Selectophore
2-[2-(9-Acridinyl)vinyl]-5-(diethylamino)phenyl stearate, chromoionophore XIV	Fluka/ Selectophore
Calcium nitrate ($Ca(NO_3)_2$)	MERCK/analytical reagent
Citric acid monohydrate ($C_6H_8O_7 \cdot H_2O$)	MERCK/ analytical reagent
Mercury nitrate ($Hg(NO_3)_2 \cdot 6H_2O$)	MERCK/ analytical reagent
Nitric acid 65% (HNO_3)	MERCK/ analytical reagent
Polyvinylchloride, PVC	Fluka/ Selectophore
Potassiumtetrakis(4-chlorophenyl)borate, KT*p*ClPB	Fluka/ Selectophore
Potassium nitrate (KNO_3)	BDH/ analytical reagent
Silver nitrate ($AgNO_3$)	BDH/ analytical reagent
Sodium hydroxide (NaOH)	MERCK/ analytical reagent
Sodium nitrate ($NaNO_3$)	CARLO ERBA/ analytical reagent
Tetrahydrofuran, THF	Fluka/Selectophore
Tris(hydroxymethyl) aminomethane ($C_4H_{11}NO_3$)	MERCK/ analytical reagent

25,27-Di(benzothiazolyl)-26,28-dihydroxy-calix[4]arene (CU1) was synthesized from our research group based on the previously published procedure [16] as follows:

3.05 g (7.2 mmol) of 22, 26, 27-tetrahydroxycalix[4]arene and 3.34 g (24.2 mmol) of potassium carbonate were dissolved in 60 mL of acetronitrile in a 100 mL two-necked round-bottomed flask. The mixture was stirred under nitrogen atmosphere at room temperature for 1 hr. Then, 2.4 mL of bromoacetonitrile was added and the mixture was heated at reflux at 90°C under nitrogen atmosphere for 7 hr. The mixture was cooled to room temperature and filtered. Then, the mixture was evaporated nearly dryness and treated with methanol. The 25, 27-bis(cyano methoxy)-26, 28-dihydroxycalix[4]arene was obtained (2.404 g, 66% yield).

The mixture of 25, 27-bis(cyanomethoxy)-26, 28-dihydroxycalix[4]arene and 0.5 mL of 4.7 mol L^{-1} aminothiophenol was stirred and heated at reflux under nitrogen atmosphere for 3 hr. Then, the mixure was subsequently cooled to room temperature. Then, dichloromethane and methanol were added to precipate 25, 27-di(benzothiazolyl)-26,28-dihydroxy-calix[4]arene (CU1) (1.081 g, 74% yield).

Silver nanoparticles was also prepared from our research group based on the previously published procedure [55] as follows:

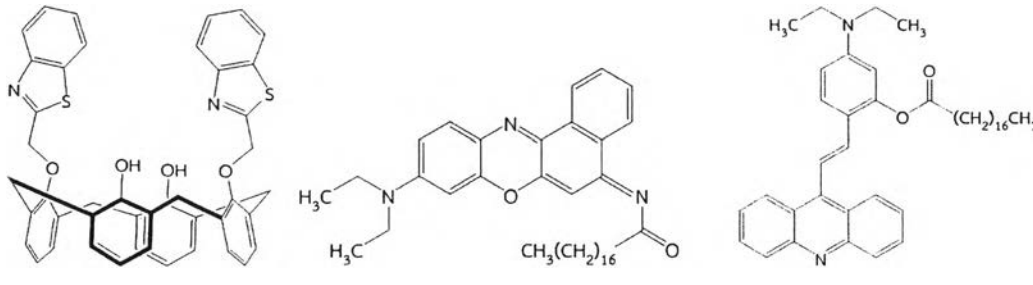
Silver nanoparticles solution was prepared by the reduction between 20 mL of 1 mmol L^{-1} $AgNO_3$ solution with 20 mL of 1 mmol L^{-1} $NaBH_4$ solution. The solution of $NaBH_4$ was cooled in an ice bath and then, $AgNO_3$ solution was added to it under vigorous stirring. Then, 100 µL of 0.1 mol L^{-1} CTAB was added in the solution in 3 min. Silver nanoparticles solution was then kept in a plastic bottle.

The absorbance measurements were recorded on a Varian Cary 50 Probe UV-Vis spectrophotometer. The pH values of the solutions were measured by a

pH/mv meter model UltraBASIC-10 (Denver). A vortex mixer was used for shaking the mixture in optode membrane preparation.

3.2. Silver selective optode membrane preparation

The amount of ionophore, ion-exchanger and chromoionophore is always kept in the order of L > R^- > C [48]. In this work, DOS was chosen to be plasticizer of optode membrane. It is one of plasticizers for preparing optode membrane which provide the best response behavior toward target ion when compared to the other plasticizer [49]. KT*p*ClPB was used as a cation exchanger which was used for membrane preparation in several works in literature [21, 40, 45]. The structures of major components of the propose optode membrane are shown in Figure 3.1.



(A) (B) (C)

Figure 3.1 Structures of (A) CU1 (B) chromoionophore I and (C) chromoionophore XIV

A mixture of the optimized composition of chromoionophore I: KT*p*ClPB: CU1 was 5.98: 7.47: 10.94 mmol kg^{-1} calculated by Equation 3.1.

$$Y = \frac{X \times 10^6}{M_{total} \times MW} \qquad (3.1)$$

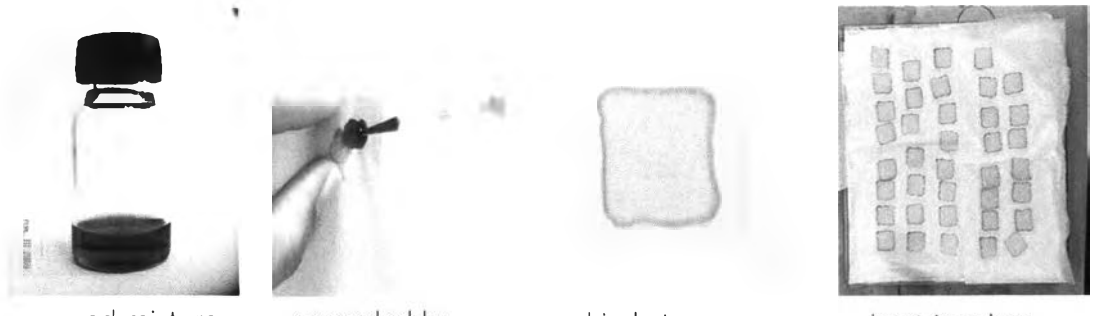
where X is the mass of each of the component (mg)

Y is the concentration of each of the component (mmol kg^{-1})

M_{total} is the mass of all of the component (mg)

MW is the molecular weight of each of the components (g mol^{-1})

The mixture was dissolved in 2 mL of THF and shaken by a vortex mixer. Then, 50 µL of the mixture solution was pipetted to spread on a square microscope cover glass (22x22) mm. The transparent and pinkish purple membranes were dried at room temperature for at least 30 min and kept in a box. The silver selective membrane preparation steps are illustrated in Figure 3.2.



prepared mixture solution | spreaded by micropipette | dried at room temperature | kept in a box

Figure 3.2 Silver selective membrane preparation

3.3. Preparation of solutions

3.3.1. Silver standard solutions

A stock solution of 0.1 and 10^{-3} mol L^{-1} $AgNO_3$ was prepared by dissolving $AgNO_3$ in Milli-Q water in an appropriate volume. Working solutions were prepared by diluting the stock solution with a buffer solution.

3.3.2. Nitric acid solution

1 mol L^{-1} nitric acid solution was prepared by direct diluting from 65% (w/w) concentrated solution.

3.3.3. Sodium hydroxide solution

1 mol L^{-1} sodium hydroxide solutions was prepared by dissolving of 10 g of NaOH in 250 mL Milli-Q water.

3.3.4. Tris buffer and citric acid solutions

10^{-3} mol L^{-1} Tris buffer and citrate buffer solutions were prepared by dissolving 0.0605 g of tris(hydroxymethyl) aminomethane or 0.0961 g of citric acid monohydrate in 500 mL Milli-Q water. The pH of solution was adjusted by 1 mol L^{-1} HNO_3 or 1 mol L^{-1} NaOH.

3.4. Steps to determine silver ion by bulk optode technique

The absorbances of the optode membrane were recorded in a range of 400–800 nm on a UV-Vis spectrophotometer using a cover glass without membrane as reference.

Step 1. Absorbance measurements for fully protonated and fully deprotonated chromoionophore membranes

A fully protonated chromoionophore membrane was prepared by immersing the membrane in a 1 mol L^{-1} HNO_3 solution for 5 min. Chromoionophore was completely changed to protonated form. The membrane changed color from pink (to obtain λ_{max} of fully protonated form) to blue (to obtain λ_{max} of fully deprotonated form) and then the membrane was rinsed with Milli-Q water. The absorbance of fully protonated chromoionophore was recorded at λ_{max} of fully protonated form (A_{pro}).

A fully deprotonated chromoionophore membrane was prepared by immersing the membrane in a 1 mol L^{-1} NaOH solution for 5 min. Chromoionophore was completely changed to deprotonated form. The color of membrane was blue. The membrane was then rinsed with Milli-Q water. The absorbance of fully deprotonated chromoionophore was recorded at λ_{max} of fully deprotonated form (A_{depro}).

Step 2. Absorbance measurements for silver determinated membranes

A fully protonated chromoionophore membranes were immersed in a silver ion solution for 20 min. The absorbance of the optode membrane was recorded at λ_{max} of fully deprotonated form.

Step 3. Regenerating optode membrane

The optode membrane was regenerated by immersing in 1 mol L^{-1} HNO_3 for 5 min. Silver ion was eluted from the optode membrane and then the optode membrane was rinsed with Milli-Q water. This optode membrane can be reused to determine silver ion again by repeating to the second step.

Step 4 Data Processing

The data from UV-Vis measurements was processed in Microsoft Excel. The degree of protonation (1- α) was plotted against the logarithmic of activity (log a).

Steps to determine silver ion by bulk optode technique are illustrated in Figure 3.3.

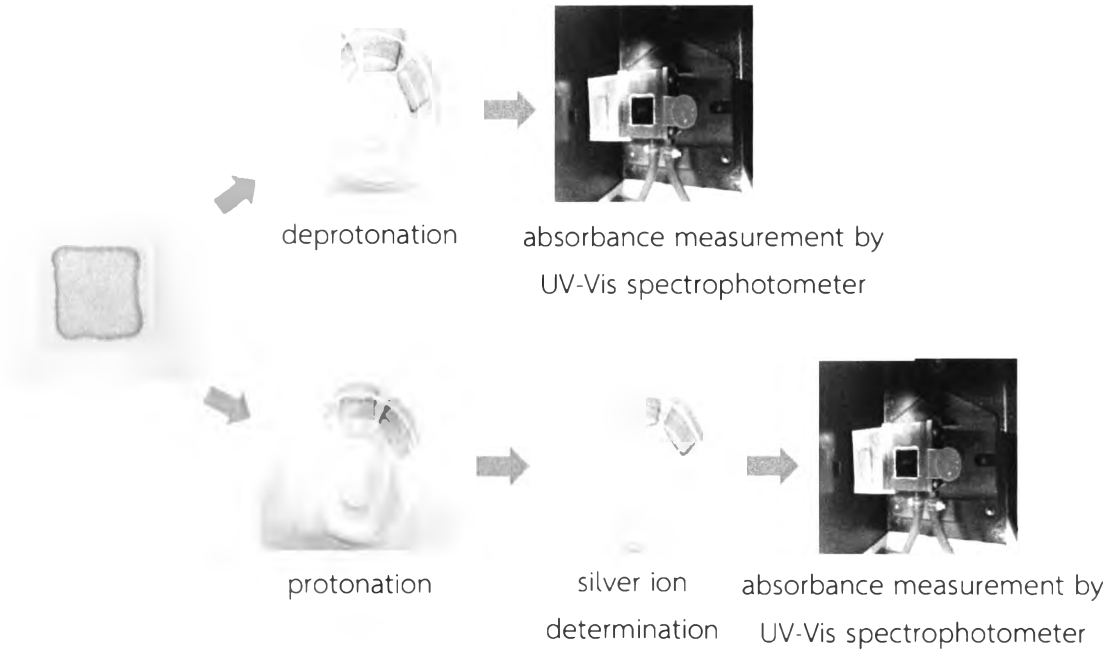


Figure 3.3 Steps to determine silver ion by bulk optode technique

3.5. Preliminary study

3.5.1. Type of chromoionophore

Chromoionophore I and chromoionophore XIV were used as a component of optode membrane. A mixture of the composition of CU1:KT*p*ClPB:chromoionophore I/ chromoionophore XIV was 10.17: 5.03: 2.50 mmol kg^{-1} [16].

A fully protonated and deprotonated chromoionophore membranes were prepared by immersing membranes in a 1 mol L^{-1} HNO_3 and 1 mol L^{-1} NaOH, respectively for 5 min and then they were rinsed with Milli-Q water. The absorbance of the optode membranes was recorded in the wavelength range of 400-800 nm.

3.5.2. Effect of pH

The fully protonated chromoionophore membranes (CU1:KT*p*ClPB:chromo ionophore I 10.17: 5.03: 2.50 mmol kg^{-1}) were immersed in the solution of 10^{-6}-10^{-3} mol L^{-1} $AgNO_3$ at pH 7.0, 8.0 and 8.5 buffering by Tris solution for 20 min. The absorbance of the optode membrane was recorded in the wavelength range of 400-800 nm.

3.6. Experiment

3.6.1. Ratio of composition of membrane

Optode membranes were prepared from a solution consisting of three components. Membranes were made with different amounts of three components which are shown in Table 3.2.

Table 3.2 Membrane compositions

Membrane	Composition				
	CU1 (mmol kg^{-1})	KT*p*ClPB (mmol kg^{-1})	chromoionophore I (mmol kg^{-1})	PVC (mg)	DOS (mg)
A	10.17	5.03	2.50	29.7	59.2
B	10.94	7.47	3.75	29.6	59.1
C	10.94	7.47	5.98	29.5	59.1

Then, the fully protonated chromoionophore membranes were immersed in 10^{-5}-10^{-2} mol L^{-1} $AgNO_3$ solution buffering by Tris solution at pH 8.0 for 20 min. The absorbance of the optode membrane was recorded in the wavelength range of 400-800 nm.

3.6.2. Effect of pH

The fully protonated chromoionophore membranes were immersed in the solution of 10^{-3} mol L^{-1} $AgNO_3$ at pH 5.0-9.0 buffering by citrate solution at pH 5.0 and 6.0 and by Tris solution at pH 7.0-9.0 for 20 min. The absorbance of the optode membranes was recorded at 545 nm.

The fully protonated chromoionophore membranes were also immersed in the solution of 10^{-5}-10^{-2} mol L^{-1} $AgNO_3$ at pH 7.0, 8.0 and 8.5 buffering by Tris solution for 20 min. The absorbance of the optode membrane was recorded in the wavelength range of 400-800 nm.

3.6.3. Response time

Response time was studied by using 10^{-2} mol L^{-1} and 10^{-5} mol L^{-1} $AgNO_3$ solutions at pH 8.5 buffering by Tris solution. The fully protonated chromoionophore membranes were immersed in the silver ion solutions. The absorbance of the optode membranes was measured at 545 nm in every 5 min for 30 min.

3.6.4. Selectivity

Cations solutions (Ag^+, Na^+, K^+, Ca^{2+} and Hg^{2+}) were prepared in Tris buffer at pH 8.5 in a range concentration of 10^{-5} to 1 mol L^{-1}. Then, the fully protonated chromoionophore membranes were immersed in these solutions for 20 min. The absorbance of the optode membranes was recorded at 545 nm.

3.6.5. Limit of detection

The standard solutions of 1×10^{-7}, 5×10^{-7}, 1×10^{-6}, 5×10^{-6}, 1×10^{-5}, 5×10^{-5}, 1×10^{-4}, 5×10^{-4}, 1×10^{-3}, 5×10^{-3} and 1×10^{-2} mol L^{-1} $AgNO_3$ were prepared in Tris buffer at pH 8.5. Then, the fully protonated chromoionophore membranes were

immersed in the $AgNO_3$ solutions for 20 min. The absorbance of the optode membranes was recorded at 545 nm.

3.6.6. Reproducibility and repeatability

Reproducibility was evaluated by measuring the absorbance of 10 optode membranes fabricated from the same cocktail solution. Then, the fully protonated chromoionophore membranes were immersed in Tris buffer solution at pH 8.5 containing 10^{-3} mol L^{-1} $AgNO_3$ for 20 min. The absorbance of optode membranes was recorded at 545 nm.

The repeatability of the optode membrane was studied by measuring the absorbance of one optode membrane. Then, the fully protonated chromoionophore membranes were 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} $AgNO_3$ for 20 min. The absorbance of the optode membrane was recorded at 545 nm. This optode membrane was regenerated by immersing in 1 mol L^{-1} HNO_3 for 5 min and then immersing in the silver ion solution again for 3 replicates.

3.6.7. Lifetime

The absorbance of one optode membrane kept in a box was recorded at 545 nm every 2 days until 18 days.

3.6.8. Real sample

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

(a) Sample A

Ingredient list:

Water, Butylene Glycol, Methyl Glucereth-20, Polysorbate 20, Poloxamer 184, Panthenol, Sodium Bonzoate, Benzyl Alcohol, Citric Acid, Sodium Citrate, Fragrance, Portulaca Oleracea Extract

(b) Sample B

Ingredient list:

Propylene Glycol N-Butyl Ether	0.9 % w/w
Ammonium Hydroxide	0.057 % w/w
Linear alkyl polybenzene sulfonate, sodium salt	0.038 % w/w
C8-C10 Alkyl polyglycoside	0.3 % w/w
C10-C16 Alkyl polyglycoside	0.06 % w/w

The calibration curve was prepared by diluting $AgNO_3$ in the concentration range of 10^{-5}-10^{-2} mol L^{-1} by Milli-Q water and real sample.

The stock real sample solution was prepared by diluting 50 mL of sample A (sample B) by Tris buffer solution at pH 8.5 in a 500 mL of a volumetric flask. Then, 0.05 mL and 0.25 mL of 0.1 mol L^{-1} $AgNO_3$ solutions were diluted by the stock real sample solution in the 50 mL of volumetric flasks. The obtained concentrations of $AgNO_3$ in the sample solutions were 1×10^{-4} mol L^{-1} and 5×10^{-4} mol L^{-1}, respectively.

Then, the fully protonated chromoionophore membranes were immersed in the real sample solution for 20 min. The absorbance of the optode membranes was recorded at 545 nm. The calibration curve prepared by Milli-Q water was used to determine silver ion in sample A and sample B

3.6.9. Silver nanoparticles solution

- Silver nanoparticles solution

Silver nanoparticles solution was prepared by diluting 5 mL and 25 mL of silver nanoparticles solutions by Tris buffer solution at pH 8.5 in the 50 mL of volumetric flasks. Then, 50 µL of 6% H_2O_2 was added into the solutions and stirred for 1 hr. Finally, the pH of the stock silver nanoparticles solutions was adjusted to pH 8.5 by 1 mol L^{-1} HNO_3 or 1 mol L^{-1} NaOH solutions.

Then, the fully protonated chromoionophore membranes were immersed in the stock silver nanoparticles solution for 20 min. The absorbance of the optode membrane was recorded at 545 nm.

- Silver nanoparticles solution diluted by Milli-Q spiked with silver ion

Silver nanoparticles solutions (5 mL and 25 mL) and 0.5 mL of 10^{-3} mol L^{-1} $AgNO_3$ solution was diluted by Tris buffer solution at pH 8.5 in the 50 mL of volumetric flasks. The obtained concentrations of $AgNO_3$ in the sample solutions were 10^{-5} M. Then, 50 µL of 6% H_2O_2 50 µL was added into the solution and stirred for 1 hr. Finally, the pH of the silver nanoparticles solution was adjusted to pH 8.5 by 1 mol L^{-1} HNO_3 or 1 mol L^{-1} NaOH solutions.

Then, the fully protonated chromoionophore membranes were immersed in the solution for 20 min. The absorbance of the optode membrane was recorded at 545 nm.

- Silver nanoparticles solution diluted by sample A and sample B spiked with silver ion

The stock real sample solution was prepared by diluting 50 mL of sample A or sample B by Tris buffer solution at pH 8.5 in a 500 mL of volumetric flask. Then, 0.5 mL of silver nanoparticles solutions and 0.05 mL of 0.01 mol L^{-1} $AgNO_3$ solution were diluted by the stock real sample solution in the 50 mL of

volumetric flasks. The obtained concentrations of $AgNO_3$ in the sample solutions were 10^{-4} mol L^{-1}.

Then, the fully protonated chromoionophore membranes were immersed in the solution for 20 min. The absorbance of the optode membrane was recorded at 545 nm.