

CHAPTER III

EXPERIMENTAL

3.1 Chemicals and Instruments

3.1.1 Chemicals

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|-----|----------------------------------------------------------------------------------------------------------------------|-------------------------------------------|
| 1. | Tetraethylammonium perchlorate | Analar grade, Fluka, Switzerland |
| 2. | Tetrabutylammonium trifluoromethanesulfonate | Electrochemical grade, Fluka, Switzerland |
| 3. | Tetraethylammonium hydroxide 1.5 M solution in methanol | Analar grade, Fluka, Switzerland |
| 4. | Tetrabutylammonium hydroxide 1.0 M solution in methanol | Analar grade, Aldrich, U.S.A. |
| 5. | Tetraethylammonium chloride | Analar grade, Fluka, Switzerland |
| 6. | Copper (II) trifluoromethanesulfonate | Analar grade, Aldrich, U.S.A. |
| 7. | Zinc (II) trifluoromethanesulfonate | Analar grade, Aldrich, U.S.A. |
| 8. | Cobalt (II) perchlorate | Analar grade, Fluka, Switzerland |
| 9. | Nickel (II) perchlorate | Analar grade, Fluka, Switzerland |
| 10. | Potassium hydrogenphthalate | Analar grade, Carlo Erba, Italy |
| 11. | Perchloric acid 70-72 % | Analar Grade, Merck, Germany |
| 12. | Methanol | Gradient grade, Merck, Germany |
| 13. | 25,27-{2,2'-[2,2'-((2,5,8-triaza)nonyl)diphenoxy]diethyl}- <i>p-tert</i> -butyl calix[4]arene (L) (synthetic ligand) | |
| 14. | Argon gas | Ultra high purity grade |
| 15. | Nitrogen gas | Analytical grade |

3.1.2 Instruments

1. Automatic titrator, Mettler, Model DL 25, Switzerland
2. Thermostat, Heto, Model DT-2, Denmark
3. Combined glass electrode (Ingold), Mettler, Switzerland
4. Combined pH electrode, Mettler, Model DG 113-SC, Switzerland
5. Personal Computer, Model 486 / DX2

3.2 Preparation of the Solution

For the basicity study 25,27-{2,2'-[2,2'-((2,5,8-triaza)nonyl)diphenoxy]diethyl}-*p-tert*-butylcalix[4]arene (**L**) in the methanolic solution of 5.0×10^{-2} M Et_4NClO_4 , methanol with very low water content was used without further purification. Solution of inert background electrolyte, of which concentration 5.0×10^{-2} M was obtained by dissolution of a weighed quantity of tetraethylammonium perchlorate (Et_4NClO_4), recrystallized twice from methanol, in methanol. A solution of the acid form of the ligand **L**, of which concentration about 1.0×10^{-3} M was made by dissolution of a weighed quantity of the acid form of the ligand **L** (**L.3HCl**) (Figure 1.7) in a methanolic solution of 5.0×10^{-2} M Et_4NClO_4 . Tetraethylammonium hydroxide (Et_4NOH), of which concentration about 4.0×10^{-2} M made by dilution of the commercial solution (1.5 M solution in methanol) in methanol with ionic strength of 5.0×10^{-2} M Et_4NClO_4 was used as the titrant base. The primary standard solution of potassium hydrogen phthalate (KHP) was prepared by dissolution of a weighed quantity of KHP in water. The stock solution of perchloric acid (HClO_4) was made by dilution of the commercial concentrated solution (70-72 % in water) in methanol. The pH standard solutions of pH = 2.0 and pH = 3.0 in the methanolic solution of 5.0×10^{-2} M Et_4NClO_4 were prepared by dilution of the stock solution of perchloric acid respectively. The standard solution of 5.0×10^{-2} M HClO_4 in the methanolic solution of 5.0×10^{-2} M Et_4NClO_4 was prepared by dilution of the stock solution of perchloric acid.

For the basicity study of 25,27-{2,2'-[2,2'-((2,5,8-triaza)nonyl)diphenoxy]diethyl}-*p-tert*-butylcalix[4]arene (**L**) and its complexation study with transition metal ions (Co^{2+} , Ni^{2+} , Cu^{2+} and Zn^{2+}) in the methanolic solution of 5.0×10^{-2} M $\text{Bu}_4\text{NCF}_3\text{SO}_3$, solution of another inert background electrolyte, of which concentration 5.0×10^{-2} M was obtained by dissolution of a weighed quantity of tetrabutylammonium trifluoromethanesulfonate ($\text{Bu}_4\text{NCF}_3\text{SO}_3$) in methanol. A solution of the acid form of the ligand **L**, of which concentration about 1.0×10^{-3} M was made by dissolution of a weighed quantity of the acid form of the ligand **L** (**L.3HCl**) in a methanolic solution of 5.0×10^{-2} M $\text{Bu}_4\text{NCF}_3\text{SO}_3$. Tetrabutylammonium hydroxide (Bu_4NOH), of which concentration about 5.0×10^{-2} M made by dilution of the commercial solution (1.0 M solution in methanol) in methanol with ionic strength of 5.0×10^{-2} M $\text{Bu}_4\text{NCF}_3\text{SO}_3$ was used as the titrant base. The stock solution of perchloric acid (HClO_4) was made by dilution of the commercial concentrated solution (70-72 % in water) in methanol. The pH standard solutions of pH = 2.0 and pH = 3.0 in the methanolic solution of 5.0×10^{-2} M $\text{Bu}_4\text{NCF}_3\text{SO}_3$ were prepared by dilution of the stock solution of the perchloric acid respectively. The standard solution of 5.0×10^{-2} M HClO_4 in the methanolic solution of 5.0×10^{-2} M $\text{Bu}_4\text{NCF}_3\text{SO}_3$ was prepared by dilution of the stock solution of perchloric acid. The solutions of transition metal ions, Co^{2+} , Ni^{2+} , Cu^{2+} , and Zn^{2+} were prepared by dissolution of weighed quantities of $\text{Zn}(\text{CF}_3\text{SO}_3)_2$, $\text{Cu}(\text{CF}_3\text{SO}_3)_2$, $\text{Co}(\text{ClO}_4)_2 \cdot 6\text{H}_2\text{O}$ and $\text{Ni}(\text{ClO}_4)_2 \cdot 6\text{H}_2\text{O}$ in the methanolic solution of 5.0×10^{-2} M $\text{Bu}_4\text{NCF}_3\text{SO}_3$ respectively. The concentration of each metallic solution were approximately 1.0×10^{-2} M.

3.3 The Calibration of Electrode

For the basicity study 25,27-{2,2'-[2,2'-((2,5,8-triaza)nonyl)diphenoxy]diethyl}-*p-tert*-butylcalix[4]arene (**L**) in the methanolic solution of 5.0×10^{-2} M Et_4NClO_4 , the electrode was calibrated at pH = 2.0 with the pH standard solution of pH = 2.0 in the presence of 4.0×10^{-2} M Et_4NClO_4 in methanol by adjustment of the Nernstian slope as defined the ratio between pH and potential in units of millivolt based on the

isopotential point of pH 8.30 = 0.0 millivolt. As the junction potentials of electrode vary exponentially with $-\log [H^+]$, the pH of the solution can be corrected by using the following equation⁽¹⁵⁾.

$$pH_{\text{corrected}} = pH_{\text{observed}} + a + b [H^+]_{\text{observed}}$$

The constants a and b were determined from a measurement of pH of a pH standard solution of pH = 3.0 in the presence of 4.9×10^{-2} M Et_4NClO_4 in methanol.

For the basicity study of 25,27-{2,2'-[2,2'-((2,5,8-triaza)nonyl)diphenoxy]diethyl}-*p-tert*-butylcalix[4]arene (**L**) and its complexation study with transition metal ions (Co^{2+} , Ni^{2+} , Cu^{2+} and Zn^{2+}) in the methanolic solution of 5.0×10^{-2} M $Bu_4NCF_3SO_3$, the electrode was calibrated at $pH = -\log[H^+] = 2.0$ with a pH standard solution of pH = 2.0 in the methanolic solution of 5.0×10^{-2} M $Bu_4NCF_3SO_3$ by same procedure. The constants a and b were calculated from a measurement of pH of a pH standard solution of pH = 3.0 in the methanolic solution of 5.0×10^{-2} M $Bu_4NCF_3SO_3$.

3.4 Potentiometric Titration

For the basicity study 25,27-{2,2'-[2,2'-((2,5,8-triaza)nonyl)diphenoxy]diethyl}-*p-tert*-butylcalix[4]arene (**L**) in the methanolic solution of 5.0×10^{-2} M Et_4NClO_4 , the concentration of free hydrogen ions was measured by an Ingold combined electrode connected to the automatic titrator at 25 °C. The external reference solution filled in the combined electrode was a mixture of methanolic solution of 1.0×10^{-2} M tetraethylammoniumchloride (Et_4NCl) and 4.0×10^{-2} M Et_4NClO_4 saturated with AgCl. The titrant base was standardized against potassium hydrogen phthalate. For the standard solution of 5.0×10^{-2} M $HClO_4$ in the methanolic solution of 5.0×10^{-2} M Et_4NClO_4 was standardized against titrant base again. The standard solution of acid was used to adjust the pH of the solution of the ligand **L**. The working solutions were made up as follows: 20 ml of a stock solution of acid form of the ligand **L** in the methanolic solution of 5.0×10^{-2} M Et_4NClO_4 was added to an amount of excess standard solution of acid be varied in 25 ml flask. The 10 ml aliquots of these solutions

were titrated with 4.0×10^{-2} M Et_4NOH in the methanolic solution of 5.0×10^{-2} M Et_4NClO_4 . The titration cell was kept at 25.00 ± 0.05 °C throughout the experiment and performed under an atmosphere of argon, saturated by the same inert background electrolyte solution.

For the basicity study of 25,27-{2,2'-[2,2'-((2,5,8-triaza)nonyl)diphenoxy]diethyl}-*p-tert*-butylcalix[4]arene (L) and its complexation study with transition metal ions (Co^{2+} , Ni^{2+} , Cu^{2+} and Zn^{2+}) in the methanolic solution of 5.0×10^{-2} M $\text{Bu}_4\text{NCF}_3\text{SO}_3$ have been studied by potentiometric titration at 25 °C. The concentrations of free hydrogen ion $[\text{H}^+]$ in observed solution were measured by a combined electrode of Mettler DG 113-SC connected to an automatic titrator, Mettler DL 25 at 25 °C. The titrant base was standardized against potassium hydrogen phthalate. For the standard solution of 5.0×10^{-2} M HClO_4 in the methanolic solution of 5.0×10^{-2} M $\text{Bu}_4\text{NCF}_3\text{SO}_3$ was standardized against titrant base again. The standard solution of acid was used to adjust the pH of the solution of the ligand L. All potentiometric titrations were carried out at 25 °C with deviation of ± 0.1 °C, regulated by external circulation of Heto DT-2 Thermostat. The titrations were performed under argon atmosphere by flowing argon gas saturated by 5.0×10^{-2} M $\text{Bu}_4\text{NCF}_3\text{SO}_3$ solution through the titration beaker. In each titration, 10 ml of a stock methanolic solution of acid form of ligand L and excess the standard solution of acid be varied were used and titrated with 5.0×10^{-2} M Bu_4NOH in methanolic solution of 5.0×10^{-2} M $\text{Bu}_4\text{NCF}_3\text{SO}_3$ for the study of basicity of the ligand L. For the study of its complexation with each metal ions, the different R values defined as the metal to ligand mole ratio at least 5-6 titrations were varied between 1:1 to 2:1. Each titration, 10 ml of the stock methanolic solution of 1.0×10^{-3} M of the acid form of ligand was mixed by the metallic solution within the concentration of 8.4×10^{-4} M to 1.7×10^{-3} M. These solution were titrated with 5.0×10^{-2} M Bu_4NOH in methanolic solution of 5.0×10^{-2} M $\text{Bu}_4\text{NCF}_3\text{SO}_3$.

3.5 Calculation

Protonation constants of 25,27-{2,2'-[2,2'-((2,5,8-triaza)nonyl)diphenoxy]diethyl}-*p-tert*-butylcalix[4]arene (**L**) were obtained from titration of the acidic form of the ligand **L** in the absence of metal. Each titration was observed within pH range 2.80-12.08. The pH range and initial concentration of acid form of the ligand **L** which was studied in the methanolic solution of 5.0×10^{-2} M Et_4NClO_4 and 5.0×10^{-2} M $\text{Bu}_4\text{NCF}_3\text{SO}_3$ used in refinement for determination of protonation constants of the ligand **L** are shown in Table 3.1 and Table 3.2 respectively.

Table 3.1 Experimental data used in computer simulations for determining the protonation constants of 25,27-{2,2'-[2,2'-((2,5,8-triaza)nonyl)diphenoxy]diethyl}-*p-tert*-butylcalix[4]arene (**L**) in the methanolic solution of 5.0×10^{-2} M Et_4NClO_4 at 25 ± 0.05 °C.

Titration	Initial concentration (mM)		pH range	Data points
	L	Proton		
1	0.769	2.307	3.41-12.01	45
2	0.727	3.014	2.94-11.98	47
3	0.727	3.014	2.95-12.08	47
4	0.673	3.580	2.83-11.96	41
5	0.757	3.034	2.94-11.90	48

Table 3.2 Experimental data used in computer simulations for determining the protonation constants of 25,27-{2,2'-[2,2'-((2,5,8-triaza)nonyl)diphenoxy]diethyl}-*p-tert*-butylcalix[4]arene (L) in the methanolic solution of 5.0×10^{-2} M $\text{Bu}_4\text{NCF}_3\text{SO}_3$ at 25 ± 0.1 °C.

Titration	Initial concentration (mM)		pH range	Data points
	L	Proton		
1	0.833	2.499	3.23-11.83	55
2	0.800	4.474	2.96-11.83	50
3	0.769	6.294	2.81-11.98	46

Stability constants of the complexes of 25,27-{2,2'-[2,2'-((2,5,8-triaza)nonyl)diphenoxy]diethyl}-*p-tert*-butylcalix[4]arene (L) with Co^{2+} , Ni^{2+} , Cu^{2+} and Zn^{2+} were evaluated from potentiometric titration data. The pH range and initial concentrations of acid form of the ligand L and metal ions which were studied in the methanolic solution of 5.0×10^{-2} M $\text{Bu}_4\text{NCF}_3\text{SO}_3$ used in refinement for determination of stability constants of the complexes of the ligand L with Co^{2+} , Ni^{2+} , Cu^{2+} and Zn^{2+} are shown in Table 3.3, 3.4, 3.5, 3.6 respectively.

Table 3.3 Experimental data used in computer simulations for determining the stability constants of 25,27-{2,2'-[2,2'-((2,5,8-triaza)nonyl)diphenoxy]diethyl}-*p-tert*-butylcalix[4]arene with Co^{2+} in the methanolic solution of 5.0×10^{-2} M $\text{Bu}_4\text{NCF}_3\text{SO}_3$ at 25 ± 0.1 °C.

Titration	Initial concentration (mM)			pH range	Data points
	L	Proton	Co^{2+}		
1	0.833	2.499	0.837	2.99-8.65	40
2	0.833	2.499	0.837	3.29-8.82	38
3	0.800	2.400	1.205	3.19-8.72	36
4	0.800	2.400	1.205	3.05-8.86	37
5	0.833	2.499	1.673	3.06-9.13	45
6	0.833	2.499	1.673	3.13-8.79	36

Table 3.4 Experimental data used in computer simulations for determining the stability constants of 25,27-{2,2'-[2,2'-((2,5,8-triaza)nonyl)diphenoxy]diethyl}-*p-tert*-butylcalix[4]arene with Ni²⁺ in the methanolic solution of 5.0 x 10⁻² M Bu₄NCF₃SO₃ at 25 ± 0.1 °C.

Titration	Initial concentration (mM)			pH range	Data points
	L	Proton	Ni ²⁺		
1	0.833	2.499	0.835	3.43-7.89	51
2	0.800	2.400	1.202	3.25-7.75	54
3	0.833	2.499	1.670	3.16-7.71	51

Table 3.5 Experimental data used in computer simulations for determining the stability constants of 25,27-{2,2'-[2,2'-((2,5,8-triaza)nonyl)diphenoxy]diethyl}-*p-tert*-butylcalix[4]arene with Cu^{2+} in the methanolic solution of 5.0×10^{-2} M $\text{Bu}_4\text{NCF}_3\text{SO}_3$ at 25 ± 0.1 °C.

Titration	Initial concentration (mM)			pH range	Data points
	L	Proton	Cu^{2+}		
1	0.833	2.499	0.839	3.15-11.31	47
2	0.909	2.727	0.915	3.08-11.22	46
3	0.820	2.460	0.990	3.08-11.22	64
4	0.813	2.439	1.064	3.12-11.34	59
5	0.833	2.499	1.678	3.02-10.51	59
6	0.833	2.499	1.678	3.03-10.80	59

Table 3.6 Experimental data used in computer simulations for determining the stability constants of 25,27-{2,2'-[2,2'-((2,5,8-triaza)nonyl)diphenoxy]diethyl}-*p-tert*-butylcalix[4]arene with Zn^{2+} in the methanolic solution of 5.0×10^{-2} M $Bu_4NCF_3SO_3$ at 25 ± 0.1 °C.

Titration	Initial concentration (mM)			pH range	Data points
	L	Proton	Zn^{2+}		
1	0.833	2.499	0.831	3.08-11.82	67
2	0.842	2.526	0.831	3.17-11.91	73
3	0.800	2.400	1.197	3.07-11.90	71
4	0.800	2.400	1.197	3.06-11.87	68
5	0.842	2.526	1.662	3.19-12.26	84

At least 35 data points of potentiometric titration for each titrations were used in optimization process of computer refinements. The experimental data were evaluated by a computer programme of SUPERQUAD which refines the logarithm of overall equilibrium constants ($\log \beta$). For the determination of stability constants of the ligand **L** with transition metal ions, these stability constants were calculated together with protonation constants of the ligand **L** which were previously obtained from the titration of acid form of the ligand **L** in the absence of metal ions. The protonation constants of the ligand **L** were settled as constant during the refinement procedure. The metal methoxide species, $M(CH_3O)^+$ and autoprotolysis constant of methanol (K_{MeOH}) were also included in the computer model of the computational process in order to precisely obtain the stability constants of the complexes system during the computer refinement procedure.