

A COMPARATIVE STUDY OF THE COMPLEXING ABIL-
ITIES AND THE PARTITION COEFFICIENTS OF
THREE MOLECULAR WEIGHT FRACTIONS OF
ETHYLENE-MALEIC ACID COPOLYMER

By

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TABLE I
Solubilities of EMA in Organic Liquids

	Anhydride	Acid	Sodium Salt
Carbon tetrachloride	I	I	I
Methyl ethyl ketone	I	I	I
Acetone	S	I	I
Dioxane	I	S	I
Pyridine	S	I	I
Dimethylformamide	S	S	I
Benzene	I	I	I
Hexane	I	I	I
Ethyl acetate	I	I	I
Ethanol	I*	I	I

S - Soluble I - Insoluble

*Dissolves with half-ester formation after 2-3 hours at reflux.

Ethylene-maleic acid copolymer and water are completely soluble in each other. When the anhydride is stirred with water at 95°, it hydrolyzes to the acid form of the polymer in approximately ten minutes. EMA solutions are used extensively to change the viscosity of solutions, dispersions, and emulsions.

Following is a list of various fields in which EMA copolymers have found application: adhesives, control of crystal growth, detergents, foundry core binders, non-flammable paint removers, non-woven fabrics, oil well drilling muds, paper sizes and finishes, photographic film,

pigment dispersants, polymerization aids, resin thickeners, cosmetics, toiletries, suspending agents, cleaning compounds, lubricants, ceramics, textile sizes, textile coatings, and tumor inhibitors (3). The use of EMA as tumor inhibitors attracted our interest and led to this research.

Hydrolyzed maleic anhydride copolymers are known to form chelates with alkaline earth metals. Morawetz, Kotliar, and Mark (15) studied the chelation of alkaline earth ions with hydrolyzed copolymers of maleic anhydride with vinyl ethyl ether and with styrene in the presence of 1 N potassium nitrate at 25⁰; chelate formation constants varied from log 1.36 to log 2.45. More recently Monobe (16) investigated copolymers of hydrolyzed maleic acid with vinyl acetate and with styrene at low and high ionic strengths and determined formation constants for the chelates with barium and calcium ions. The polymers gave very different curves on titration, the difference being attributed to the tighter coiling of the styrene copolymer.

Gregor, Luttinger, and Loebel (17) measured the formation constants for polyacrylic acid-copper complexes by potentiometric titrations. The complexes have greater stabilities than those of glutaric acid (a monomeric analog). Wall and Gill (18) investigated the interaction of cupric ions with polyacrylic acid spectroscopically, polarographically, and by pH titrations, and found some evidence for the formation of a complex between copper and polyacrylic acid. Kotliar and Morawetz (19) found evidence of a single copper chelate of polyacrylic acid and of polymethacrylic acid which persisted

over a wide range of conditions; the chelate seemed to involve four carboxylate groups (20). Gregor, Luttinger, and Loebl (21) measured the complexity constants for polyacrylic acid with magnesium, calcium, manganese, cobalt, and zinc ions. The complexes of the alkaline earth metals are less stable than the others, but none are as stable as the copper complexes.

Jacobson (22) has shown that the binding of magnesium ions to polyacrylic acid is dependent on configurational effects of the polymer. Divalent cations such as copper and magnesium have different affinities for different forms of polymethacrylic acid. The isotactic polymer has the higher affinity for copper(II) ions, while magnesium(II) ions are bound more strongly to the syndiotactic species (23).

INTRODUCTION

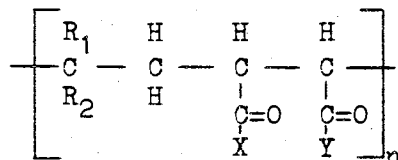
Considerable research has been done in the past few years in the field of cancer chemotherapy. Included among the compounds studied are several different molecular weight fractions of various derivatives of EMA. Table II shows data for the inhibition of a mouse tumor, sarcoma 180, by some such derivatives of EMA (3). The doses shown for each compound give maximum inhibition of the tumor without being toxic. The higher molecular weight fractions show greater toxicity and greater tumor inhibition on a weight basis. Our chief interest was to find some property of the various molecular weight fractions (2-3,000, 20-30,000, and 60-70,000) of HEMA (hydrolyzed ethylene-maleic anhydride copolymer) which might be related to their abilities to inhibit tumors.

Since the structure of these polymers allows them to form chelates readily with metals, a comparative study was made of the relative stability constants* of several molecular weight fractions for the metal chelates of copper(II), cobalt(II), and nickel(II). These metals were chosen

*The term relative stability constant as used here does not refer to a true stability constant, but to the ability of the three molecular weight fractions to complex the metals mentioned. Further discussion of this point will be found on page 24.

TABLE II

Inhibition of Sarcoma 180 by Poly(Ethylene-Maleic Acid) and Derivatives*



	R ₁ = H, R ₂ = H, X = OH, Y = OH		R ₁ = H, R ₂ = H, X = NH ₂ , Y = ONH ₄		R ₁ = H, R ₂ = H, X = NH ₂ , Y = OH		R = H, R ₂ = CH ₃ X = NH ₂ , Y = ONH ₄	
Molecular Weight Fraction	Dose, mg./kg.	Inhibition, %	Dose, mg./kg.	Inhibition, %	Dose, mg./kg.	Inhibition, %	Dose, mg./kg.	Inhibition, %
2-3,000	200	54	300	80	400	70	400	59
20-30,000	100	72	50	81	100	78	50	67
60-70,000	10	55	50	65	100	69	50	59
80-100,000	4	46	75	83	25	58		
120,000 and up	4	61	75	72	25	51		

*W. Regelson, S. Kukar, M. Tunis, J. Johnson, J. Fields, and E. Gluesenkamp, Nature, 186, 778 (1960)

because they had shown antitumor activity when used with 2-keto-3-ethoxybutyraldehyde bis(thiosemicarbazone) (KTS) (4). The metal derivatives of this compound, particularly that of copper(II), showed greater inhibition of the growth of tumors than the ligand itself. These particular metal ions have important functions in biological systems (5). The stability constants of the metal complexes were determined potentiometrically. The determination of stability constants by this method requires the calculation of the ionization constants of the acids. The calculations for both the ionization constants and the relative stability constants were performed on an IBM 7040 computer (Appendix).

The calculation of the ionization constants was made by the method of Noyes (6) as modified by Albert and Serjeant (7) for the ionization constants of a dibasic acid with overlapping pK_a values. In order to permit the calculation, the following concentrations must be considered:

- C, the total concentration of all species of the acid being titrated,
- B, the concentration of the added alkali (if no reaction occurred), and
- H, the hydrogen ion concentration.

If one lets

$$X = [H^+](B - C + [H^+]),$$

$$Y = 2C - (B + [H^+]), \text{ and}$$

$$Z = [H^+]^2(B + [H^+]),$$

then it follows that

$$K_{a_1} = \frac{Y_1 Z_2 - Y_2 Z_1}{X_1 Y_2 - X_2 Y_1} \quad \text{and} \quad K_{a_2} = \frac{X_1 Z_2 - X_2 Z_1}{Y_1 Z_2 - Y_2 Z_1}$$

where X_1 , Y_1 , and Z_1 refer to values of X , Y , and Z calculated from pH values obtained with less than one equivalent of titrant added and X_2 , Y_2 , and Z_2 refer to values of X , Y , and Z calculated from pH values obtained with more than one equivalent of titrant added. Pairs of readings are selected from either side of the mid-point; the selection is preferably, but not necessarily, symmetrical. We chose symmetrical values for our calculations. The equations for the determination of C , B , and H may be found in the 7040 IBM program for ionization constants in the appendix.

The calculation of the stability constants by the potentiometric method (8) requires the calculation of two functions, $[L^-]$ and \bar{n} . $[L^-]$ is the concentration of the free chelating species and \bar{n} is the average number of molecules of ligand bound by one atom of metal. The following is a development of the equations for $[L^-]$ and \bar{n} . The concentration of free and combined hydrogen ions $[H_0^+]$ can be expressed in two ways:

$$[H_0^+] = [H^+] + [LH^-] + 2[LH_2] - [OH^-] \quad (1)$$

$$[H_0^+] = [L_0] + [HClO_4] - [KOH] \quad (2)$$

where $[L_0]$ is the total concentration of ligand added. From equation 1 and equation 2 we get

$$\begin{aligned}
 [L_0] + [HClO_4] - [KOH] &= [H^+] + \\
 [LH^-] + 2[LH_2] - [OH^-] &
 \end{aligned}
 \tag{3}$$

From the mass action equations

$$[LH_2] = \frac{[H^+]^2[L^-]}{K_{a_1} K_{a_2}} \quad \text{and} \quad [LH^-] = \frac{[H^+][L^-]}{K_{a_2}}$$

the values of $[LH]$ and $[LH^-]$ may be found. When these are substituted into equation (3), we get

$$\begin{aligned}
 [L_0] + [HClO_4] - [KOH] &= [H^+] + \\
 \frac{[H^+][L^-]}{K_{a_2}} + \frac{2[H^+]^2[L^-]}{K_{a_1} K_{a_2}} - [OH^-] &
 \end{aligned}
 \tag{4}$$

Solving equation 4 for $[L^-]$ gives

$$[L^-] = \frac{[L_0] + [HClO_4] - [KOH] - [H^+] + [OH^-]}{\frac{[H^+]}{K_{a_2}} + \frac{2[H^+]^2}{K_{a_1} K_{a_2}}} \tag{5}$$

$$[L_0] = [L^-] + [LH^-] + [LH_2] + [LM^+] + 2[L_2M] \tag{6}$$

By definition

$$\bar{n} = \frac{[LM^+] + 2[L_2M]}{[M_0]}$$

where $[M_0]$ is the total concentration of metal added.

Therefore

$$[L_0] = [L^-] + [LH^-] + [LH_2] + \bar{n}[M_0] \quad (7)$$

Solving equation (7) for \bar{n} gives

$$\bar{n} = \frac{[L_0] - [L^-] - [LH^-] - [LH_2]}{[M_0]} \quad (8)$$

Substituting the values of $[LH^-]$ and $[LH_2]$ gives

$$\bar{n} = [L_0] - [L^-] - \frac{[H^+]^2[L^-]}{K_{a_1} K_{a_2}} - \frac{[H^+][L^-]}{K_{a_2}} \quad (9)$$

The stability constants (K_1 and K_2) for the 2:1 complex are found by the use of Bjerrum's summation equation which relates \bar{n} and $[L^-]$ to the stability constants as follows:

$$\sum_{n=0}^N (\bar{n}-n) \beta_n [L^-]^n = 0 \quad (10)$$

where n represents various small numbers, N is the largest value of n , \bar{n} has the usual meaning, and β_n is the product of all of the constants from K_1 to K_n .

The correct values of K_1 and β_2 can be found by the summation of equation 10 over $n = 0, 1, \text{ and } 2$ for the bidentate complex of a bivalent metal (11). This yields the equation:

$$\frac{\bar{n}}{(\bar{n} - 1)[L^-]} = \frac{(2 - \bar{n})[L^-]}{(\bar{n} - 1)} \beta_2 - K_1 \quad (11)$$

Equation 11 is the equation of a straight line of which the slope is β_2 and the intercept is $-K_1$. From the computer program for stability constants, values of \bar{n} , X, and Y were obtained for each pH reading taken from the titrations on metal solutions, where

$$X = \frac{(2 - \bar{n}) [L^-]}{(\bar{n} - 1)} \quad \text{and} \quad Y = \frac{\bar{n}}{(\bar{n} - 1)[L^-]}$$

These values for X and Y were placed in a 7040 computer program set up to solve the simultaneous equations for the least squares (Appendix) which are:

$$\begin{aligned} \sum Y &= na + b \sum X \\ \sum XY &= a \sum X + b \sum X^2 \end{aligned}$$

where n equals the number of observations, a is $-K_1$ and b is β_2 . Values of X and Y corresponding to \bar{n} values between 0.95 and 1.05 were rejected because values in this range are too sensitive to experimental error.

A study was made of the partition coefficients between water and 1-octanol for each of the three molecular weight fractions of the acids. Hansch (13) has shown that partition between 1-octanol and water is helpful in relating the biological activities of several series of compounds to their structures. This partition function may be related to the transport of the compounds in biological systems.

The chelates of the copper(II), cobalt(II), and nickel(II) ions for the three molecular weight fractions were prepared and sent to the Cancer Chemotherapy National

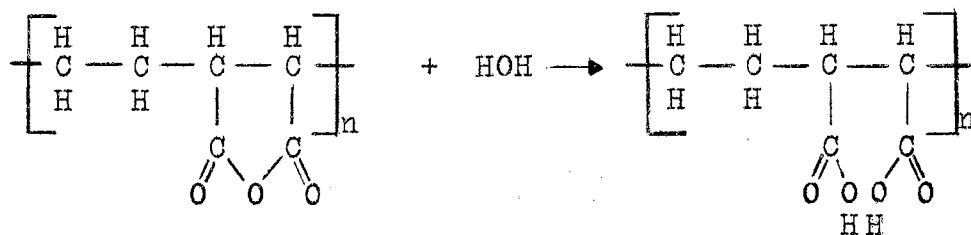
Service, National Institutes of Health, Bethesda, Maryland for determination of their antitumor activities. A preliminary study was made of the structure of the chelates formed.

EXPERIMENTAL AND RESULTS

All titrations were made on a Beckman model 1019 research pH meter. The temperature was controlled by a Brinkman model 550005 Thermocool unit and a Haake constant temperature circulator model Fe. The titrations were made under a nitrogen atmosphere at 25°. The titrant used was 0.1N carbonate-free potassium hydroxide made up according to the method outlined by Albert and Serjeant (8). Barium hydroxide was added to a solution of potassium hydroxide (analytical grade). The precipitate of barium carbonate was allowed to settle and the excess barium ions removed by passing the solutions through a column of ion-exchange resin (Dowex 50-WX8) which was in the potassium form. The eluate was a solution of pure potassium hydroxide free from carbonate. Prior to the titrations, the pH meter was calibrated and standardized against Beckman buffer solutions of pH 4.00 and 8.68.

Ionization Constants

Solutions of 0.02N acid were prepared from the anhydride forms by refluxing in water for 2 hr. During this time the following reaction occurred quantitatively.



Since one unit of the acid has a formula weight of 144 and contains two carboxyl groups, the equivalent weight of the acid is 72. To make 700 ml. of a 0.02N solution 0.882 g. of each molecular weight fraction of the anhydride was weighed and hydrolyzed. Fifty milliliters of each solution was titrated with 0.1003N base in 0.50-ml. additions with a pH determination after each addition. Figures 1-3 show the acid-base titrations. The volume of base added and each pH reading were then used in the computer program for ionization constants along with the number of points, initial volume, molecular weight, sample weight, and normality of the base to determine the values for pK_{a1} and pK_{a2} for each acid. The results are listed in Table III. The K_{a1} values corresponding to the addition of 2.00, 2.50, and 3.00 ml. of base were averaged to obtain the value listed in Table III and the K_{a2} values corresponding to the addition of 7.00, 7.50, and 8.00 ml. of base were averaged to obtain the value given in Table III for K_{a2} .

This experiment was repeated in a solution of constant ionic strength achieved by the addition of 6.123 g. of sodium perchlorate to each 50-ml. aliquot, giving a solution which was about 1 M with respect to this compound. The

titrations were made in the same way as before; data are listed in Table IV. Plots of the acid-base titration in the presence of added salt are shown in Figures 4-6.

TABLE III

Ionization Constants of Poly(Ethylene-Maleic Acid)
in 0.02N Solutions at 25°

Molecular Weight	$K_{a_1} \times 10^5$	$K_{a_2} \times 10^9$
2-3,000	1.47	1.54
20-30,000	1.40	3.13
60-70,000	1.21	2.40

TABLE IV

Ionization Constants of Poly(Ethylene-Maleic Acid)
in 0.02N Solutions with 1M Sodium Perchlorate at 25°

Molecular Weight	$K_{a_1} \times 10^4$	$K_{a_2} \times 10^7$
2-3,000	7.10	3.59
20-30,000	7.26	6.49
60-70,000	8.34	5.29

Stability Constants

Solutions of the perchlorates of copper(II), cobalt(II), and nickel(II) were prepared according to Serjeant (10). An ion-exchange resin column was loaded with Dowex 50-WX8 resin (cation exchanger) and flushed with water until the elutant had the same pH as the entering water. The column was completely loaded with the metal ion and then 12.2 g. of barium perchlorate dissolved in water was flushed through the column with enough excess water to give 500 ml. of the perchlorate of the metal. Nickel(II) chloride, copper(II) sulfate, and cobalt(II) nitrate were used. When 12.2 g. of barium perchlorate was passed through the column, 500 ml. of the perchlorate of the metal with a molarity of approximately 0.0625 was obtained. The molarity of each solution was checked by placing 2 ml. of the metal solution on a column loaded with Dowex 50-WX8 resin which had been flushed with water to remove any excess hydrogen ion on the column and then passing enough water through the column to give a constant pH reading of the elutant (approximately 300 ml.). This solution was then titrated with 0.0993N carbonate-free potassium hydroxide using phenolphthalein as an indicator. The hydrogen equivalents released by the metal solutions were calculated from the titrations and converted to the molarities of the metal solutions. Three titrations were made for each metal solution and the results averaged to obtain the following values:

copper(II) perchlorate, 0.0621 M;
cobalt(II) perchlorate, 0.0608 M; and
nickel(II) perchlorate, 0.0512 M.

Three 50-ml. aliquots were taken from the HEMA solutions which had previously been prepared for determination of ionization constants. To one 50-ml. aliquot from each molecular weight fraction of HEMA, 4 ml. of the copper(II) perchlorate solution was added to give a 2:1 ligand-to-metal ratio. These solutions were then titrated with 0.1003N carbonate-free potassium hydroxide. The corresponding pH values for each addition were determined. The pH readings and the corresponding volumes of base added were placed in the computer program along with the number of points read, molecular weight of ligand, sample weight of ligand, initial volume of solution titrated, molarity of metal solution, volume of metal solution added, pK_{a_1} and pK_{a_2} of the ligand. Values of X , Y , and \bar{n} are obtained from the stability constant program for each pH recorded. X , Y , and \bar{n} are defined in the introductory discussion on the stability constants. X and Y values were then submitted to the least-squares program and values of β and K_1 were obtained from which K_2 values were calculated by dividing β by K_1 . These results are listed in Table V.

The above experiment was repeated with the solutions all made about 1 molar with respect to sodium perchlorate by the addition of 6.125 g. of this salt to each 50-ml.

TABLE V

Stability Constants of Chelates of Poly(Ethylene-Maleic Acid) with
Copper(II), Cobalt(II), and Nickel(II) at 25°

Molecular Weight	Copper(II)		Cobalt(II)		Nickel(II)	
	log K ₁	log K ₂	log K ₁	log K ₂	log K ₁	log K ₂
2-3,000	9.27	3.32	7.79	2.55	7.84	2.93
20-30,000	9.21	3.20	7.47	2.95	7.51	2.86
60-70,000	9.39	3.03	7.72	2.92	7.78	3.06

TABLE VI

Stability Constants of Chelates of Poly(Ethylene-Maleic Acid) with
Copper(II), Cobalt(II), and Nickel(II) in 1 M
Sodium Perchlorate at 25°

Molecular Weight	Copper(II)		Cobalt(II)		Nickel(II)	
	log K ₁	log K ₂	log K ₁	log K ₂	log K ₁	log K ₂
2-3,000	5.60	2.14	3.11	1.54	3.14	1.75
20-30,000	5.73	2.30	3.09	1.97	3.11	1.98
60-70,000	5.80	----	3.02	1.59	3.05	2.29

aliquot of acid. Results of these experiments are in Table VI.

Comparisons of Chelating Abilities

The potentiometric method of determining stability constants is based on a change in pH upon chelation; therefore plots of the differences in pH show the relative amount of chelation of the copper(II), cobalt(II), and nickel(II) complexes of the three molecular weight fractions (Figures 7-12). Maxima in these curves occur at the points of maximum complexing. These data are summarized in Table VII.

TABLE VII
Maximum Values of Δ pH for Copper(II),
Cobalt(II), and Nickel(II) Complexes

Molecular Weight	Δ pH		
	Copper(II)	Cobalt(II)	Nickel(II)
2-3,000	2.804	1.921	2.054
20-30,000	2.628	1.821	2.005
60-70,000	2.702	1.892	2.034

Partition Coefficients

Water was saturated with 1-octanol by placing 50 ml. of 1-octanol and 360 ml. of water in a flask and shaking for 24 hours. This solution was allowed to stand for 12 hours. 1-Octanol was saturated with water by placing 50 ml.

of water and 360 ml. of 1-octanol in a flask and shaking for 24 hours. This solution was allowed to stand for 12 hours.

The anhydrides were hydrolyzed to the acids by heating 0.126 g. of the three molecular weight fractions for one hour at 95° in 60 ml. of the 1-octanol-saturated water solution. These solutions were diluted to 100 ml. with the 1-octanol-saturated water, giving solutions that were 0.02N in the polymeric acid. Five-ml. aliquots of these solutions were titrated with 0.01N potassium hydroxide to determine the pH of the first end-point.

Twenty-milliliter aliquots of the acid solutions were added to 100-ml. portions of the 1-octanol which had been previously saturated with water. These solutions were shaken for 72 hours and allowed to stand for 24 hours. Three 5-ml. aliquots of the aqueous phase were titrated on a Radiometer-Copenhagen type TTTIC titrator to the pH of the first end-point of the acid solutions. Five milliliters of base were required to titrate 5 ml. of the original acid solution to the first end-point. The results of the experiment are shown in Table VIII.

TABLE VIII
 Partition of Poly(Ethylene-Maleic Acid)
 Between 1-Octanol and Water

Molecular Weight Fraction	Base Required for 5 ml. of the Aqueous Phase, ml.	Ratio of Acid, Water/1-octanol
2-3,000	5.08	620
	4.87	
	4.95	
20-30,000	4.82	120
	4.77	
	4.80	
60-70,000	4.75	91
	4.72	
	4.74	

Preparation of Complexes

Copper(II) Complexes. - Six and three-tenths grams of each of the three molecular weight fractions of EMA was hydrolyzed by boiling in 800 ml. of a 7:1 mixture of water and dimethylformamide. After boiling for 30 minutes to insure complete hydrolysis, 6.04 grams of copper(II) nitrate was added to the solution. The pH of the solution was raised to 4.5 by the addition of potassium hydroxide. Upon the formation of the copper complex, the solution tended to gel but by diluting the solution with water as it cooled, gelling was prevented. The solution was agitated continuously during the operation by means of a magnetic stirrer. The complexes were separated in a centrifuge and

dried on a steam bath.

Nickel(II) Complexes. - Six and three-tenths grams of each of the three molecular weight fractions of EMA was hydrolyzed by boiling for 30 minutes in a mixture of 100 ml. of water and 300 ml. of dimethylformamide. Then 5.95 g. of nickel(II) chloride was added to this solution and the pH adjusted to 6.0 by the addition of potassium hydroxide. The solutions were agitated on a magnetic stirrer-hot plate combination. No appreciable complex was visible but upon the evaporation of the solvent to approximately one-third of the original volume and addition of carbon tetrachloride the complex was obtained in the form of a gummy substance which was separated and dried on a steam bath.

Cobalt(II) Complexes. - Six and three-tenths grams of each of the three molecular weights of EMA was hydrolyzed by boiling for 30 minutes in a mixture of 200 ml. of dimethylformamide and 200 ml. of water. After boiling, 5.95 grams of cobalt(II) chloride was added to this solution and the pH adjusted to 6.0 by the addition of potassium hydroxide. The solutions were agitated on a magnetic stirrer-hot plate combination. Each solution was evaporated to approximately one half of the original volume and carbon tetrachloride added to precipitate the cobalt complex. These complexes were separated by decantation and dried in a desiccator.

Investigation of the Structure of a Chelate

An effort was made to determine the structure of the copper(II) complexes of HEMA, molecular weight 2-3,000. Solutions were made 0.02N in acid and 0.01N in metal so as to give a 2:1 molar ratio of ligand to metal. The pH of one solution was adjusted to 4.53 and the other to 8.66. These are the pH values which gave maxima in the Δ pH plot. Samples of these solutions were studied with a Cary 14 recording spectrophotometer; spectra for the two solutions were obtained over the wavelength range of 4500 to 7300⁰Å. Both solutions had maximum absorption at 7100⁰Å.

The nuclear magnetic resonance spectra of the solutions were scanned over the range of 0 to 1000 cps. with a Varian A-60 spectrometer. Absorption by a carboxyl proton normally appears between 500-1000 cps.; however, no peak was found in this range. Peaks corresponding to the protons on the carbon chain of the polymer were present at 310 cps. (δ 5.17) and 182 cps. (δ 3.03) along with the water peak at 250 cps. (δ 4.17).

DISCUSSION OF RESULTS

Ionization Constants

The ionization constants of the polymeric acids were not as reproducible as those of a simple acid. As the titration proceeded K_{a1} and K_{a2} decreased. This variation is normal for a polymer owing to the change in charge density on the chain as the protons are removed. The variation in pK_a values can be minimized by the addition of a neutral salt, which raises the ionic strength of the solution. The increase in ionic strength prevents the polymer from changing configurations during the titration (14). This variation in pK_a has the same magnitude for all three molecular weights; therefore, at the same degree of neutralization the pK_a of each molecular weight fraction could be compared with the others.

Stability Constants

The method used to calculate the stability constants of the metal chelates does not yield a true stability constant in the case of a polymer because of the change in pK_a values with increasing neutralization. It was not our intention to measure the true stability constants for these complexes, but to find the stabilities of the metal complexes of one

molecular weight fraction relative to those of the other molecular weight fractions. By this method, we could determine whether the degree of complexing between the different molecular weight fractions corresponded to the antitumor activities of the polymeric acids. This method makes it possible to compare the complexing ability of one molecular weight fraction to that of a different molecular weight fraction.

The values obtained from the solutions which were about 1 molar with respect to sodium perchlorate cannot be used in a comparison of the amount of complexing. Since precipitation of the metal complex in the solutions containing sodium perchlorate occurred, the values so obtained have no meaning.

Comparison of Chelating Abilities

For solutions in which the ionic strength does not remain constant (solutions without added salt) the change in ionic strength upon the addition of the base will cause an apparent deviation in the pH; however, since all solutions were titrated identically, the change in pH due to change in ionic strength will be the same for each metal solution and we may still use the differences in pH as a valid comparison of complexing abilities. The plots indicate the following order of strengths of complexes formed: $\text{Cu} > \text{Ni} > \text{Co}$. This sequence is the same as that given by Irving and Williams (12). The corroboration of this known sequence is

an indication of the validity of this method of comparison.

Partition Coefficients

The partition study showed that the relative solubility of the polymeric acid in the 1-octanol increased with increasing molecular weight of the polymeric acid. This fact indicates that the difference in the antitumor activities of the molecular weight fractions may be related to their transport in the animal.

Preparation of Complexes

In the preparation of the complexes for testing, it was noted that making the ligand metal ratio greater than 2:1 speeds the redissolving of the metal hydroxide which forms when potassium hydroxide is added to adjust the pH. The metal hydroxides precipitate immediately upon the addition of the potassium hydroxide and then dissolve as chelation takes place. The solution is due to the greater stability of a chelate over that of a linear type complex. The pH was adjusted to values at which the Δ pH values were greatest in the base titration of the metal solutions. Since the difference in pH values of the metal complex solutions and the acid solution is due to protons liberated by the formation of complex, the maximum difference in pH occurs at the point of greatest complexing with the metal.

Chelate Structure

The Δ pH plot of each metal has two maxima. These indicate two major types of complexes which would be formed at different hydrogen ion concentrations. These should show two peaks in the absorption spectra of the solutions. However, the spectra showed only the absorption peak at $7100\overset{\circ}{\text{A}}$. Although the solutions were diluted and restudied the peak was not resolved.

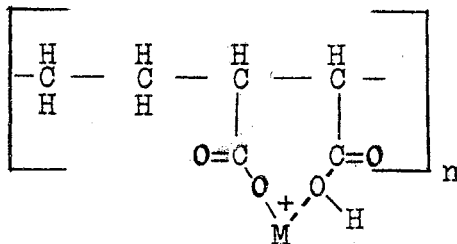
The nuclear magnetic resonance spectra of the solutions did not show any absorption corresponding to a carboxyl proton. This was probably due to a very rapid exchange of the proton between the aqueous solvent and the acid. It was hoped that by a comparison of the spectra of the free acid and the chelated form, the number of carboxyl protons existing per mole of chelate at the points of maximum complexing could be determined and thus several possible structures of the chelate could be eliminated. Since no peak was obtained for the carboxyl proton of the acid, this could not be done in an aqueous solvent.

SUMMARY

Three molecular weight fractions of ethylene-maleic acid copolymer have ionization constants which are similar in magnitude; no relationship between the ionization constants and antitumor activities of the fractions was found.

The stability constants of the copper(II), cobalt(II), and nickel(II) complexes of three different molecular weight fractions of the polymeric acid are somewhat different, but are not related directly to the antitumor activities of the polymeric acids.

The maximum amount of complexing with these metals occurs at half neutralization of the acid. A charged complex represented by the following formula is indicated;



This structure could not be fully verified by the data obtained from the n.m.r. and U.V. spectra.

When these acids are partitioned between water and 1-octanol, a greater amount enters the 1-octanol phase as the molecular weight increases. Since their antitumor activities also increase with molecular weight, the antitumor

activities of the different molecular weight fractions may be related to their transport in the biological system.

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APPENDIX

Notation for Computer Programs

CHION	
or	= Hydrogen Ion Concentration
CONH	
CONLH	= $[L_0]$
CONL	= $[L^-]$
CONOH	= $[OH^-]$
CONKOH	= $[KOH]$ (if no reaction had occurred)
INVOL	= Initial Volume of Acid Solution Titrated
MWLH	= Molecular Weight of Ligand
SWLH	= Sample Weight of Ligand
NOROH	= Normality of Base
VOLOH	= Volume of Base
MOLME	= Molarity of Metal
VOLME	= Volume of Metal Used
CONME	= Concentration of Metal
NBAR	= \bar{n} (average number of ligand molecules attached to each metal ion)


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$ID          S-666
$JOB         IONIZATIONCONST          2211-40004
$IBJOB
$IBFTC      NODECK
REAL INVOL,MOLWT,SAMWT,NOROH,VOLOH,PH,C,B,CHION,X,Y,KA1,KA2,
1 PKA1,PKA2
INTEGER NOPTS,NPTS1,ONE,TWO,NPT12
DIMENSION VOLOH(50),PH(50),C(50),B(50),CHION(50),X(50),Y(50),
1 Z(50),A(10)
2 FORMAT(1H1)
3 FORMAT(I2,F5.2,F6.2,2F6.4)
4 FORMAT(/2X,17HNUMBER OF POINTS=,I2,3X,16HINIT VOL OF H2O=,F6.2,
13X,7HMOL WT=,F7.2,3X,10HSAMPLE WT=,F7.4,3X,10HN OF BASE=,F7.4//)
5 FORMAT(2X,10A6)
6 FORMAT(3X,5HML OH,5X,2HPPH,8X,1HC,8X,1HB,7X,5HH ION,11X,1HX,
111X,1HY,11X,1HZ,8X,4HPKA1,8X,4HPKA2//)
7 FORMAT(2X,F7.2,4X,F7.3,2(2X,F8.4),4(2X,E10.3))
8 FORMAT(76X,4(6X,F7.3))
9 FORMAT(2X,F7.2,4X,F7.3,2(2X,F8.4),4(2X,E10.3)//)
10 READ(5,5)(A(I),I=1,10)
    READ(5,2)NOPTS,INVOL,MOLWT,SAMWT,NOROH
    IF(NOPTS.EQ.99)CALLEXIT
    WRITE(6,1)
    WRITE(6,5)(A(I),I=1,10)
    WRITE(6,4)NOPTS,INVOL,MOLWT,SAMWT,NOROH
    DO 11 I=1,NOPTS
    READ(5,3) VOLOH(I),PH(I)
11 CONTINUE
    NPTS1=NOPTS-1
    DO 12 I=2,NPTS1
    C(I)=((SAMWT*1000.)/MOLWT)/(INVOL+VOLOH(I))
    B(I)=(VOLOH(I)*NOROH)/(INVOL+VOLOH(I))
    CHION(I)=EXP(-2.3025851*PH(I))
    X(I)=CHION(I)*(B(I)-C(I)+CHION(I))
    Y(I)=2.0*C(I)-(B(I)+CHION(I))
    Z(I)=CHION(I)**2*(B(I)+CHION(I))
12 CONTINUE
    ONE=2
    TWO=NPTS1
    WRITE(6,6)
13 KA1=(Y(ONE)*Z(TWO)-Y(TWO)*Z(ONE))/(X(ONE)*Y(TWO)-X(TWO)*Y(ONE))
    PKA1= ALOG(ABS(KA1))/(-2.3025851)
    KA2=(X(ONE)*Z(TWO)-X(TWO)*Z(ONE))/(Y(ONE)*Z(TWO)-Y(TWO)*Z(ONE))
    PKA2= ALOG(ABS(KA2))/(-2.3025851)
    WRITE(6,7)VOLOH(ONE),PH(ONE),C(ONE),B(ONE),CHION(ONE),X(ONE),
1Y(ONE),Z(ONE)
    WRITE(6,8)PKA1,PKA2
    WRITE(6,9)VOLOH(TWO),PH(TWO),C(TWO),B(TWO),CHION(TWO),X(TWO),
1Y(TWO),Z(TWO)
    NPT12=NPTS1/2
    IF(ONE.GE.NPT12) GO TO 10
    ONE=ONE+1
    TWO=TWO-1
    GO TO 13
END
$ENTRY

```

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SID          S-666
$JOB        STABILITYCONST          2211-40004
$IBJOB
$IBFTC      NODECK
REAL INVOL,MWLH,SWLH,NOROH,VOLOH,PH,MOLME,VOLME,CONME,CONOH,
1CONH,CONL,KA1,KA2,NBAR,X,Y,PKA1,PKA2,CONKOH
INTEGernoPTS
DIMENSIONVOLOH(50),PH(50),CONME(50),CONOH(50),CONH(50),
-- 1CONL(50),NBAR(50),X(50),Y(50),CONLH(50),CONKOH(50),A(10)
1 FORMAT(1H1)
2 FORMAT(I2,F5.2,F6.2,F6.4,2F8.6,F5.2,2F6.3)
3 FORMAT(/2X,17HNUMBER OF POINTS=,I2,3X,16HMOL WT OF COMPD=,F7.2,
13X,5HPKA1=,F7.3,3X,5HPKA2=,F7.3)
4 FORMAT(2X,8HVOL BASE,3X,2HPH,5X,5HCONME,5X,6HCONKOH,5X,5HCONLH,5X,
14HCONH,7X,5HCONOH,6X,4HCONL,6X,4HNBAR,7X,1HY,9X,1HX//)
5 FORMAT(F5.2,F6.3)
6 FORMAT(2X,F6.2,3X,F7.3,3(2X,F9.6),3(2X,E9.3),2X,F7.3,2(2X,E9.3))
7 FORMAT(2X,10A6)
8 FORMAT(2X,13HINIT VOL H2O=,F6.2,3X,10HSAMPLE WT=,F7.4,3X,
110HN OF BASE=,F9.6,3X,16HM OF METAL SOLN=,F9.6,3X,
218HVOL OF METAL SOLN=,F6.2//)
9 FORMAT(3E14.8,F6.2)
W=1.
10 READ(5,7)(A(I),I=1,10)
READ(5,2)NOPTS,INVOL,MWLH,SWLH,NOROH,MOLME,VOLME,PKA1,PKA2
IF(NOPTS.EQ.99)CALLEXIT
WRITE(6,1)
WRITE(6,7)(A(I),I=1,10)
WRITE(6,3)NOPTS,MWLH,PKA1,PKA2
WRITE(6,8)INVOL,SWLH,NOROH,MOLME,VOLME
WRITE(6,4)
DO11I=1,NOPTS
READ(5,5)VOLOH(I),PH(I)
CONME(I)=(MOLME*VOLME)/(INVOL+VOLME+VOLOH(I))
CONKOH(I)=(NOROH*VOLOH(I))/(INVOL+VOLME+VOLOH(I))
CONLH(I)=(SWLH*1000.)/(MWLH*(INVOL+VOLME+VOLOH(I)))
CONH(I)=EXP(-2.3025851*PH(I))
CONOH(I)=(6.808E-15)/CONH(I)
KA1=EXP(-2.3025851*PKA1)
KA2=EXP(-2.3025851*PKA2)
CONL(I)=(2.*CONLH(I)+CONOH(I)-CONH(I)-CONKOH(I))/((CONH(I)/KA2)+
12.*(CONH(I)**2.)/(KA1*KA2))
NBAR(I)=(CONLH(I)-(CONL(I)*CONH(I)/KA2)-(CONL(I)*(CONH(I)**2.)/
1(KA1*KA2))-CONL(I))/CONME(I)
Y(I)=NBAR(I)/((NBAR(I)-1.)*CONL(I))
X(I)=((2.-NBAR(I))*CONL(I))/(NBAR(I)-1.)
WRITE(6,6)VOLOH(I),PH(I),CONME(I),CONKOH(I),CONLH(I),CONH(I),
1CONOH(I),CONL(I),NBAR(I),Y(I),X(I)
WRITE(7,9)Y(I),X(I),W,VOLOH(I)
11 CONTINUE
GOTO10
END

```

```

0 01BFTC          NODECK
C LEAST SQUARES FIT ( M TERM POLYNOMIAL USING WEIGHTED POINTS)
1 DIMENSION X(50),Y(50),W(50),B(20),A(20,21),F(50),DF(50),NAME(16),
  VLOH(50),AK(50)
2 COMMON,M,N2
3 1 FORMAT(16A5)
4 2 FORMAT(1HL,25X,16A5////)
5 3 FORMAT(1HL,7,1X,37HSTANDARD DEVIATION OF ORDINATE POINTS,E17.8)
6 5 FORMAT(1HL,52HSTANDARD DEVIATION OF K(1) ASSUMING BETA IS CONSTANT
  I,E17.8)
7 6 FORMAT(1HL,24HINDIVIDUAL VALUES FOR K1,/(2X,8E16.8))
10 723 FORMAT(16)
11 700 FORMAT (216)
12 701 FORMAT (6F12.8)
13 715 FDRMAT(40X,6H 1 J,22X,7H A(1,J),4X,17HCOEFFICIENT MATRIX/)
14 716 FDRMAT(40X,13,13,18X,E15.8)
15 717 FDRMAT(1HL,40X,3H J ,23X,5H B(1J),11X,13HR,H,S. MATRIX/)
16 719 FDRMAT(40X,13,21X,E15.8)
17 713 FDRMAT(1HL,40X,2H 1,24X,5H B(1),11X,13HANSWER MATRIX/)
20 705 FDRMAT(40X,13,21X,E15.8)
21 706 FDRMAT(1HL,9X,5HVLOH,14X,5H X(1),23X,11H Y MEASURED,20X,13H Y CAL
  ICULATED,10X,9HDEVIATION/)
22 707 FDRMAT(10X,F6,2,8X,F15.8,16X,E15.8,18X,E15.8,5X,E15.8)
23 4 FDRMAT(3E14,8,F5,2)
C N1 IS THE NUMBER OF SETS OF DATA WHICH ARE TO BE FIT FORMAT IS 16
24 READ(5,723)N1
C NAME IS READ FROM THE SECOND CARD AND APPEARS AT THE TOP OF THE P
C PAGE AS A HEADING ALL 80 CHARACTERS CAN BE USED
26 721 READ(5,1)NAME
30 WRITE(6,2)NAME
C N IS THE NUMBER OF DATA POINTS WHICH FOLLOW, M IS THE NUMBER OF T
C TERMS IN THE EQUATION TO BE FIT BOTH ARE FORMATTED 16
31 READ(5,700)N,M
C EACH POINT CONSISTS OF Y, X, W AND VLOH
C Y,X AND W ARE READ AS E14.8, VLOH IS READ AS F5.2
34 DD709 I=1,N
35 709 READ(5,4)Y(I),X(I),W(I),VLOH(I)
37 SUM=0.0
40 DO 703 J=1,M
41 B(1J)=0.0
42 DO 703 J=1,M
43 703 A(1,J)=0.0
46 DO 725 J=1,M
47 DO 725 I=1,N
50 IF(I,J.EQ.1)GOTO750
53 GOTO724
54 750 B(J)=B(1J)*W(I)*Y(I)
55 GOTO725
56 724 B(J)=B(1J)*W(I)*Y(I)*X(I)**(J-1)
57 725 CONTINUE
62 DO 751 J=1,M
63 DO 751 K=J,M
64 DO 751 I=1,N
65 IF(I,K.EQ.2)GOTO752
70 GOTO711
71 752 A(J,K)=A(1J,K)*W(I)
72 GOTO751
73 711 A(J,K)=A(1J,K)*W(I)*X(I)**(J-K-2)
74 751 CONTINUE
100 DO 712 J=1,M
101 L=J+1
102 DO 712 K=L,M
103 712 A(K,J)=A(1J,K)
106 WRITE(6,715)
107 DO 714 I=1,M
110 DO 714 J=1,M
111 714 WRITE(6,716)I,J,A(1,J)
114 WRITE(6,717)
115 DO 718 J=1,M
116 718 WRITE(6,719)J,B(1J)
120 N2=M+1
121 DO101 I=1,M
122 10 A(1,N2)=B(1I)
124 CALL INVERT
125 DO11 I=1,M
126 11 B(1I)=A(1,N2)
130 WRITE(6,713)
131 WRITE(6,705)I1,B(1I),I=1,M1
136 DO 702 I=1,N
137 DF(I)=0.0
140 F(1)=0.0
141 DO 756 J=1,M
142 IF(J.EQ.1)GOTO755
145 GOTO708
146 755 F(1)=B(1J)+F(1)
147 GOTO756
150 708 F(1)=B(1J)*X(1)**(J-1)+F(1)
151 756 CONTINUE
153 DF(1)=F(1)-Y(1)
154 702 SUM=SUM+DF(1)*DF(1)
156 STDEV=SQR(SUM/FLOAT(N))
157 WRITE(6,706)
160 WRITE(6,707) VLOH(1),X(1),Y(1),F(1),DF(1),I=1,N)
165 WRITE(6,3)STDEV
166 SUM=0.0
167 DD770 I=1,N
170 AK(I)=Y(1)-X(1)*B(2)
171 770 SUM=SUM+AK(I)
173 ANEAN=SUM/FLOAT(N)
174 SUM=0.0
175 DD771 I=1,N
176 771 SUM=SUM+(ANEAN-AK(I))**2
200 STDEV=SQR(SUM/FLOAT(N-1))
201 WRITE(6,6) AK(1),I=1,N)
206 WRITE(6,5)STDEV
207 N1=N1-1
210 IF(N1.GT.0)GOTO721
213 720 CALL EXIT
214 END

```

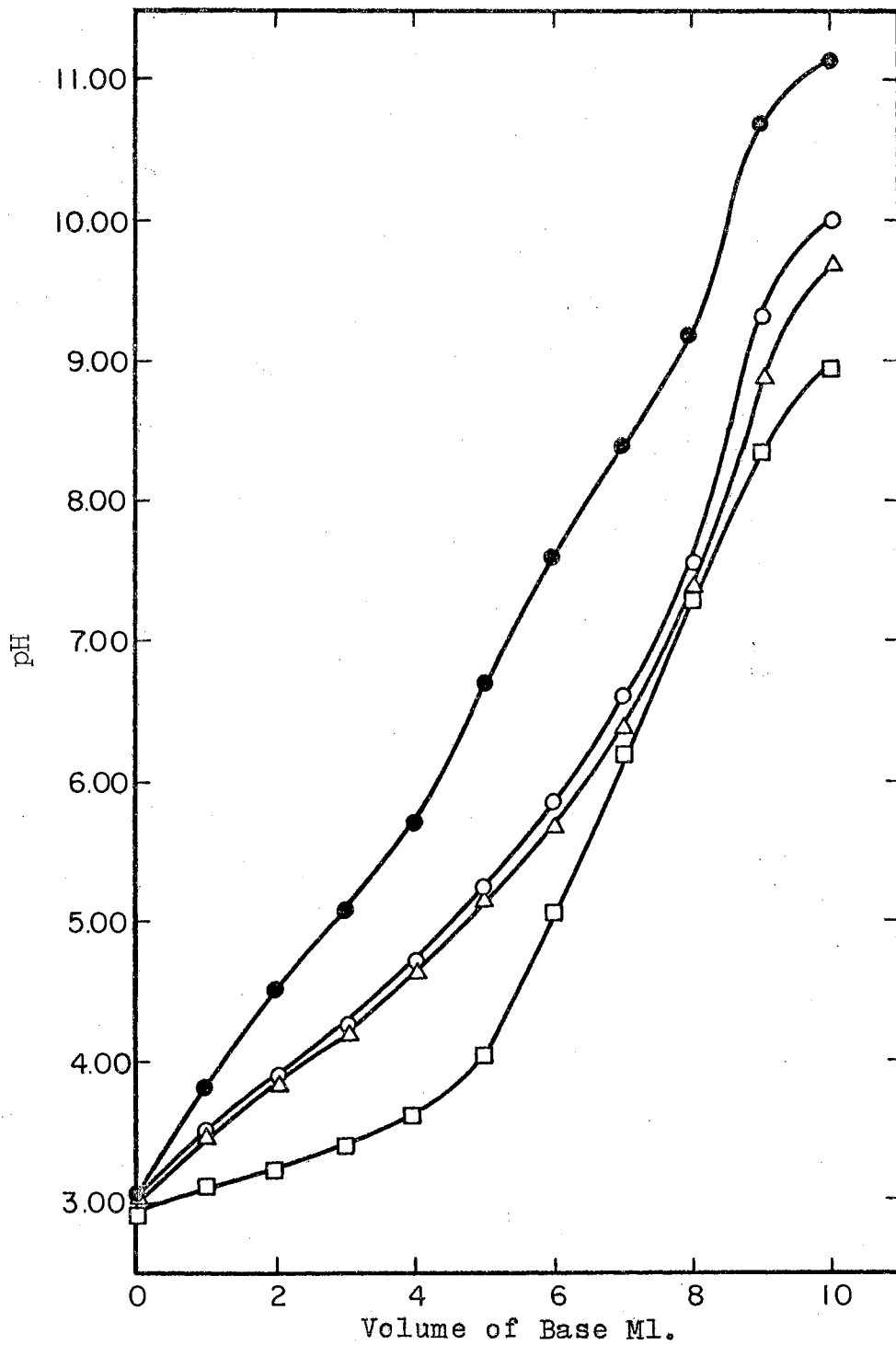


Figure 1. Titration of 0.02N Polymeric Ethylene-Maleic Acid (Molecular Weight 2-3,000) with 0.1 N Base: in the Absence of Metal Ions, ●; in 0.005 M $\text{Cu}(\text{ClO}_4)_2$, ◻; in 0.005 M $\text{Ni}(\text{ClO}_4)_2$, △; in 0.005 M $\text{Co}(\text{ClO}_4)_2$, ○.

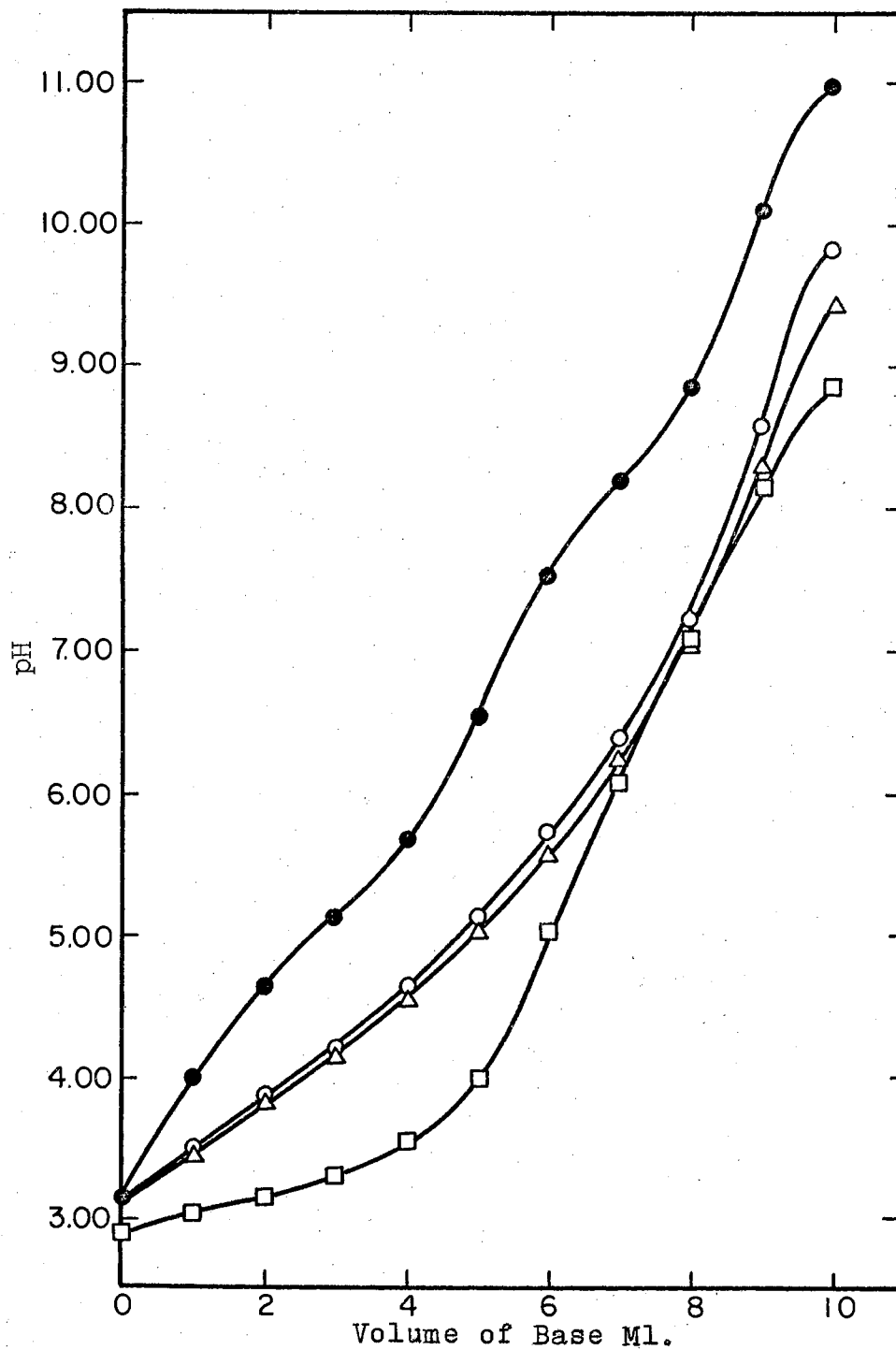


Figure 2. Titration of 0.02N Polymeric Ethylene-Maleic Acid (Molecular Weight 20-30,000) with 0.1 N Base: in the Absence of Metal Ions, ●; in 0.005 M $\text{Cu}(\text{ClO}_4)_2$, □; in 0.005 M $\text{Ni}(\text{ClO}_4)_2$, Δ; in 0.005 M $\text{Co}(\text{ClO}_4)_2$, ○.

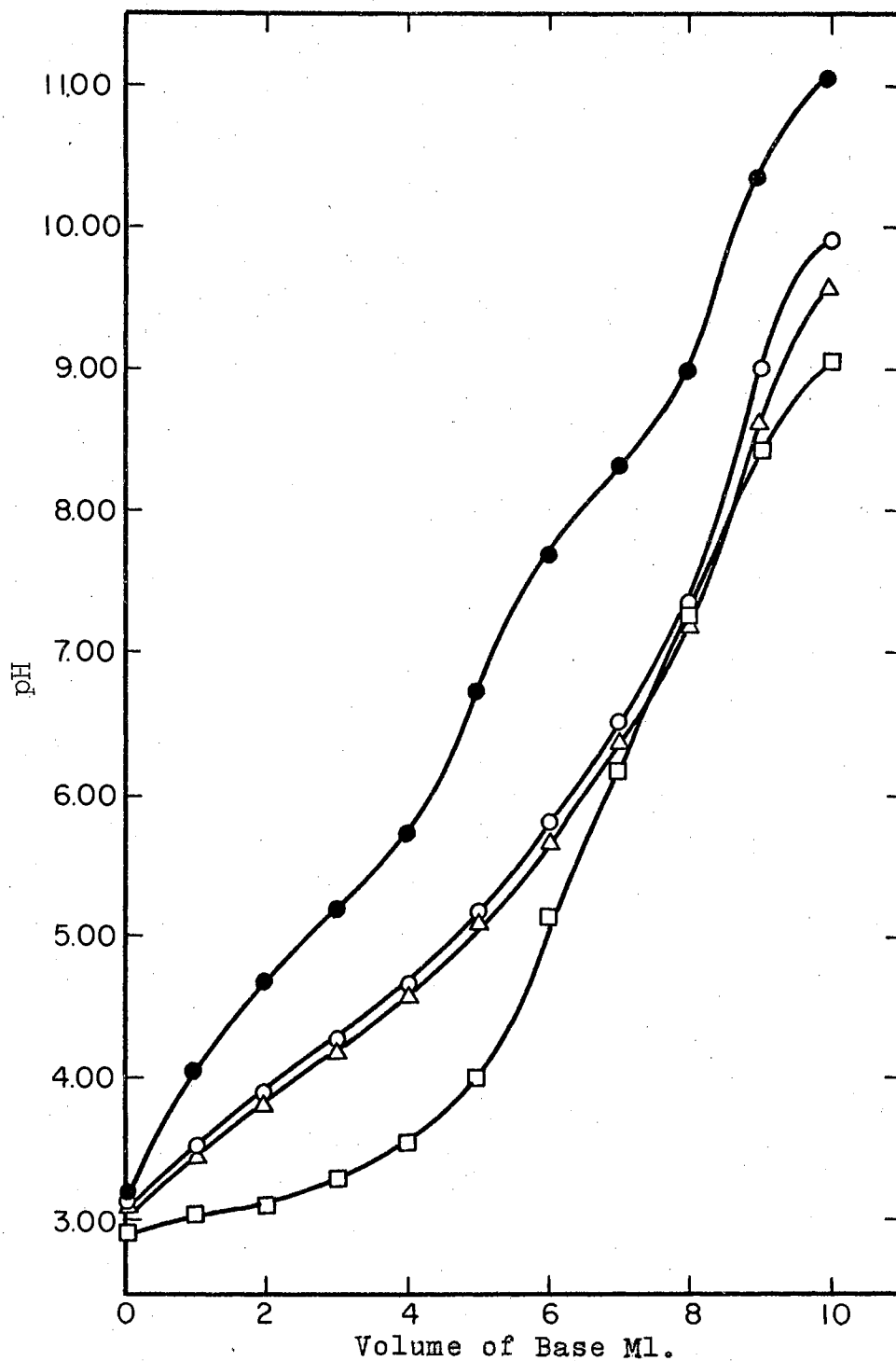


Figure 3. Titration of 0.02N Polymeric Ethylene-Maleic Acid (Molecular Weight 60-70,000) with 0.1 N Base: in the Absence of Metal Ions, ●; in 0.005 M $\text{Cu}(\text{ClO}_4)_2$, □; in 0.005 M $\text{Ni}(\text{ClO}_4)_2$, Δ; in 0.005 M $\text{Co}(\text{ClO}_4)_2$, ○.

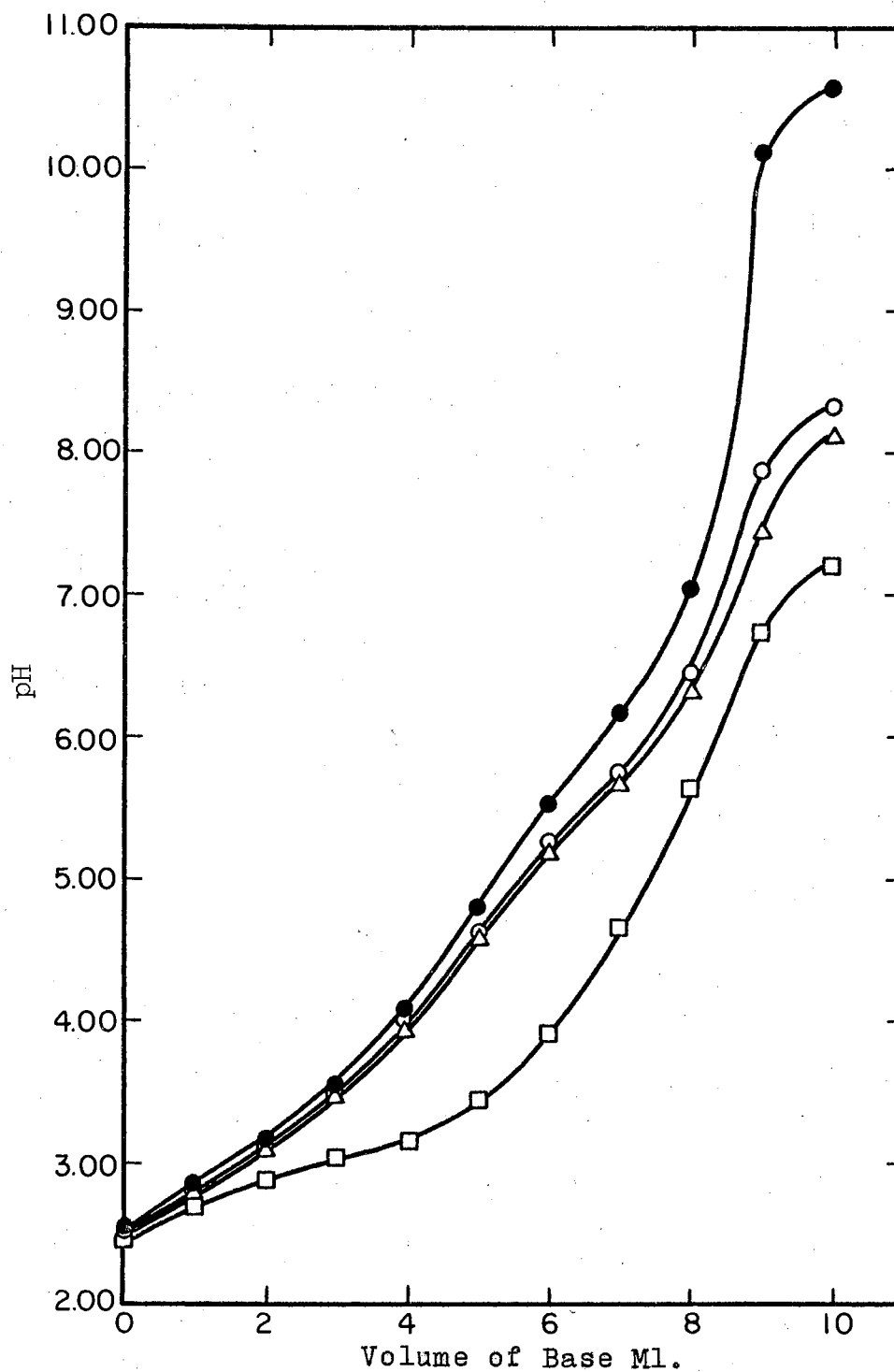


Figure 4. Titration of 0.02 N Polymeric Ethylene-Maleic Acid (Molecular Weight 2-3,000) with 0.1 N Base in 1 M NaClO_4 : in the Absence of Metal Ions, ●; in 0.005 M $\text{Cu}(\text{ClO}_4)_2$, □; in 0.005 M $\text{Ni}(\text{ClO}_4)_2$, Δ; in 0.005 M $\text{Co}(\text{ClO}_4)_2$, ○.

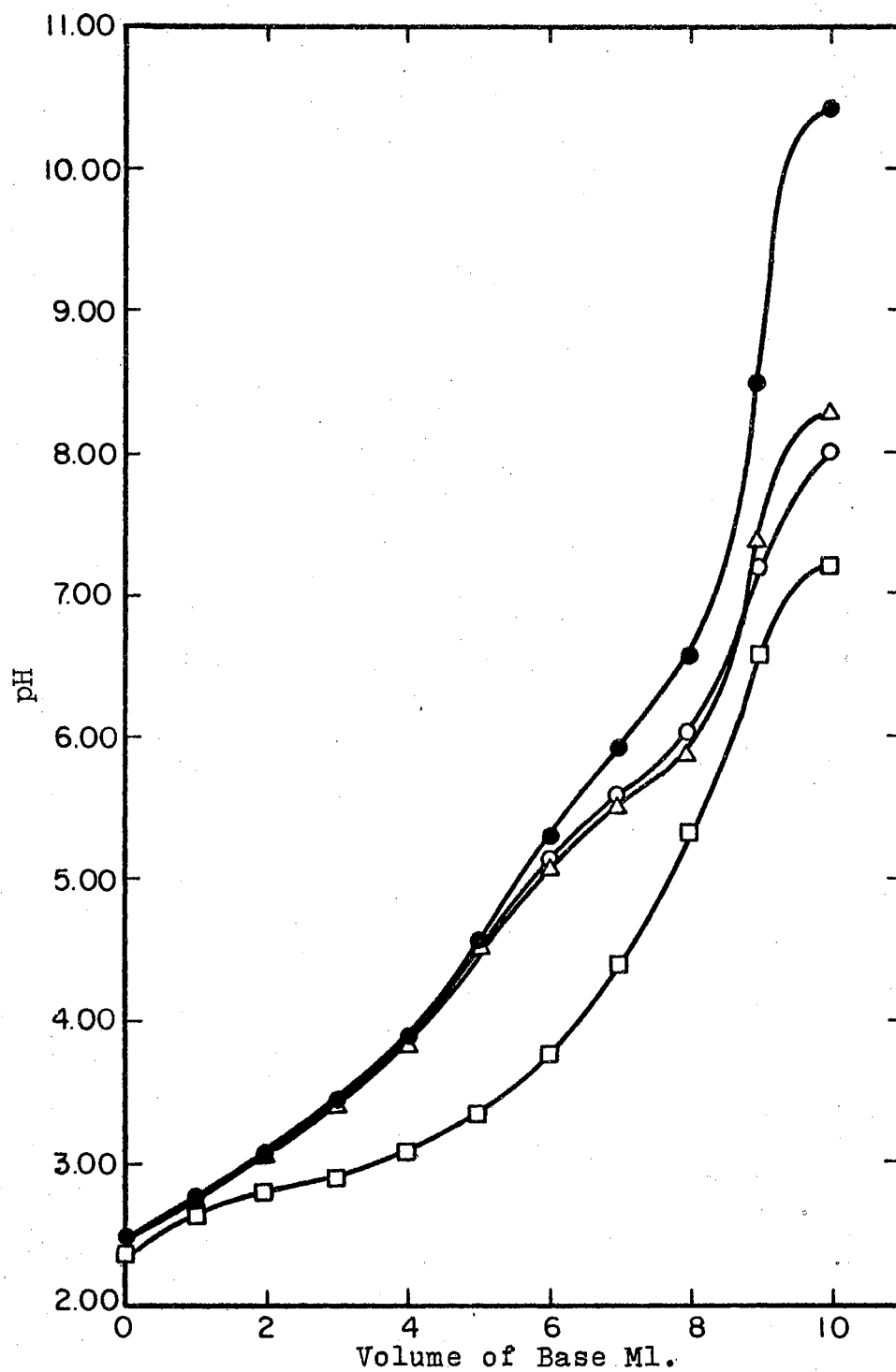


Figure 5. Titration of 0.02 N Polymeric Ethylene-Maleic Acid (Molecular Weight 20-30,000) with 0.1 N Base in 1 M NaClO_4 : in the Absence of Metal Ions, ●; in 0.005 M $\text{Cu}(\text{ClO}_4)_2$, □; in 0.005 M $\text{Ni}(\text{ClO}_4)_2$, Δ; in 0.005 M $\text{Co}(\text{ClO}_4)_2$, ○.

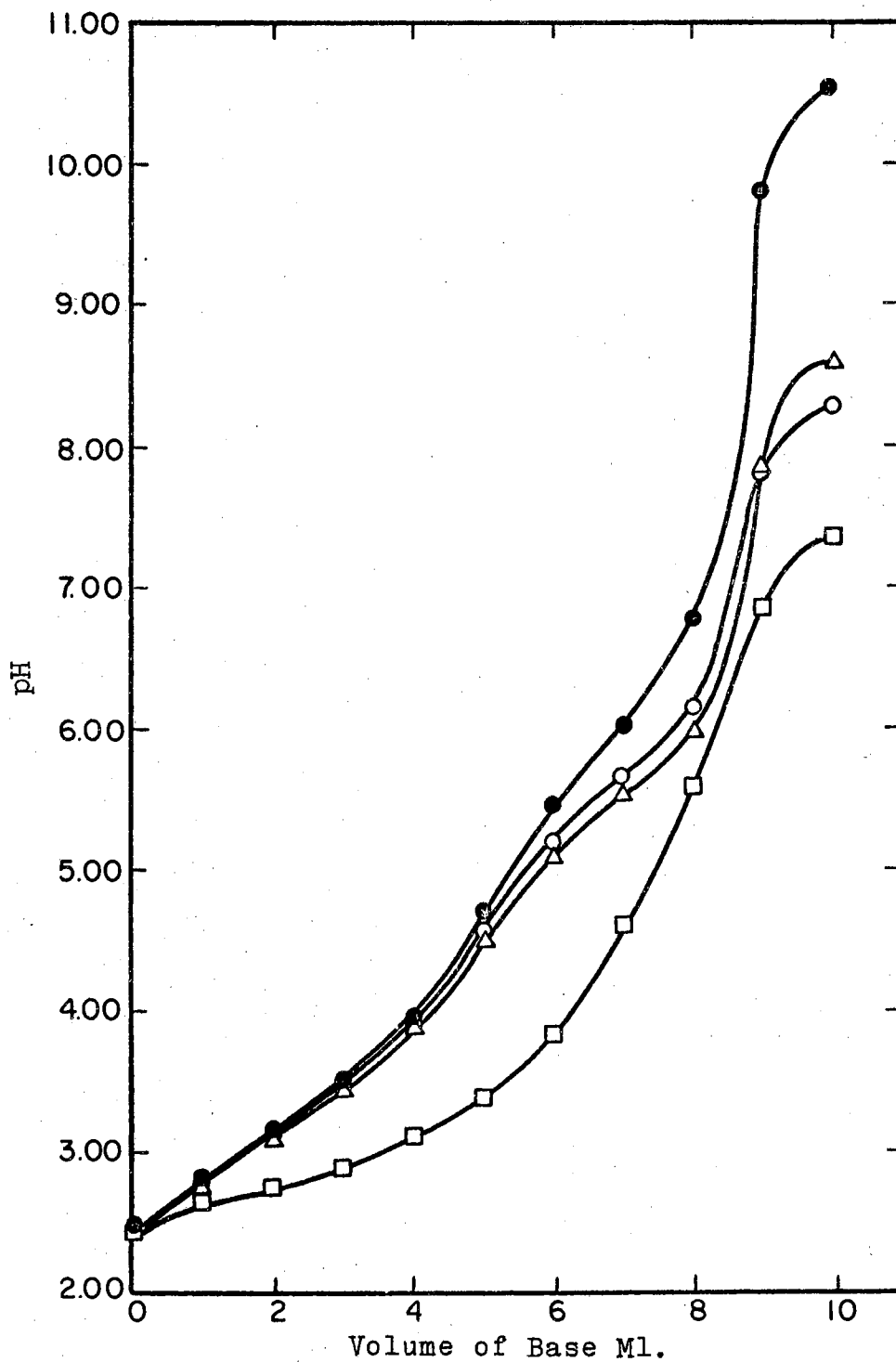


Figure 6. Titration of 0.02 N Polymeric Ethylene-Maleic Acid (Molecular Weight 60-70,000) with 0.1 N Base in 1 M NaClO₄: in the Absence of Metal Ions, ●; in 0.005 M Cu(ClO₄)₂, □; in 0.005 M Ni(ClO₄)₂, △; in 0.005 M Co(ClO₄)₂, ○.

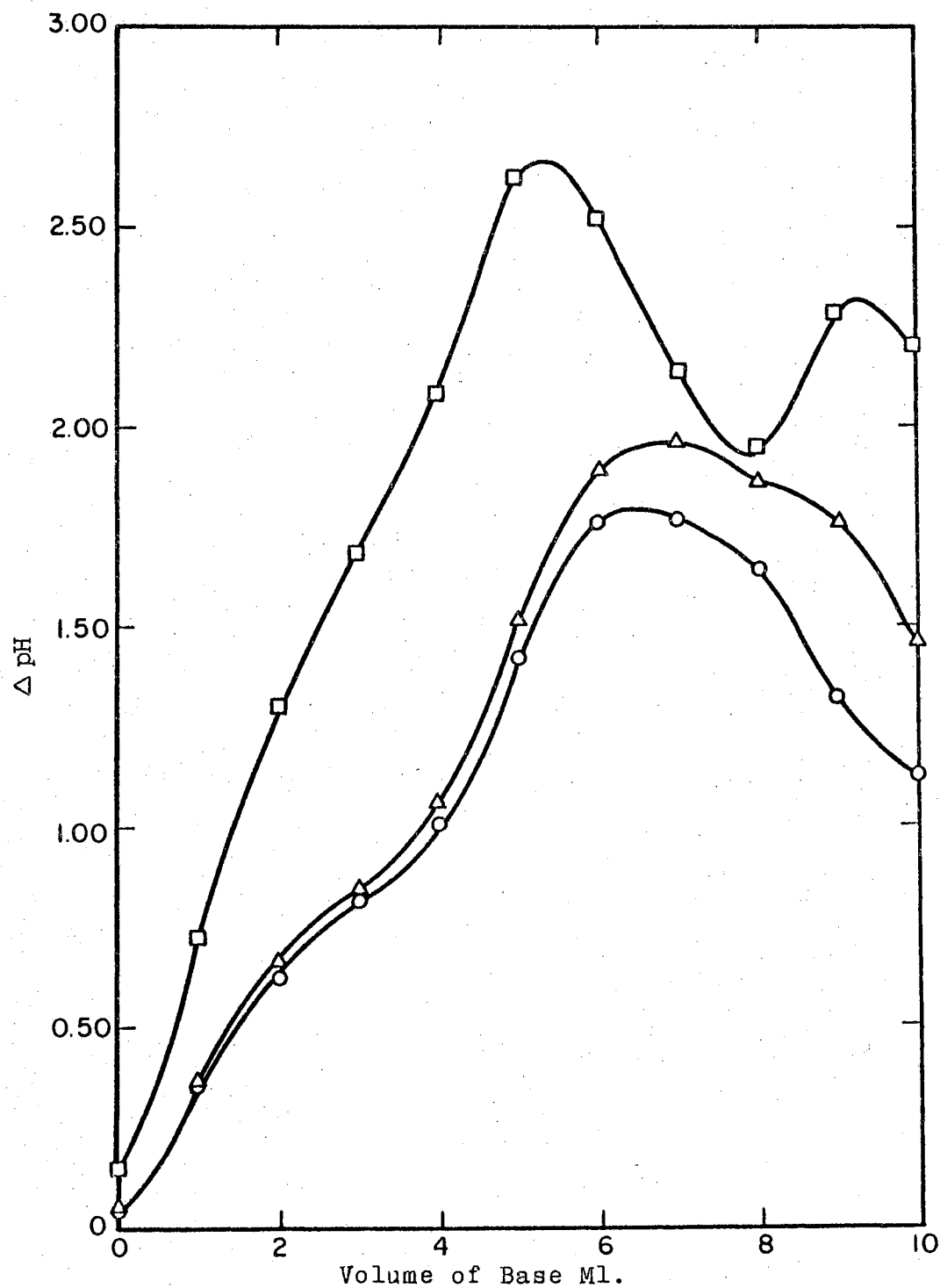


Figure 7. Differences in pH of 0.02 N Solution of Polymeric Ethylene-Maleic Acid (Molecular Weight 2-3,000) when Titrated with 0.1 N Base, First Alone and then in the Presence of: 0.005 M Cu(ClO₄)₂, □; 0.005 M Ni(ClO₄)₂, Δ; 0.005 M Co(ClO₄)₂, ○.

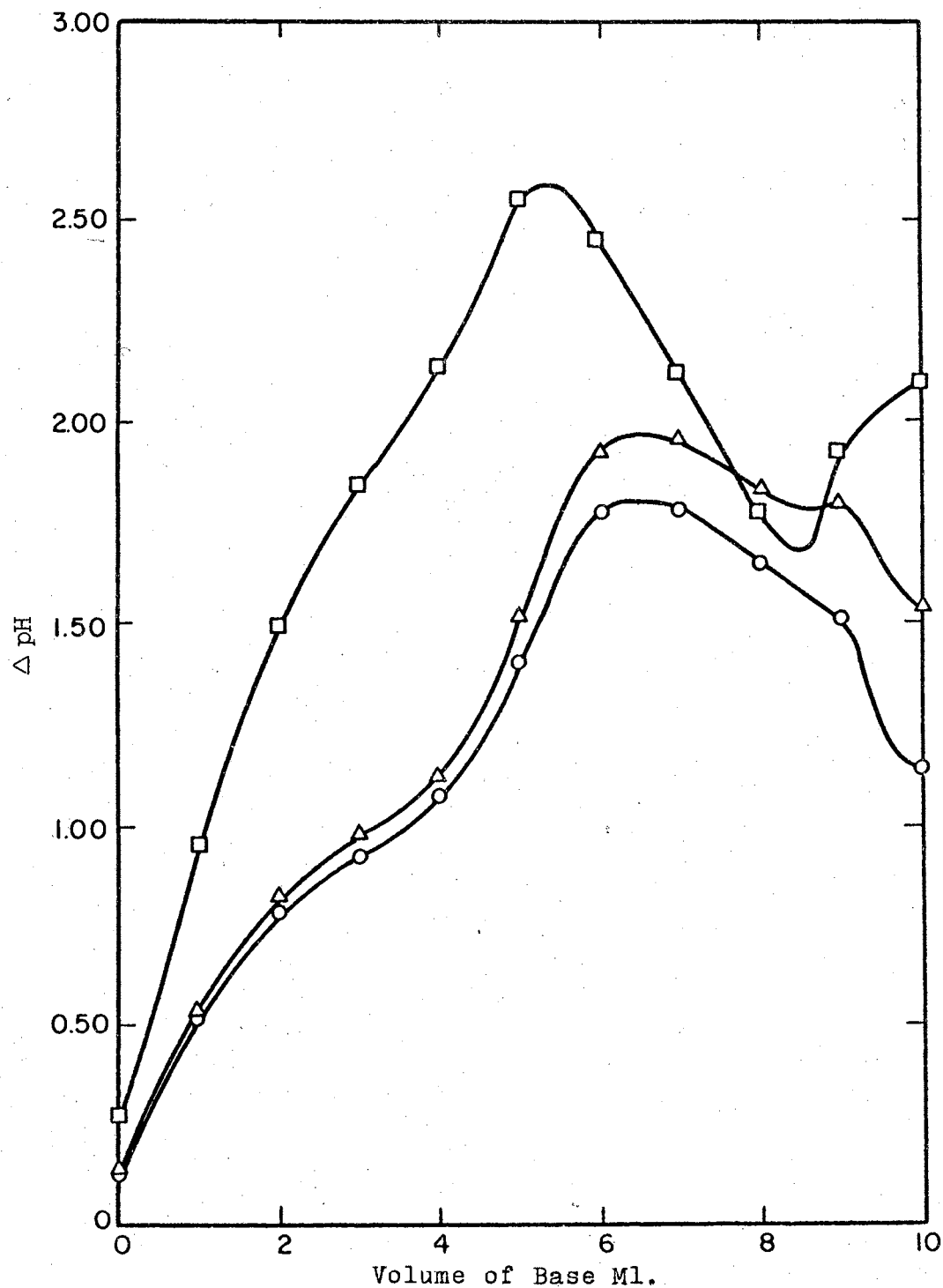


Figure 8. Differences in pH of 0.02 N Solution with Polymeric Ethylene-Maleic Acid (Molecular Weight 20-30,000) when Titrated with 0.1 N Base, First Alone and then in the Presence of: 0.005 M $\text{Cu}(\text{ClO}_4)_2$, \square ; 0.005 M $\text{Ni}(\text{ClO}_4)_2$, Δ ; 0.005 M $\text{Co}(\text{ClO}_4)_2$, \circ .

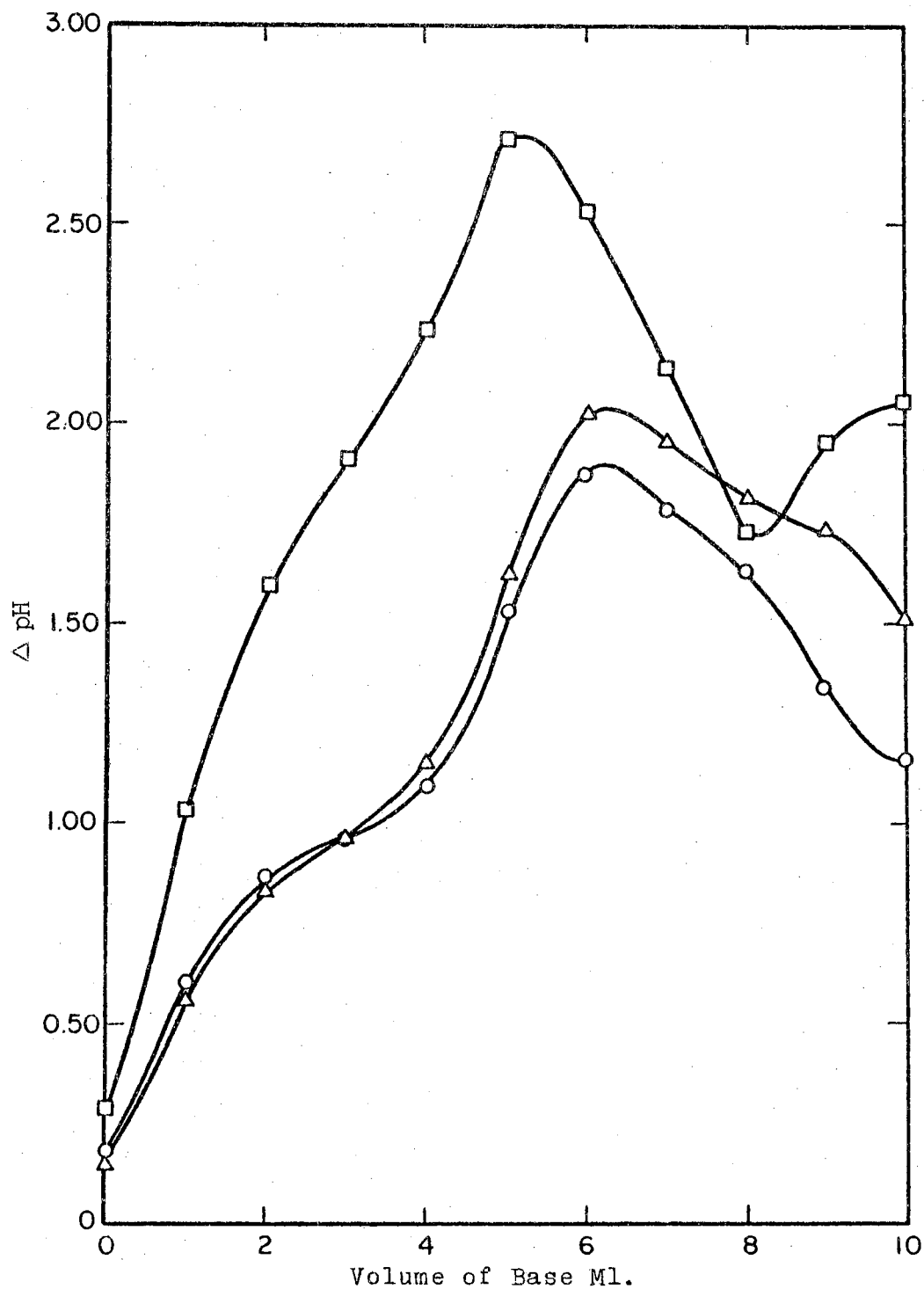


Figure 9. Differences in pH of 0.02 N Solution with Polymeric Ethylene-Maleic Acid (Molecular Weight 60-70,000) when Titrated with 0.1 N Base, First Alone and then in the Presence of: 0.005 M $Cu(ClO_4)_2$, \square ; 0.005 M $Ni(ClO_4)_2$, \triangle ; 0.005 M $Co(ClO_4)_2$, \circ .

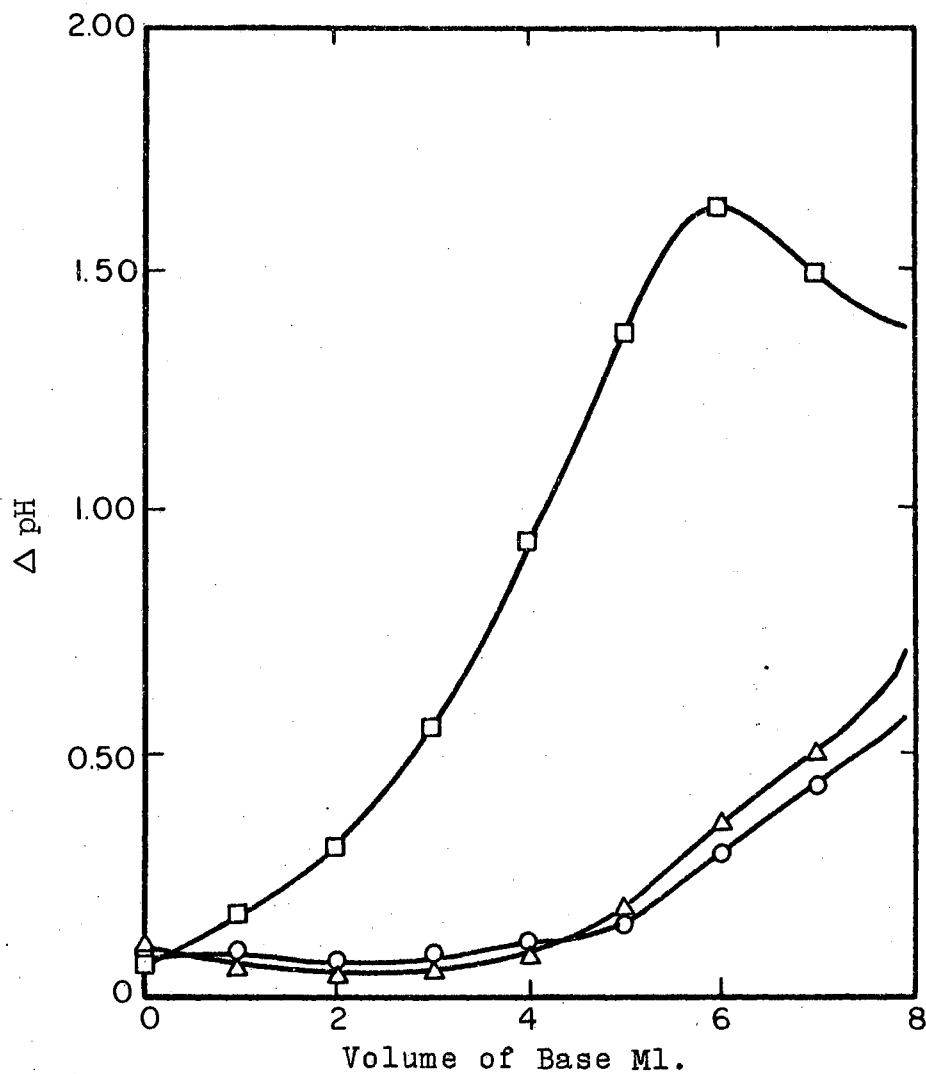


Figure 10. Differences in pH of 0.02 N Solution of Polymeric Ethylene-Maleic Acid (Molecular Weight 2-3,000) when Titrated in 1 M NaClO₄ with 0.1 N Base, First Alone and then in the Presence of:
 0.005 M Cu(ClO₄)₂, □; in
 0.005 M Ni(ClO₄)₂, Δ; in
 0.005 M Co(ClO₄)₂, ○.

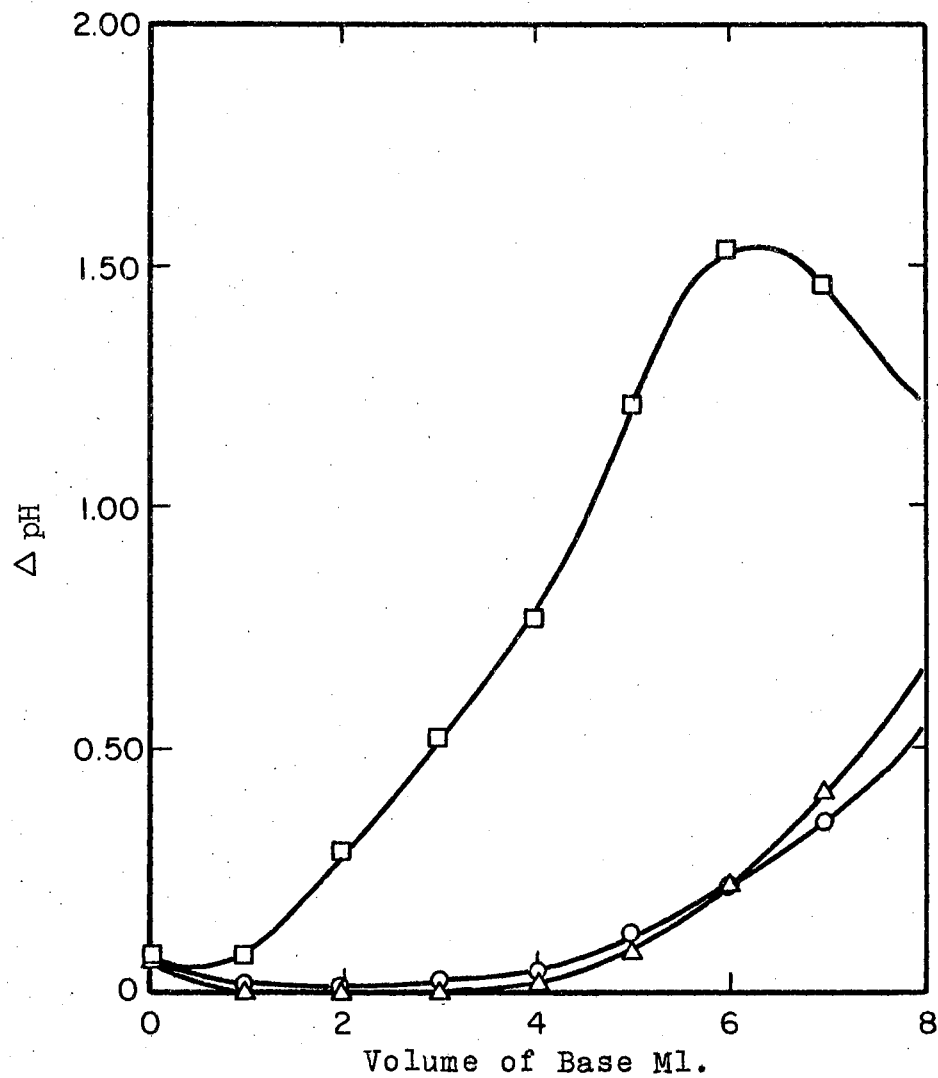


Figure 11. Differences in pH of 0.02 N Solution of Polymeric Ethylene-Maleic Acid (Molecular Weight 20-30,000) when Titrated in 1 M NaClO₄ with 0.1 N Base, First Alone and then in the Presence of:
 0.005 M Cu(ClO₄)₂, □; in
 0.005 M Ni(ClO₄)₂, Δ; in
 0.005 M Co(ClO₄)₂, ○.

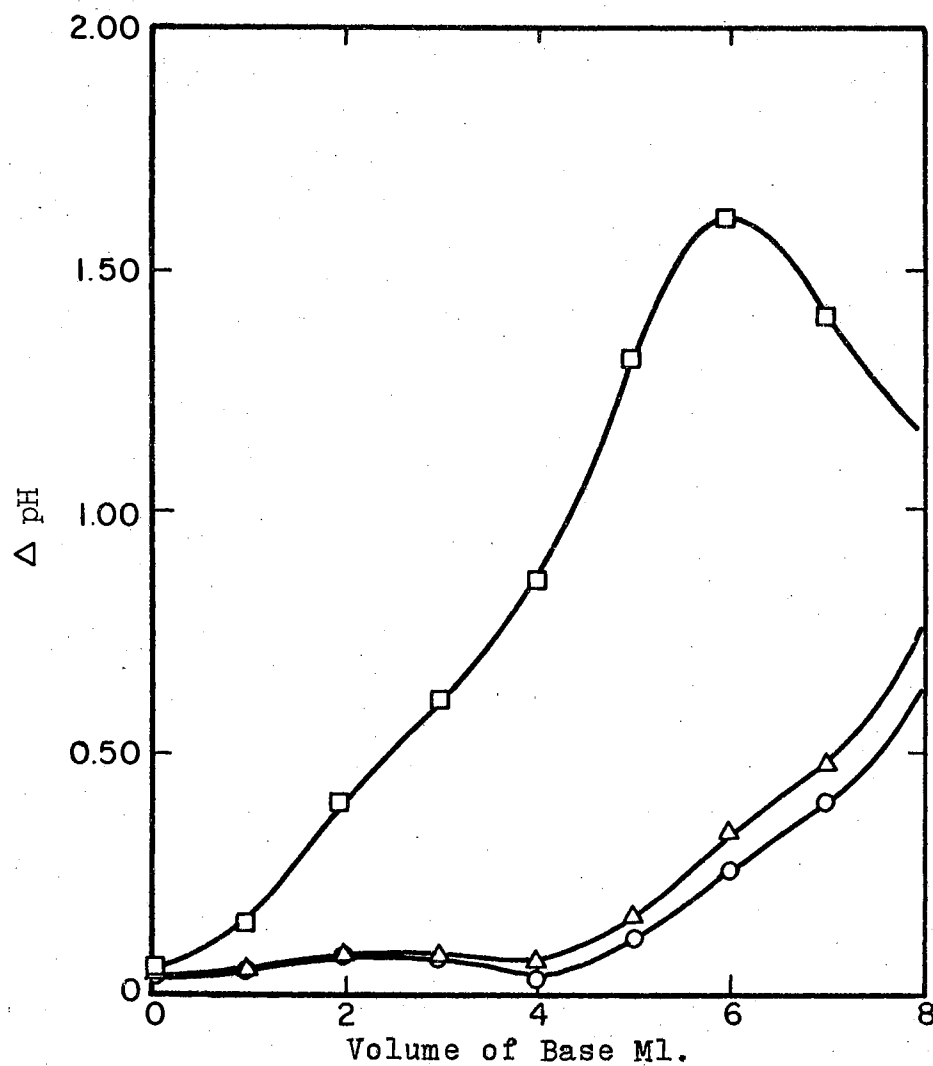


Figure 12. Differences in pH of 0.02 N Solution of Polymeric Ethylene-Maleic Acid (Molecular Weight 60-70,000) when Titrated in 1 M NaClO_4 with 0.1 N Base, First Alone and then in the Presence of:
 0.005 M $\text{Cu}(\text{ClO}_4)_2$, □; in
 0.005 M $\text{Ni}(\text{ClO}_4)_2$, Δ; in
 0.005 M $\text{Co}(\text{ClO}_4)_2$, ○.

VITA

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Candidate for the Degree of

Master of Science

Thesis: A COMPARATIVE STUDY OF THE COMPLEXING ABILITIES
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WEIGHT FRACTIONS OF ETHYLENE-MALEIC ACID COPOLYMER

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