PREPARATION, CHARACTERIZATION, AND STUDY OF SOME NEW POLYCARBOXYLIC ACIDS AS POTENTIAL TUMOR INHIBITORS

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CHAPTER I

INTRODUCTION

The general effects of synthetic polyelectrolytes on living cell systems was reviewed in 1964 by Katchalsky[1], who stated that "the electrical properties of synthetic polyelectrolytes endow them with powerful adhesive properties and with easy orientability which may play a role in the organization of biological structures".

Besides effecting the agglutination of bacteria and animal cells and affecting the activities of vital enzymes (such as ribonuclease[2,3] and deoxyribonuclease[4,5]), some naturally occurring as well as some synthetic polyelectrolytes have shown the ability to inhibit tumor growth[6-10]. Although polyelectrolytes include polyacids, polybases, and polyampholytes, polyacids have shown the greatest degree of tumor inhibition. Among the polyacids that have been tested, those that have shown antitumor activities are polyethenesulfonic acids[7], polycarboxylic acids[11-20], and polynucleotides[21-24].

The tumor inhibition of some naturally occurring polyelectrolytes is known to be due to their ability to induce the production of interferon. Some interferons thus produced have been separated and characterized chromatographically[7,25,26]. Since it was suspected that the synthetic polyelectrolytes which were effective against tumors might possess this same ability <u>in vivo</u>, this postulation was examined. Merigan[12] in 1967 observed interferon induction by a series of copolymers of maleic anhydride with divinyl ether, vinyl methyl ether, vinyl acetate, and styrene. The "pyran copolymer" (maleic anhydride-divinyl ether copolymer) was found to induce interferon in man by Regelson[13] and in mice by De Clercq *et al.*[14] Some polynucleotides were also shown to have the same ability to induce the production of interferon[14,21,22,23,24]. Work done by De Clercq and De Somer[15] in 1968 further proved that the injection of poly(acrylic acid) prior to inoculation with tumor cells had the same effect in prolonging the animal life as the prior injection of interferon, i.e., each can protect the animals from being affected by the tumor for a certain period of time.

Studies of antiviral activities were also carried out by varying the charge distribution and composition[11,12,23], the secondary structure[22], and the effect of thermal activation[24] of the polyelectrolyte structures.

De Somer *et al.*[16,17] in 1968 compared the antiviral activities of poly(acrylic acid) and poly(methacrylic acid) with those of other types of synthetic polyelectrolytes (e.g., dextran sulfate, and polyphloroglucinol) *in vitro* and *in vivo*. They found that the first two synthetic polyacids are more antivirally active than other polyanions. The enhanced action was attributed to both the direct interaction between these two polyacids and the virus cells and to the interferon production.

The conclusions about the structures of polyanions and their antitumor activities can be summarized as follows: (1) an ionized acid group is necessary[11], (2) a high molecular weight compound is necessary[11], (3) the activity is greatly reduced if the hydrophobic content of the polyelectrolyte increases[12], and (4) charge density may play a role[11].

The goals of this study were to synthesize some carboxylic acid polymers with varied structural parameters, to examine the chemical properties (as to their content of anion groups, molecular weights, and binding abilities) as well as their biological activities, and to correlate these with their ability to inhibit the growth of tumors.

CHAPTER II

HISTORICAL

Potentiometric titrations of synthetic polyelectrolytes were carried out as early as 1938 by Kern[27]. In 1947 Katchalsky and Spitnik[28] started the theoretical interpretation of the results of the titrations of polyelectrolytes. They found that when pH was plotted against $\log(1-\alpha)/\alpha$ (where α is the degree of ionization of the acid), in the case of either a monobasic acid or a polyacid, a straight line was obtained for values of α near 0.5. While the slope of this straight line in a monobasic acid is -1, slopes of less than -1 with values approaching -2 were observed for polyelectrolytes. They rationalized this behavior by expanding the Henderson-Hasselbalch equation from $pH = p\underline{K}_o - \log(1-\alpha)/\alpha$ to $pH = p\underline{K}_{av} - \underline{n}\log(1-\alpha)/\alpha$, where $\underline{n}$ denotes the deviation of slope from 1 and $p\underline{K}_o$ is the negative logarithm of the intrinsic ionization constant.

The reason for this deviation of the values of $\underline{n}$ from 1 for polymeric acids is that an additional amount of work is required to remove protons from the polymer molecule during the dissociation. This additional energy requirement is due mainly to the electrostatic interaction between (1) the protons and other cations and (2) the charges on the polyelectrolyte chain[29-36] and is due partially to the necessity for steric alteration (i.e., the conformational energy change)[36-39] of the polymer.

The total energy change (ΔG_t) required for the loss of a proton in order to establish the following equilibrium:

$$-COOH \rightleftarrows -COO^- + H^+ \tag{1}$$

is then the sum of the standard free energy change (ΔG_o) and the additional energy change (ΔG_{el} is usually used to denote this additional energy change since the electrostatic interaction causes the major difference).

$$\Delta G_t = \Delta G_o + \Delta G_{el} \tag{2}$$

At equilibrium:

$$\Delta G_t = \underline{R}\underline{T} \ ln\underline{K} \tag{3}$$

$$p\underline{K} = 0.434 \ \Delta G_t/\underline{R}\underline{T} \tag{4a}$$

$$= 0.434 \ (\Delta G_o + \Delta G_{el})/\underline{R}\underline{T} \tag{4b}$$

$$= p\underline{K}_o + 0.434 \ \Delta G_{el}/\underline{R}\underline{T} \tag{4c}$$

where R is the gas constant, and T is the absolute temperature. When this pK is substituted into the Henderson-Hasselbalch equation, the following potentiometric titration equation for polyacids is obtained:

$$pH = p\underline{K}_o - \log(1-\alpha)/\alpha + 0.434 \ \Delta G_{el}/\underline{R}\underline{T} \tag{5}$$

Since the ΔG_{el} is found to be a function of α, the $\log(1-\alpha)/\alpha$ and 0.434 $\Delta G_{el}/\underline{RT}$ can then be combined as a function of α to give the expanded Henderson-Hasselbalch equation.

The theoretical evaluation of ΔG_{el} has been carried out by many workers. Different models of polyelectrolyte molecules have been proposed to permit the calculation of charge distribution and density and thus the interactions. A spherical model was proposed[31,35,40] as well as a coil-thread or rodlike model[30,32,37,41-49] for this purpose.

It is generally agreed[36,47] that the rodlike model is more appropriate for explaining ionization phenomena in polyelectrolytes than the spherical model. However, it is very unlikely that the polyelectrolyte molecule will remain in a fixed or defined conformation during the whole titration process. Changes in conformation of polyelectrolytes as α changes have been established through viscosity studies[1]. The polyelectrolyte molecules will expand as the degree of ionization increases. This expansion will reach its maximum degree at values of α around 0.8. The calculated potentials based on the rodlike model were found to fit the experimental result only at certain ranges of α and the modes of change in conformation were realized in plots of potential vs α[47,48,49]. This conformational change was also observed in recent colorimetric studies of polyelectrolytes[50].

When the reduced viscosity, $\eta_{sp}/\underline{c}$, of a solution of a noncharged polymer is plotted against concentration, a straight line is obtained. This linear relationship can be expressed by the Huggins equation[51,52]:

$$\eta_{sp}/\underline{c} = [\eta] + \underline{k}[\eta]^2\underline{c} \qquad (6)$$

where $[\eta]$ is the intrinsic viscosity, $\underline{c}$ is the concentration of the polymer solution (usually in g/100 ml), and $\underline{k}$, the Huggins constant, is related to the shape of polymer molecules and their degree of association. The ideal value of $\underline{k}$ for a spherical molecule is 2.0 - 2.26, for a dumbbell or rigid rod is 0.77, and for a centrosymmetric flexible molecule is 0.60.

Polyelectrolyte molecules in a non-ionizing solvent behave according to the Huggins equation. However, when the viscosity of polyelectrolytes is measured in an ionizing solvent, in the absence of added salt, an abnormal behavior is noted. Fuoss[53] reported in 1948 that the reduced viscosity of polyelectrolytes decreases first sharply as the concentration of the polymer solution increases, then reaches a limiting value as c approaches infinity.

Various empirical equations were proposed to relate this abnormal change in concentration and viscosity. These equations are introduced briefly below.

The Fuoss equation[41,53-58] is

$$Z = A/(1 + B\sqrt{c}) + D \tag{7}$$

where Z represents η_{sp}/c, A is a constant that depends on the molecular weight (it is a measure of the extent to which the polymer coil can spread out at infinite dilution as a consequence of the intramolecular coulomb repulsion between charges attached to the chain), B is a constant that measures the electrostatic forces and increases with decreasing dielectric constant of the solvent, and D is a parameter primarily for linearizing the plot.

Equation (7) can be rearranged to read as follows:

$$1/(Z - D) = 1/A + B\sqrt{c}/A \tag{8}$$

By substituting a proper value of D, a straight line can be obtained from a plot of $1/(Z-D)$ vs $\sqrt{c}$. All other constants can then be obtained from the plot.

Schaefgen and Trivisonno[59] in 1952 proposed the following equation:

$$Z = A/(1 + Bc) + D \tag{9}$$

where $\underline{D}$ is a constant for the adjustment of linearity (its value is intrinsic for the polymer itself and is not influenced by the solvent), $\underline{A}$ is a constant depending on the uncoiling of polymer chain due to repulsion between unneutralized charges along the chains, and $\underline{B}$ is a measure of the electrostatic forces between the polyion and its counterions. According to Eq. (9), the plot of $1/(\underline{Z}-\underline{D})$ vs $\underline{c}$ will give a straight line and constants $\underline{A}$ and $\underline{B}$ may be obtained thereafter.

Liberti and Stivala[60-63] in 1966 proposed the following equation:

$$\underline{Z} = \underline{A}/[1 + \underline{B}(\sqrt{\underline{c}} - \underline{k}'\underline{c})] \qquad (10)$$

where $\underline{k}'$ is a measure of the deviation from linearity and $\underline{A}$ and $\underline{B}$ are constants and found to be functions of the molecular weight of the polymer. A plot of $1/\underline{Z}$ vs $(\sqrt{\underline{c}} - \underline{k}'\underline{c})$ according to Eq. (10) will give a straight line from which the values of the constants $\underline{A}$ and $\underline{B}$ can be obtained.

Recently, Yuan, Dougherty and Stivala[64] proposed the following equation:

$$\eta_{sp}/\underline{c} = [\eta]_\infty\, [1 + (\underline{k}''/\sqrt{\underline{c}})] \qquad (11)$$

where $[\eta]_\infty$ is the intrinsic viscosity of a swamped polyelectrolyte (i.e., the polyelectrolyte molecules are swamped or shielded by added ions to such an extent that the macromolecules behave as a non-charged chain) and $\underline{k}''$ is a parameter which represents the effect of electrostatic interaction.

When the viscosity of polyelectrolytes is measured in the presence of free salt, the result is found to follow the Huggins equation[53,65,66]. Also, it is realized that in extremely dilute solution, the polyelectrolyte molecules behave in the same way as non-charged polymers[67]. In the former case, the counterions from the neutral salt can neutralize the charges on the polyions. The shielded polyelectrolyte molecules will thus act as a non-charged polymer. In very dilute solution, as soon as the polyelectrolyte molecules reach the final state of expansion in salt-free solution, interactions between charged groups are at a minimum and the polyelectrolyte molecules will then act like a non-charged polymer.

For cases between these two extremes, where the interaction of charged groups on the polymeric chain can not be ignored, the expansion of the polymer chain during dilution causes an increase in viscosity. At least three theories have been developed to explain this phenomenon[68]. The "folding chain theory" explains the increase in viscosity during dilution by the stretching of the polyelectrolyte molecules due to the electrostatic repulsion[68]. The "electrical theory"[69-72] attributes the viscosimetric properties of polyelectrolytes to the classical electroviscous effect. The "swarm theory"[73] explains that the change in degree of association of the polyions occurs through the sharing of the ionic atmosphere by the molecules.

The interaction between metal ions and the polyanions has long received extensive attention[1,74,75]. The phenomenon of association of metal ions by polyanions was first observed through osmotic pressure studies[76]. This was later confirmed by a series of transference studies using radioactive metal ions[77-81]; certain percentages of the metal ions were carried by the polyanions toward the anode. Studies by using other means of measurements were also noted. Katchalsky and Zwick[82] recorded the decreased swelling of crosslinked poly(methacrylic acid) by metal ions. Gregor and Frederick[83,84] observed the shift in pK_{av} of polyacids when different alkali metals and quaternary ammonium bases were used in titration, and the extents of binding of different cations to the polyanions were compared. Turbidity measurements were also used for studying the critical concentration of cations to bring about the precipitation of polyanions[85-88]. Electrical transport measurements were employed for the determination of counterion association in salt-free polyelectrolyte solutions[89]. Also the potentiometric method was used to determine the polyanion-metal ion complex formation[90,91,92].

For the purpose of our study, we were particularly interested in metal ion association through turbidity measurements.

Wall and Drenan[85] found that though the charge on the metal ion still plays the major role in the nature of association, this phenomenon is not due entirely to coulombic charges since different divalent metal ions differ in their effectiveness in the association.

Michaeli[86] studied the association effect of calcium, barium, and magnesium ions with poly(methacrylic acid) neutralized to different degrees with sodium hydroxide. He observed that the critical concentration of the precipitating ion is directly related to the concentration of the polymeric charged groups. The precipitation occurs when about 80% of the carboxylate groups are associated with divalent counterions. He proposed that the precipitation is caused mainly by a change in the solubility properties of the single polymer molecule rather than by crosslinking.

Ikegami and Imai[87,93] studied the precipitation of poly(acrylic acid) (which was neutralized to different degrees with tetra-*n*-butylammonium hydroxide) by sodium, magnesium, barium, zinc, aluminum, and calcium ions. They proposed that two types of binding may occur. One is the L-type, which occurs at high α. The other is the H-type, which appears at low α.

Constantino et al.[88] found that metal binding is independent of the molecular weight of poly(methacrylic acid), but is influenced by the stereoregularity of the polymer molecules.

An equation was proposed to related the metal ion concentration, polyanion concentration, and the bound metal ion concentration[86,88,94]. This equation reads as follows:

$$(M^{+2})_t = (M^{+2})_f + \beta(PA)_t \qquad (12)$$

where $(M^{+2})_t$ is the total divalent metal ion concentration, $(M^{+2})_f$ is the free divalent metal ion concentration, β is the equivalents of divalent metal ions bound per acid group, and $(PA)_t$ is the total concentration of acid groups. A plot of $(M^{+2})_t$ vs $(PA)_t$ gives a straight line. The intercept of this straight line gives the $(M^{+2})_f$ and the slope gives the value of β.

CHAPTER III

EXPERIMENTAL

Materials

Acetone (J. T. Baker). Reagent-grade acetone was used as a solvent without further purification for some purposes. For use in viscosity and molecular weight determinations reagent-grade acetone was further purified by refluxing through a Soxhlet extractor filler with 4A Molecular Sieves and was then distilled.

Acrylic acid (Aldrich). Lot #14723-0, assaying 99 weight %, was distilled under reduced pressure over *p*-methoxyphenol before use.

Benzene (Fisher Scientific). Reagent-grade benzene was distilled over sodium metal before being used as a solvent in polymerization reactions. For other purposes it was used without purification.

N,*N*-Dimethylaminoethyl methacrylate (Rohm and Haas). Lot #36373, industrial-grade, 94 weight % containing 2000 ppm *p*-methoxyphenol, was distilled under reduced pressure before use.

Isobutyl vinyl ether (Eastman). The practical-grade monomer was distilled over anhydrous calcium hydride before use.

Isooctyl vinyl ether (General Aniline and Film). The practical grade monomer was distilled over anhydrous calcium hydride before use.

Itaconic acid (Chas. Pfizer and Co.). The refined-grade compound was crystallized from acetone before use.

Nitrogen (Linde). Lamp-grade nitrogen was dried by passing it through a trap of concentrated sulfuric acid, then through anhydrous calcium chloride.

The following reagents were used without further purification: acetone-$\underline{d}_6$ (Bio-Rad, Control #52123), aluminum sulfate (Fisher Scientific, reagent-grade), benzoyl peroxide (Fisher Scientific, reagent-grade), calcium hydride (Fisher Scientific, purified-grade), carbon tetrachloride (Fisher Scientific, reagent-grade), chloroform (Fisher Scientific, reagent-grade), chloroform-$\underline{d}_3$ (Diaprep, Lot #680701), deuterium oxide (Diaprep, Lot #680602), dimethyl sulfoxide-$\underline{d}_6$ (Bio-Rad, Control #81115), N,N'-dinitroso-N,N'-dimethylterephthalamide (Du Pont, Du Pont EXR-101 with inert filler), 1,4-dioxane (J. T. Baker, reagent-grade), 2-(2-ethoxyethoxy)ethanol (Eastman, practical-grade), ethyl acetate (Fisher Scientific, reagent-grade), ethyl ether (Fisher Scientific, anhydrous), formic acid (Mallinckrodt Chemical Work, analytical-grade), hexanes (Fisher Scientific, reagent-grade), ligroin (Skelly, Skelly B, boiling point range 146 - 157° F), methanol (Fisher Scientific, reagent-grade), p-methoxyphenol (Eastman, reagent-grade), n-pentane (Fisher Scientific, reagent-grade), potassium peroxydisulfate (Fisher Scientific, Cat. #P-281), sodium polyacrylate (Nalco Chemical Co., RB-124-66), sodium polyacrylate (Nalco Chemical Co., NX-23-67), tetrahydrofuran (Fisher Scientific, reagent-grade), tetramethylsilane (Thompson-Packard, NMR-grade).

Instrumentation

Infrared spectra were obtained with a Beckman IR-5A spectrophotometer. Proton magnetic resonance spectra were obtained on a Varian Associates Model A-60 or an XL-100 Analytical Nuclear Magnetic Resonance

Spectrometer.

Potentiometric titrations were performed with a Beckman Research pH Meter equipped with a Brinkman Instrument Heater-Circulator and Thermoelectric Cooler and a magnetic stirrer by Precision Scientific Co.

Viscosity measurements were made with a Cannon-Fenske Viscometer (size 50 or 100) in a constant temperature bath regulated with a Sargent Thermonitor.

Turbidity studies were performed with a Cary Model 14 Spectrophotometer and a "Titration Head", a specially designed motor-driven buret of variable and reproducible rate of titrant delivery and a special cell holder comprising an air-driven stirrer. The apparatus was designed and constructed by Dr. H. A. Mottola, Mr. H. Hall, and Mr. B. E. Simpson, of this department.

Number average molecular weight determinations were made using a Coleman 115 Molecular Weight apparatus.

Elemental Analysis

Elemental analyses were determined by M-H-W Laboratories, P.O. Box 326, Garden City, Michigan.

Preparation of Polymers

Preparation of Poly(acrylic acid) (I). The method used for this preparation was adapted from the patent by Barrett[95]. Acrylic acid (5.00 g), benzoyl peroxide (0.005 g), and benzene (100 ml) were added to a 500-ml three-neck round-bottom flask equipped with a condenser and a mechanical stirrer. The mixture, under dry nitrogen, was heated with an oil bath at 82°. After polymerization started, a mixture of 36.0 g acrylic acid, 0.30 g benzoyl peroxide, and 110 ml benzene was added continuously through a dropping funnel to the reaction flask during 12 hr. The poly(acrylic acid), which precipitated from the benzene solution, was collected by filtration. The product was washed in a blender three times each with 200 ml of benzene and filtered out each time. The polymer was then dried under reduced pressure at room temperature. The yield was 36.3 g, 89.0% of theory.

Preparation of Acrylic Acid-Isobutyl Vinyl Ether Copolymer (II). Acrylic acid (58.2 g), isobutyl vinyl ether (20.2 g), and benzoyl peroxide (0.726 g) were added to 200 ml of benzene in a 1000-ml three-neck flat-bottom reaction flask equipped with a condenser and a mechanical stirrer. The polymerizing mixture was kept under dry nitrogen and heated to 82° with an oil bath. The initiation time was about twenty minutes and the reaction was completed after stirring and heating for one hour. The mixture was filtered and the solid transferred to a blender where it was washed three times each with 200 ml ligroin. The solid was then dried under reduced pressure at room temperature. The yield was 56.0 g, 72.1 % of theory.

Preparation of Acrylic Acid-Isobutyl Vinyl Ether Copolymer (III). Acrylic acid (30.0 g), isobutyl vinyl ether (21.3 g), benzoyl peroxide (1.40 g), and 220 ml of benzene were added to a 500-ml three-neck round-bottom flask equipped with a condenser and a mechanical stirrer. Polymerization was carried out under dry nitrogen and at 70° for eight hours. The product was isolated and purified like II. The yield was 26.9 g, 52.4% of theory.

Preparation of Acrylic Acid-Isobutyl Vinyl Ether Copolymer (IV). Acrylic acid (43.1 g), isobutyl vinyl ether (39.9 g), benzoyl peroxide

(0.729 g), and 200 ml benzene were caused to react as for II. The initiation time was 25 min. at 82° and the reaction was completed during forty minutes of heating at this temperature. Acetone (500 ml) was added to the flask to dilute the viscous slurry. Then this acetone-benzene solution of polymer was added slowly in 125-ml portions to 1000-ml portions of ligroin in a 2000-ml beaker under vigorous agitation with a mechanical stirrer. The mixture was filtered and the solid dissolved in 500-ml of acetone. The polymer was precipitated by dividing the solution into four equal portions and adding slowly each portion to 1000-ml of ligroin under stirring. The solid collected after filtration was dried under reduced pressure at room temperature. The polymer was then ground to a white powder. The yield was 56.9 g, 68.7 % of theory.

<u>Preparation of Acrylic Acid-Isobutyl Vinyl Ether Copolymer (V)</u>. Acrylic acid (28.9 g), isobutyl vinyl ether (60.1 g), benzoyl peroxide (0.726 g), and 200-ml of benzene were copolymerized as for II, initiation taking fifteen minutes at 82°. The reaction was terminated after thirty-five minutes of heating. Acetone (600-ml) was added to the kettle to dilute the viscous solution. This benzene-acetone polymer solution was added slowly in 125-ml portions with mechanical stirring to 1400-ml portions of 0.05 N hydrochloric acid solution in a 2000-ml beaker. The polymer collected through filtration was dissolved in 1000 ml of methanol. Each 125-ml of this methanol solution was added slowly to 1400 ml of 0.05 N hydrochloric acid solution to reprecipitate the polymer. The polymer was dissolved in 250 ml of 1,4-dioxane. The final precipitation of polymer was done by adding one volume of this dioxane solution to 10 volume of water. The polymer was collected and dried as usual, and ground to a white powder. The yield was 54.2 g, 60.5% of

theory.

Preparation of Acrylic Acid-Isobutyl Vinyl Ether Copolymer (VI). Acrylic acid (15.1 g) and isobutyl vinyl ether (80.3 g) were copolymerized with benzoyl peroxide (0.728 g) in 200 ml of benzene as for II. The reaction was terminated after one hour of heating. The viscous benzene solution of the polymer was diluted with 1000 ml of acetone. This acetone-benzene solution was added slowly with agitation in 125-ml batches to 1500-ml portions of a methanol-water mixture (1:4 in volume) at pH 2 (with the addition of hydrochloric acid). The solid was collected and was dissolved in acetone. Precipitation was repeated by dissolving the polymer in 1000 ml of acetone and adding this solution slowly to the methanol-water mixture previously described. The final polymer was collected, dried, and ground to powder as before. The yield was 39.8 g, 41.7% of theory.

Preparation of Poly(isobutyl vinyl ether)[96] (VII). The catalyst for this polymerization was prepared by dissolving 10.0 g of aluminum sulfate in the minimum amount of water. Concentrated sulfuric acid (3.30 g) was added and the solution was evaporated to dryness. The residue was left in an oven at 170° for four hours. A sample of the dried salt (0.20 g) was suspended in 10.0 ml of mineral oil (to give a 2% suspension of $Al_2(SO_4)_3 \cdot 3H_2SO_4 \cdot 7H_2O$).

Isobutyl vinyl ether (25 ml) and 150 ml of *n*-pentane were placed in a 16-oz pressure bottle. The air in the bottle was replaced by dry nitrogen and 0.5 ml of the catalyst suspension was added through a syringe. The mixture was heated in a water-bath at 35° for forty-seven hours. The polymer was precipitated in methanol in the presence of a small amount of *N*-phenyl-2-naphthylamine. The precipitate was washed in

150 ml methanol three times in a blender. The solid collected through filtration was dissolved in benzene and then freeze-dried. The yield was 15.3 g, 79.0% of theory.

<u>Preparation of Poly(itaconic acid)(VIII).</u>[97] Itaconic acid (50.0 g), potassium peroxydisulfate ($K_2S_2O_8$) (0.25 g), and 125 ml 0.5 N hydrochloric acid were added to a 12-oz beverage bottle. The air in the bottle was replaced by dry nitrogen. Polymerization was carried out at 50° by heating in an oil bath under stirring. The total reaction time was six days and three and one-half hours. The polymer was precipitated by adding the water solution dropwise to 1400 ml acetone with vigorous stirring. The mixture was filtered and the polymer collected. The final solid was dried under reduced pressure at 50° and ground to a powder. The yield was 14.5 g, 29.0% of theory.

<u>Preparation of Acrylic Acid-Itaconic Acid Copolymer (IX).</u>[97] Acrylic acid (20.0 g), itaconic acid (20.0 g), and potassium peroxydisulfate (0.20 g) were dissolved in 100 ml of water and transferred to a 16-oz pressure bottle. The air in the bottle was replaced by dry nitrogen and the bottle was shaken with a mechanical shaker at room temperature for eight days. The polymer was precipitated by adding the viscous water solution slowly to acetone (volume ratio = 1:8) with stirring. The polymer was redissolved in water and precipitated again in acetone. The polymer was then dried in a rotary evaporator under reduced pressure at 50°. The residue was ground to powder. The yield was 27.5 g, 68.8% of theory.

<u>Preparation of Acrylic Acid-N,N-Dimethylaminoethyl Methacrylate Copolymer (X).</u> Acrylic acid (25.0 g) and N,N-dimethylaminoethyl methacrylate (25.0 g) were copolymerized with potassium peroxydisulfate

(0.500 g) in 400 ml of water under dry nitrogen at room temperature for five days and four hours. The polymer was precipitated by adding the water solution slowly to acetone (volume ratio = 1:10) with stirring. The solid was washed in a blender three times each with 200 ml of methanol. The final polymer was collected by filtration and dried under reduced pressure at room temperature. The yield was 45.8 g, 91.6% of theory.

Preparation of Acrylic Acid-Isooctyl vinyl ether Copolymer (XI). Acrylic acid (25.0 g), isooctyl vinyl ether (25.0 g), benzoyl peroxide (0.500 g), and 390 ml of benzene were kept at 53° under dry nitrogen for four hours. The precipitation of the polymer was done by adding the benzene solution slowly to ligroin (volume ratio = 1:3) with stirring. The polymer was washed three times in a blender each time with 200 ml of ligroin, filtered out, and dried under reduced pressure at room temperature. The dry polymer was a fine white powder. The yield was 32.3 g, 64.6% of theory.

Preparation of Poly(methyl acrylate)(XII).[51] Methyl acrylate (20.0 ml), benzene (50.0 ml), and 2,2-azobisisobutyronitrile (0.058 g) were added to a screw-capped vial. The air in the vial was replaced by dry nitrogen. The mixture was stirred and heated at 60° for twenty hours. The polymer was precipitated in methanol (one volume of benzene solution to ten volumes of methanol) with stirring. The polymer was redissolved in tetrahydrofuran and precipitated again in methanol (one to ten volume ratio). The polymer was dissolved in 50 ml of benzene and freeze dried under reduced pressure. The yield was 15.5 g, 81.2% of theory.

Esterification of Poly(acrylic acid) to give Poly(methyl acrylate)

(XIII). Sodium hydroxide (2.4 g) was dissolved in 20 ml of water. This solution was mixed with 50 ml of 2-(2-ethoxyethoxy)ethanol and 150 ml anhydrous ethyl ether. N,N'-Dinitroso-N,N'-dimethylterephthalamide (7.1 g) was added to the mixture. The diazomethane thus generated was distilled with ether by heating over a steam bath to a collector cooled with Dry Ice. The ether solution of diazomethane was poured into an Erlenmeyer flask containing 3.0 g of poly(acrylic acid)(I). Enough diazomethane solution was added that the yellow color persisted in the flask. The flask was warmed over a hot plate to decompose the unreacted diazomethane. All ether was then evaporated. Chloroform (30 ml) was added to dissolve the polymer. The polymer was precipitated in 450 ml of hexanes. The polymer was redissolved in 50 ml of ethyl acetate and was precipitated again in 1200 ml of methanol. The residue was dissolved in 25 ml of benzene and was freeze-dried under reduced pressure. The yield was 2.94 g, 98.0% of theory.

Infrared Spectroscopic Studies of Polymers

Infrared spectra of polymers were obtained by forming thin films on sodium chloride plates. Each polymer film was prepared by dissolving 0.1 g of polymer in 5 ml of a suitable solvent. The solution was concentrated by evaporation of solvent over a hot plate at 50°. The concentrated solution was spread evenly over a sodium chloride plate. A thin film of polymer formed on the plate when the solvent was evaporated under reduced pressure. Further drying of the film was done in an oven at 110° for four hours[98,99]. The solvents used for the preparation of polymer films are summarized in Table I.

TABLE I

SOLVENTS USED FOR FILM PREPARATIONS

Polymer	Solvent	Polymer	Solvent
I	Methanol	VIII	Methanol
II	Methanol	IX	Methanol
III	Methanol	X	Formic Acid
IV	Acetone	XI	Methanol
V	Methanol	XII	Tetrahydrofuran
VI	Methanol	XIII	Chloroform
VII	1,4-Dioxane		

Nuclear Magnetic Resonance Studies of Polymers

A solution of about 10 weight % was prepared for each polymer in a suitable solvent. Tetramethylsilane (TMS) was used as the reference standard either internally or externally.

The solvents, the model of Varian NMR spectrometer, and the method of using the TMS standard are summarized in Table II.

Potentiometric Titration of Aqueous Polymer Solution

Aqueous polymer solutions were prepared by three methods as follows: Method 1. A sample of the polymer (about 0.05 g) was weighed (to three significant figures) and dissolved in 80 ml of carbon-dioxide-free water. One sample each of polymers VIII and IX was heated at 70^{o} for three hours before being titrated. Method 2. A sample of the polymer (about 0.05 g) was weighed and dissolved in 10.0 ml of standard base solution and 20.0 ml of standard acid solution was added. The final

TABLE II

NMR STUDIES OF POLYMERS

Polymer	Solvent	Varian Model	TMS
I	DMSO-d_6	A-60	External
II	DMSO-d_6	A-60	External
	D_2O	A-60	External
III	DMSO-d_6	A-60	External
IV	DMSO-d_6	A-60	External
V	DMSO-d_6	A-60	External
VI	Acetone-d_6	A-60	Internal
VII	CCl_4	A-60	Internal
VIII	DMSO-d_6	XL-100	External
	D_2O	XL-100	External
IX	DMSO-d_6	XL-100	External
	D_2O	XL-100	External
X	HCOOH	XL-100	External
XI	DMSO-d_6	A-60	External
XII	$CDCl_3$	A-60	Internal
XIII	$CDCl_3$	A-60	Internal

volume was adjusted to 80 ml by adding carbon-dioxide-free water. Method 3. The polymer (about 0.05 g) was weighed and dissolved in 10.0 ml of standard base solution. The final volume was adjusted to 80 ml by adding carbon-dioxide-free water.

The methods of preparation of aqueous polymer solutions are summarized in Table III.

TABLE III

PREPARATION OF POLYMER SOLUTIONS FOR POTENTIOMETRIC TITRATIONS

Polymer	Methods of Preparation	Polymer	Methods of Preparation
I	1,2,3	VIII	1,2
II	1,2	IX	1,2
III	1,3	XI	1
IV	1,3	XIV[a]	2
V	3	XV[b]	2
VI	3		

[a] Sodium polyacrylate, Nalco Chemical Co., RB-124-66

[b] Sodium polyacrylate, Nalco Chemical Co., NX-23-67

All titrations were done at 25.0 ± 0.05° under nitrogen atmosphere with a Beckman Research pH Meter. Each solution was held at this temperature for ten minutes to allow attainment of temperature equilibrium before the titration was started.

Solutions prepared by Method 1 and 2 were titrated with a standard sodium hydroxide or potassium hydroxide solution. Solutions prepared by Method 3 were titrated with a standard hydrochloric acid solution. The titrant was added slowly to the polymer solution with magnetic stirring. The pH readings were made after each addition of 0.25 to 0.50 ml of titrant.

Viscosity Studies of Polymers

A stock solution containing about 1 g per 100 ml was prepared for each polymer. A series of solutions for each polymer were obtained by diluting this stock solution with the same solvent used. The measurements of the efflux times of these solutions were done in a constant temperature bath with a Cannon-Fenske viscometer. All solutions were filtered through sintered glass plates before being placed in the viscometer. Seven milliliters of a solution was put in the viscometer and ten minutes were allowed for the solution in the viscometer to reach the temperature of the bath before measurements were made. Five efflux times were measured for each solution. The average values of the efflux time were within ± 0.2%.

Aqueous polymer solutions at different pH values for viscosity studies were prepared by adding suitable amounts of standard acid or base solution to the stock solution. The effect of different amounts of sodium hydroxide to the efflux time of water was studied. Three water solutions of sodium hydroxide were prepared with concentrations ranging from 0.01 N to 1 N. The efflux times were determined. These data were used for the correction of the solvent efflux time for viscosity calculations.

The solvents used and the temperature at which the viscosities were measured for different polymers are summarized in Table IV.

TABLE IV

SOLVENTS AND TEMPERATURES FOR THE VISCOSITY STUDIES OF POLYMER SOLUTIONS

Polymer	Solvent	Temperature, ^{o}C
I	1,4-Dioxane	30
	Water	25
	Water at pH 7.0	25
	Water at pH 10.0	25
II	Water	25
	Water at pH 7.0	25
III	Water at pH 7.0	25
IV	Water at pH 7.0	25
V	Water at pH 7.0	25
VI	Water at pH 7.0	25
VIII	Water	25
	Water at pH 7.0	25
IX	Water	25
	Water at pH 7.0	25
X	Water at pH 7.0	25
XI	Water at pH 7.0	25
XII	Acetone	20
XIII	Acetone	20
XIV	Water at pH 7.0	25
XV	Water at pH 7.0	25

Number Average Molecular Weight Determinations of Polymer XII and XIII

These determinations were made with a Coleman 115 Molecular Weight Apparatus. A solution of 2.45 g per 100 ml of XII in dry acetone and a solution of 2.67 g per 100 ml of XIII in dry acetone were prepared for the measurements. The main-oven temperature of the instrument was set at 40.4° and the sub-oven temperature at 34.0°. Seven readings were made for each solution, and the readings were averaged. The molar concentrations of the polymer solutions were evaluated through a calibration curve constructed with a solution of benzil in acetone.

Turbidity Measurements of Aqueous Polymer Solutions

A standard solution of magnesium chloride was prepared by dissolving 30 g of magnesium chloride in 100 ml of deionized water (made by passing distilled water through a Deeminac Filter Model F-4, Crystal Research Laboratories, Inc.). Two milliliters of this solution was diluted to 50.0 ml in a volumetric flask. The standard calcium chloride solution was prepared by dissolving 42 g of the salt in 250 ml of deionized water. The standardization of these two chloride solutions were done by titrating with standard silver nitrate solution, potassium chromate being used as the indicator.

The polymer solutions were prepared by dissolving weighed amounts of solid in 100 ml of deionized water in a volumetric flask. These solutions were adjusted to the desired pH value for measurement by adding either sodium hydroxide solution or hydrochloric acid solution.

A series of dilutions were made to each polymer stock solution. Two milliliters of each solution thus obtained was transferred with a pipette to a Coleman Spectrophotometer Cell (Cat. #30-300) (10 mm width, 10 mm light path). The turbidity measurements were done with a double-beam Cary 14 Spectrophotometer at $25.0^{\circ} \pm 0.1^{\circ}$ at a fixed wavelength of 5050 Å. The cation solution (magnesium or calcium) was added through a "Titration Head"[100]. The rate of addition of cation solution and the rate of stirring in the sample cell were properly adjusted to the working conditions. The formation of turbidity in the polymer solution after the addition of cations was indicated by a sharp change in optical density. The critical concentration of cations, i.e., the amount of cations added just to cause turbidity formation in a particular run, was calculated from the chart speed, the rate of addition of cation solution, and the concentration of cation solution.

Screening Against L1210 Mouse Leukemia

Polymers were sent to Dr. Harry B. Wood, Jr., Chief, Drug Development Branch, Drug Research and Development, Chemotherapy, National Cancer Institute, National Institutes of Health, Bethesda, Maryland, 20014, for screening in this tumor system.

Test for Sarcoma 180

Tumor cells were obtained from Frederic A. French of Mt. Zion Hospital, San Francisco, California, through living mouse carriers.

Six female Swiss mice weighing between 20 and 24 g were used in each treatment and for controls. Single and triple treatments were performed with polymer solutions.

The polymer solutions were prepared one day prior to use by dissolving weighed amount of sample in distilled water. The polymer solutions were adjusted to the phenolphthalein end point by adding either dilute sodium hydroxide or hydrochloric acid solution.

Toxicity of each polymer solution was tested in the same manner as the antitumor properties (i.e., single-dose or triple-dose). Concentrations of the polymer solutions were adjusted so that the dose to be used in treatment was contained in 0.4 to 0.8 ml. The safe dose of each solution was established when the animals survived over six days after the last injection of a given amount of the polymer solution.

Tumor cells to be inoculated were obtained by sacrificing one of the carrier mice. The skin from the abdominal area of the animal was removed and 2 ml of ascitic fluid was withdrawn with a 10-ml syringe with a 22-gauge needle. The fluid was diluted with saline to a cell concentration of two million cells per 0.1 ml of solution. A 0.1-ml portion of this cell solution was injected into each mouse to be used in the test. All the equipment used was sterilized beforehand to minimize contamination.

For single-dose treatment, the animals were treated with the solution one day after the tumor inoculation. The animals were weighed on the first and the seventh days after inoculation. For triple-dose treatment, the first injection was made one day after inoculation, and the second and the third injections were performed on the third and the fifth days after the inoculation, respectively. Animals were weighed on the first and the ninth days after inoculation. Mice used for controls were injected with isotonic saline solution instead of the polymer solution.

The mean survival time of treated animals was calculated by averaging the survival times of the third and the fourth deaths of the six animals tested. This value was compared to the mean survival time of the control animals calculated in the same manner. The polymer is considered to be active toward Sarcoma 180 when the mean survival time of treated animals is 1.25 times that of the controls. Repetition of the treatment with polymer solutions that proved active was used to establish the reproducibility of the test.

CHAPTER IV

RESULTS AND DISCUSSIONS

Characterization of Polymer Structures

The compositions of copolymers of acrylic acid (AA) and isobutyl vinyl ether (IBVE) (II-VI) were calculated from the titration data. The total moles of acrylic acid units present per gram of each copolymer were calculated from the titers. The equivalent weights of the polymers were then calculated by dividing the weight of the polymer sample by the equivalents of COOH groups present. Hence, the moles of IBVE to AA were calculated by use of the following equation:

$$\frac{\text{eq. wt. of polymer - eq. wt. of AA}}{\text{formula weight of IBVE}} = \frac{\text{moles of IBVE}}{\text{one mole of AA}}$$

The equivalent weight, the IBVE/AA ratio of monomers used, and the ratio in polymers thus calculated, are tabulated in Table V.

Vinyl ethers are quite inert toward free-radical-initiated polymerization. Though it was reported that oligomers of vinyl ethers may be obtained from benzoyl-peroxide-initiated polymerization[101-103], attempts in this laboratory failed to attain any appreciable amount of poly(isobutyl vinyl ether) through radical initiation. However, when IBVE was present in a relatively large quantity with AA, the propagation of the polymer chain did involve the incorporation of the vinyl ether, as can be seen from Table V. Preparation of AA-IBVE copolymers was also

TABLE V

THE EQUIVALENT WEIGHTS, MONOMER RATIOS, AND COMPOSITIONS OF ACRYLIC ACID - ISOBUTYL VINYL ETHER COPOLYMERS

Copolymer	Equivalent Weight	IBVE/AA	
		In Monomer Mixture	In Polymer[d]
II	74.6[a] 76.5[b]	0.25/1.0	0.035/1.0
III	77.8[a] 75.6[c]	0.51/1.0	0.046/1.0
IV	101[a] 102[c]	0.67/1.0	0.29/1.0
V	115[c]	1.5/1.0	0.43/1.0
VI	145[c]	3.8/1.0	0.73/1.0

[a] Polymer solutions were prepared for potentiometric titration by Method 1.

[b] Polymer solutions were prepared for potentiometric titration by Method 2.

[c] Polymer solutions were prepared for potentiometric titration by Method 3.

[d] The average value of equivalent weight was used for the calculation when more than one method of solution preparation was employed for titration.

possible through other types of radical initiation, as with peroxydisulfate in water[104].

The major infrared (IR) absorptions of polymers I-VII are tabulated in Table VI. The intensity of the absorptions were compared according to Henniker[105].

A broad, and strong O-H stretching vibration centered near 3000 cm^{-1}, evidently involving H bonding, appears in all IR spectra[99,100] but that of polymer VII. Acrylic-acid-containing polymers all showed no signs of anhydride linkage, though the IR films were dried in oven at 110° for several hours[107]. The IR spectrum of VII agreed with that reported by Natta[108], except for a sharp, weak absorption at 1710 cm^{-1}. This exceptional absorption proved to be due to C = C stretching vibration, apparently the same one as in the IR spectrum of isobutyl vinyl ether. The most reasonable explanation for this discrepancy is thermal de-polymerization which occurred during drying of the IR film at 110°, since the intensity of this absorption increases with the drying times.

The nuclear magnetic resonance (NMR) spectrum of polyacrylic acid (I) in deuterium oxide shows three absorptions, a sharp singlet at δ 5.20 (τ 4.80) for the H-OD, a broad peak at $\delta \sim 2.83$ ($\tau \sim 7.17$) for the methine protons on the polymer chain, and a broad peak at $\delta \sim 2.25$ ($\tau \sim 7.75$) for the methylene protons on the polymer chain[109]. The integrations of these three types of protons gave a ratio of 1:1:2 in that order. The proton and deuterium exchange appeared to be quantitative after stirring of the solution several hours before the spectrum was taken. When DMSO-$\underline{d}_6$ was used as the solvent, the acidic proton could be seen as a broad peak at $\delta \sim 10.0$ ($\tau \sim 0.0$). The other two types of protons appeared at relatively the same positions as in deuterium oxide.

TABLE VI

INFRARED ABSORPTIONS OF POLYMERS I - VII

I cm^{-1}	II cm^{-1}	III cm^{-1}	IV cm^{-1}	V cm^{-1}	VI cm^{-1}	VII cm^{-1}	Assignment*	Description
~3060	~3030	~3010	~3050	~3000	~3010	-----	O-H	broad, strong
2920	2941	2900	2900	2924	2910	2941	C-H	sharp, strong
1704	1698	1700	1709	1704	1700	-----	C=O	sharp, strong
1433	1439	1440	1449	1447	1440	1470	C-H scissoring	broad, medium
1403	1410	1400	1420	1416	----	-----	O-H bending	broad, weak to medium
----	----	----	----	1374	1366	1380	$(CH_3)_2$-C	doublet to shoulder, weak
~1239	~1238	~1200	~1271	~1270	1270	-----	C-O	broad, strong to medium
~1175	~1176	~1170	~1176	~1180	~1170	~1168	C-C	broad, strong to weak
----	----	----	----	----	~1090	~1090	C-O-C asym.	broad, strong

*The assignments are for stretching vibrations unless specified otherwise.

The NMR spectrum of poly(isobutyl vinyl ether) (VII) in carbon tetrachloride showed a distinctive doublet at δ 0.91 (τ 9.09) with a coupling constant of 6 cps. The absorptions of other protons all merged into two broad peaks around δ 3.41 - 3.09 (τ 6.59 - 6.91) and δ 1.69 (τ 8.31)[110].

For the AA-IBVE copolymers (II-VI), a broad peak for the acidic proton was observed in DMSO-$\underline{d}_6$ at $\delta \sim 12.0$ ($\tau \sim -2.0$) and a doublet at δ 0.9 - 1.25 (τ 9.09 - 8.75), with intensity ratios corresponding to the polymer compositions. The methine and methylene protons were all observed as broad peaks near δ 3.0 - 3.5 (τ 7.0 - 6.5) and δ 1.5 - 3.0 (τ 8.5 - 7.0) respectively.

The compositions of poly(itaconic acid) (VIII) and itaconic acid-acrylic acid copolymer (IX) were calculated from their elemental analyses and then checked by NMR analysis.

The composition of VIII as calculated from its elemental analysis is $((C_5H_6O_4)_{1.0} \cdot (H_2O)_{0.4})_n$. The elemental content calculated from this formula is C, 42.08%; H, 5.19%; and O, 52.73%; the values observed are C, 40.66%; H, 5.01%; and O, 54.33%. The equivalent weight obtained from this formula is 71.3.

The NMR spectrum of VIII in deuterium oxide shown in Figure 1(A) gives the ratio of H-OD to other protons of 3.3:4.0, compared to the ratio calculated from elemental analysis of 3.4:4.0.

The difficulty of complete removal of water from poly(itaconic acid) has long been known[97]. The presence of water in VIII can also be noticed from its NMR spectrum in DMSO-$\underline{d}_6$ shown in Figure 2(A). The water absorption appeared as a broad hump near δ 5.6 (τ 4.4). The position of water proton absorption was confirmed by adding 5% of distilled water

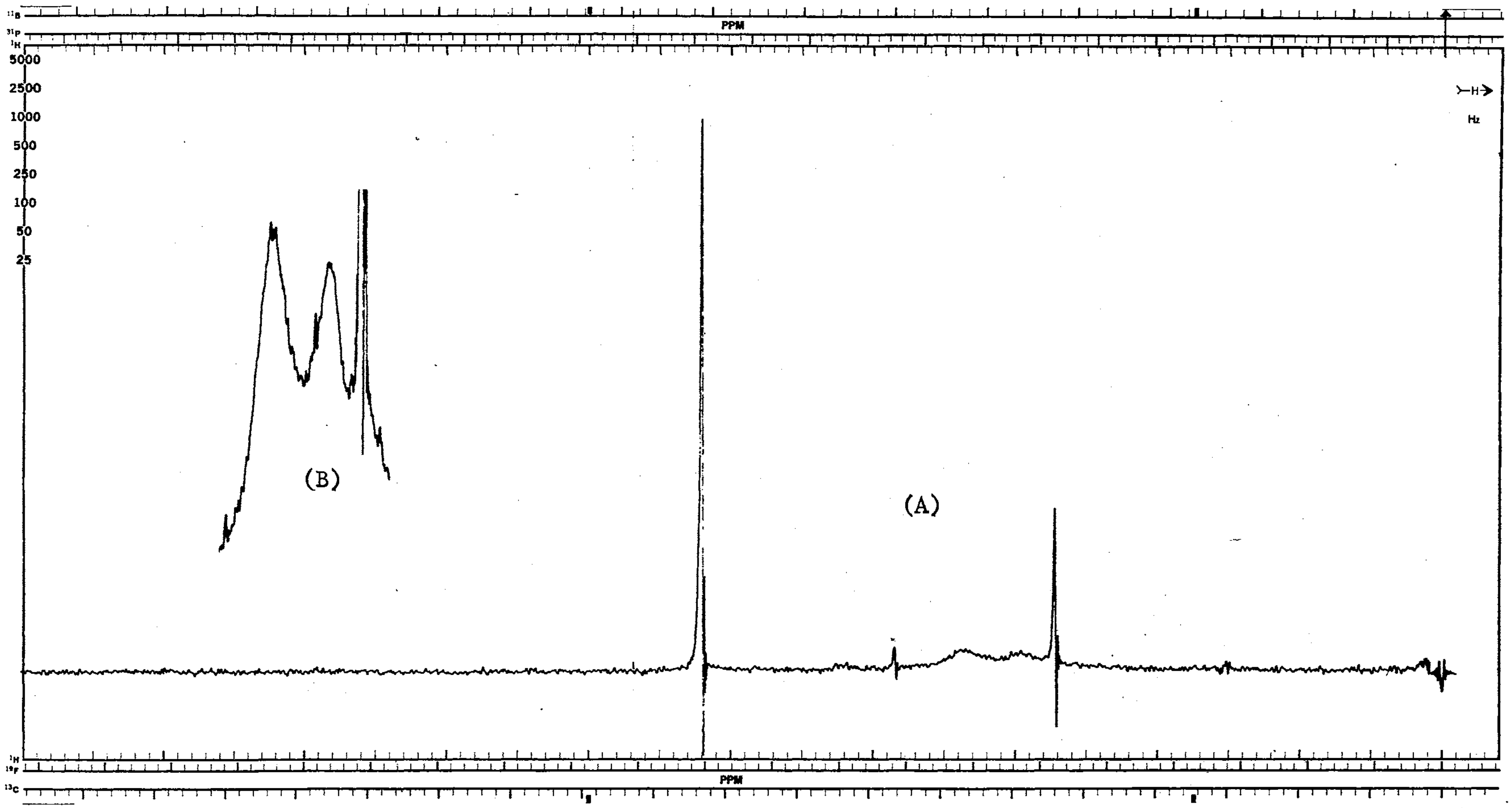


Figure 1. Proton Magnetic Resonance Spectrum of Poly(itaconic acid) (VIII) in D_2O -- 100 MHz

R.F. . . (A) 56, (B) 65 dB S.W. . . 1000 Hz S.A. . . (A) 3.2, (B) 40
F.B. . . 2.0 Hz S.T. . . (A) 250, (B) 500 sec S.O. . . 83701 Hz

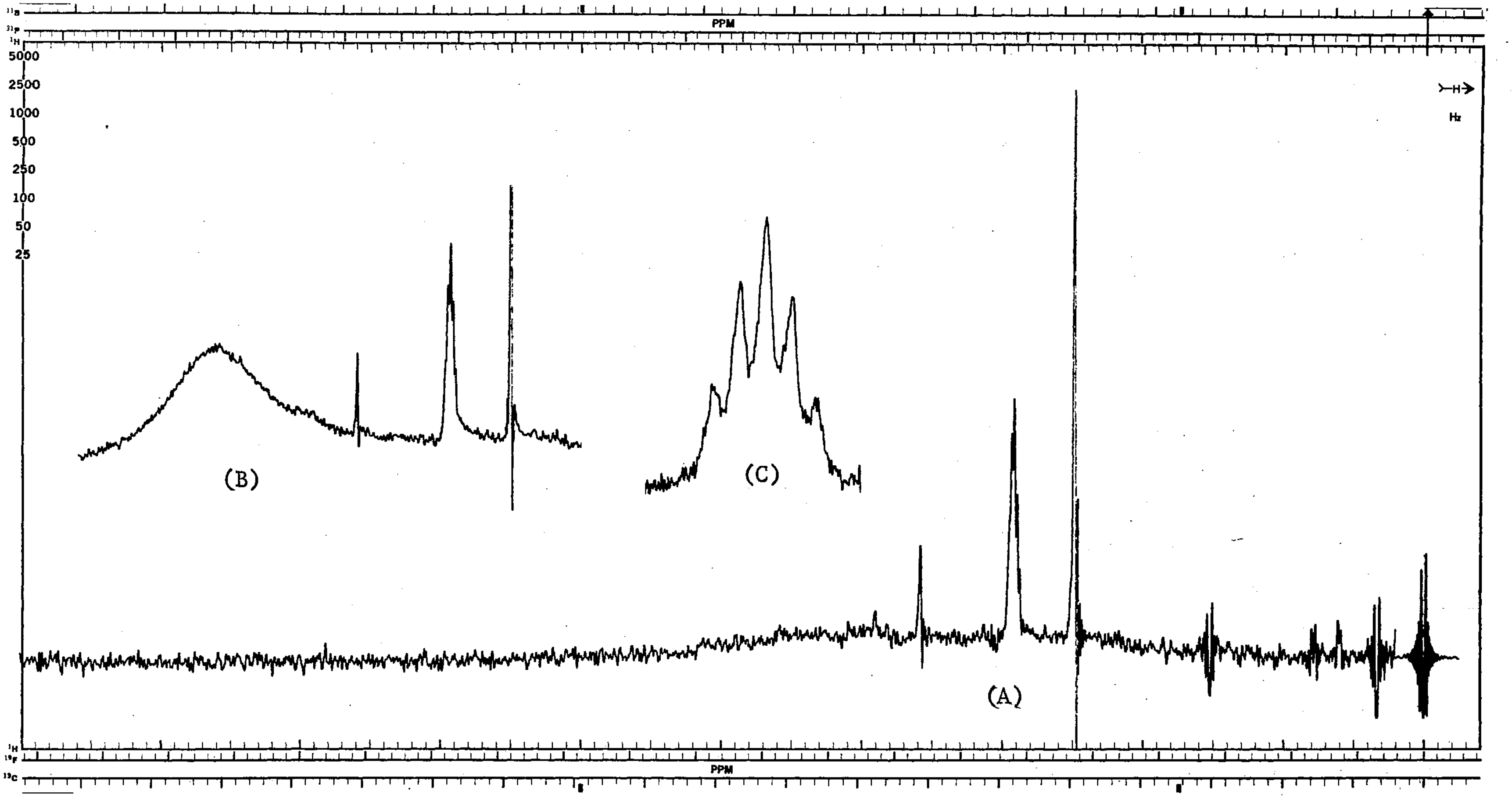


Figure 2. Proton Magnetic Resonance Spectrum of Poly(itaconic acid) (VIII) in DMSO-$\underline{d}_6$ -- 100 MHz

R.F. . . 60 dB   S.W. . . (A) and (B) 1000 Hz, (C) 100 Hz
S.A. . . (A) and (C) 40, (B) 20   F.B. . . (A) and (C) 2.0, (B) 1.0 Hz
S.T. . . (A) and (C) 250, (B) 500 sec   S.O. . . 83701 Hz

to the sample tube shown in Figure 2 (B). Integration of the spectrum shown in Figure 2(A) gave a ratio of about 1.4 water protons to 4 of other protons in the polymer sample.

The sharp singlet at δ 2.72 (τ 7.28) in deuterium oxide and δ 2.50 (τ 7.50) in DMSO-$\underline{d}_6$ of polymer VIII corresponds to the side-chain methylene protons. The two broad peaks at δ 2.96 (τ 7.04) and δ 3.36 (τ 6.64) in deuterium oxide correspond to the methylene protons on the polymer chain. This area was enlarged and is shown in Figure 1(B). These same protons appeared as a multiplet in DMSO-$\underline{d}_6$ at δ 2.92 (τ 7.08) (Figure 2(A) and (C)). These two observations strongly indicate that the polymerization was predominately by way of head-to-head propagation. Otherwise, only singlet absorption would be seen in the NMR spectrum since all methylene protons would be separated.

The IR spectrum of VIII (see Figure 3) shows weak absorptions at 1860 cm^{-1}, 1780 cm^{-1} and 970 cm^{-1} which might be due to a small amount of anhydride linkage[111]. However, the low carbon content of VIII, the NMR spectrum, and the titration result (to be discussed later) do not support anhydride formation in VIII. This phenomenon can result from drying of the IR film at 110°. Poly(itaconic acid) was reported[111] to form anhydride linkages rather easily under heating.

The composition of IX calculated from elemental analysis is $(C_3H_3O_2)_{1.0} \cdot (C_5H_6O_4)_{1.0} \cdot 0.4H_2O$. The elemental content calculated from this formula are: C, 45.89%; H, 5.16%; O, 48.95%; the values observed are: C, 46.62%; H, 5.62%; O, 47.76%. The equivalent weight obtained from this formula is 69.7.

The NMR spectrum of IX in deuterium oxide (Figure 4) gives a proton ratio of H-OD to polymer protons of 3.9 to 7 compared to 3.8 to 7 from

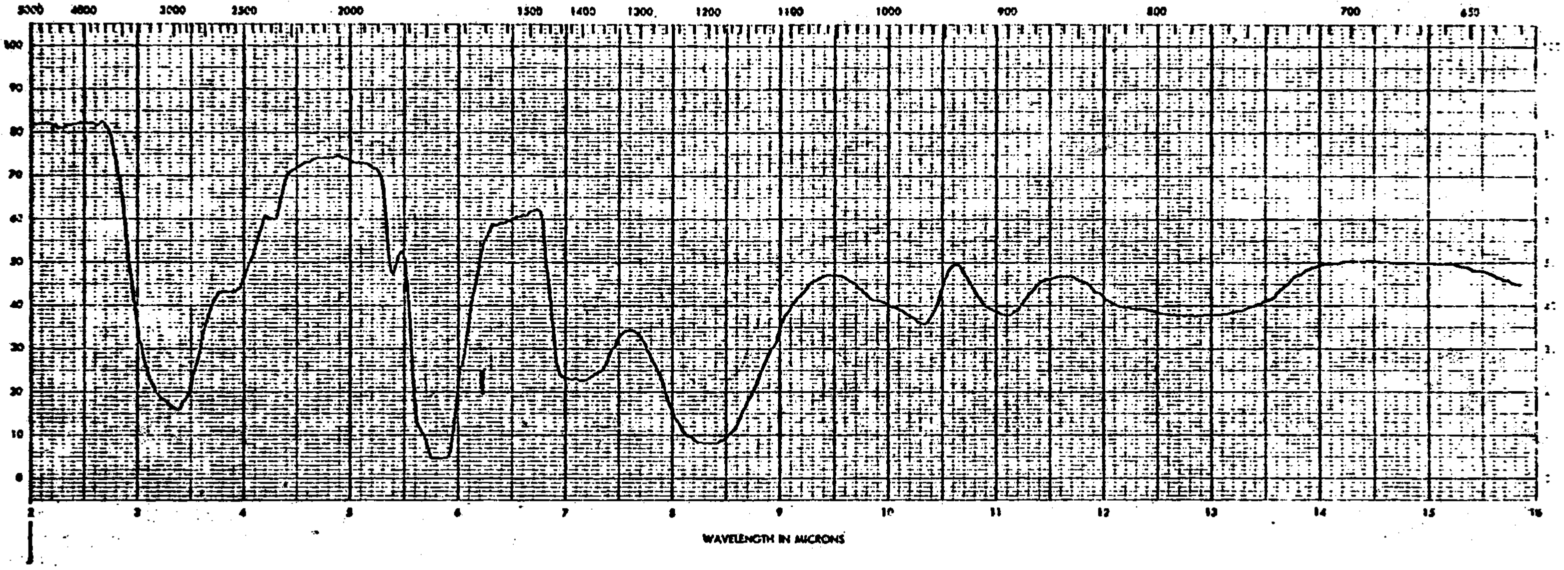


Figure 3. Infrared Spectrum of Poly(itaconic acid) (VIII)--Film on Sodium Chloride Plates

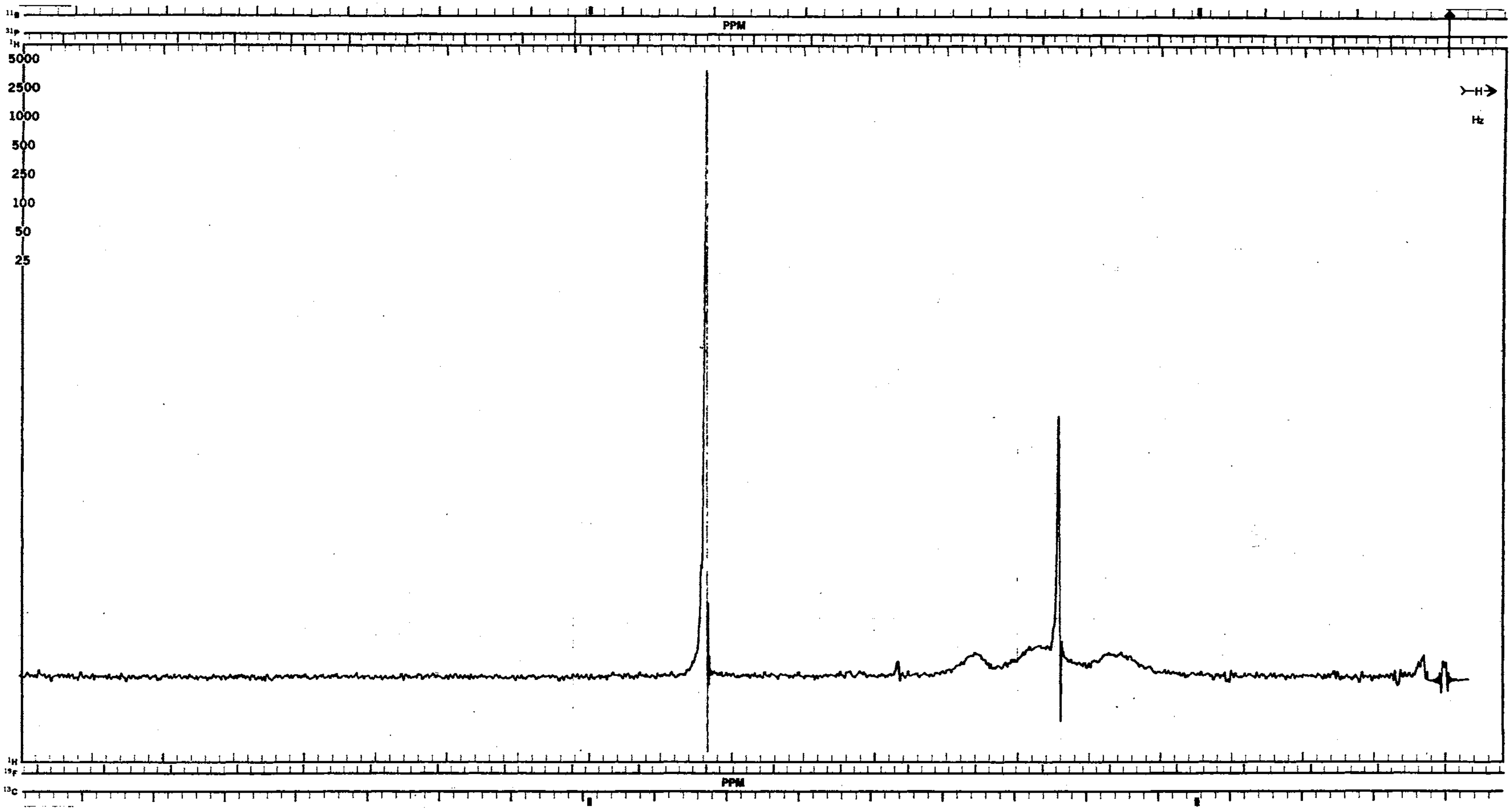


Figure 4. Proton Magnetic Resonance Spectrum of Acrylic Acid-Itaconic Acid Copolymer (IX) in D_2O -- 100 MHz

R.F.	59 dB	S.W.	1000 Hz	S.A.	32
F.B.	2.0 Hz	S.T.	250 sec	S.O. .	83701 Hz

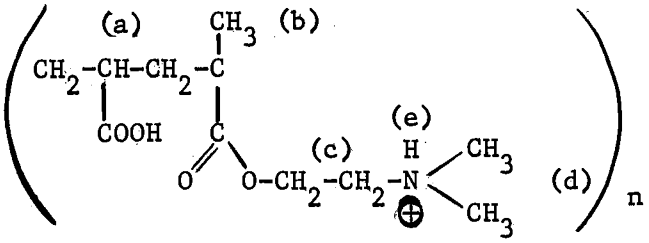
the proposed composition. A multiplet at δ 2.91 (τ 7.09) is also seen in the NMR spectrum in DMSO-$\underline{d}_6$ (Figure 5). The additional broad peak at δ ~ 2.0 for IX as compared to VIII results from the methylene protons on acrylic acid, since it was absent in the spectrum of VIII.

The IR spectrum of IX again may show a small degree of anhydride linkage (Figure 6). By the same reasoning as for VIII, this could be due to the drying of the IR film at 110°.

The composition of acrylic acid-N,N-dimethylaminoethyl methacrylate copolymer (X) was calculated from its NMR spectrum (Figure 7) in formic acid. It is found that the ratio is 1.0 to 1.0 of acrylic acid to N,N-dimethylaminoethyl methacrylate. The description of the NMR spectrum and assignments of the peaks are summarized in Table VII.[106]

TABLE VII

THE PROTON MAGNETIC RESONANCE SPECTRUM AND PEAK ASSIGNMENTS OF POLYMER X



δ	τ	Description	Integration	Assignment
1.8-2.6	7.4-8.2	broad	5 H	H_a
1.26	8.74	broad	3 H	H_b
~3.8	~6.2	broad	4 H	H_c
3.22	6.78	doublet J=9 cps	6 H	H_d
4.52	5.48	broad	~1 H	H_e

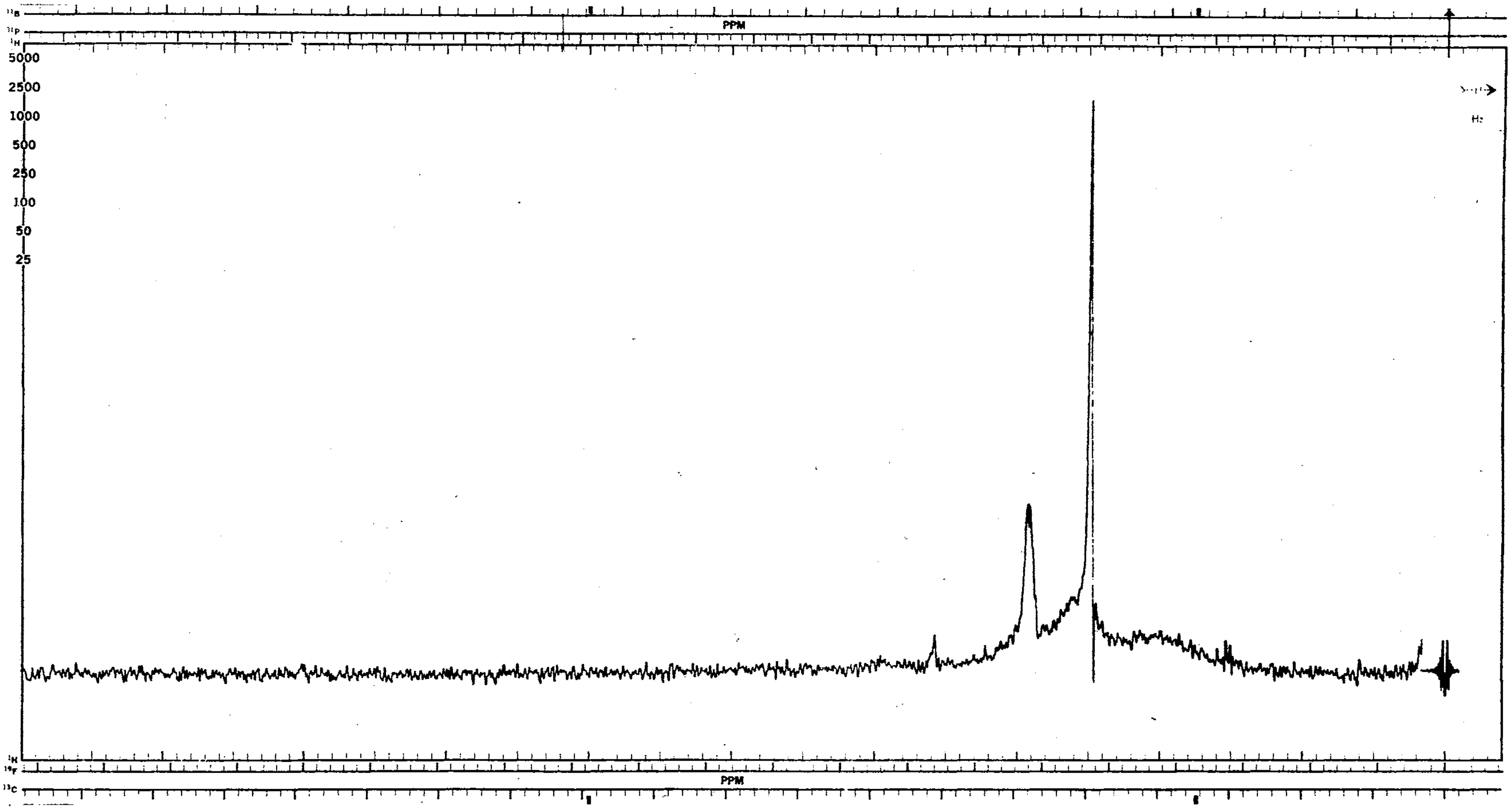


Figure 5. Proton Magnetic Resonance Spectrum of Acrylic Acid-Itaconic Acid Copolymer (IX) in DMSO-d_6 -- 100 MHz

R.F. . . 60 dB	S.W. . . 1000 Hz	S.A. . . 32
F.B. . .2.0 Hz	S.T. . . 250 sec	S.O. . . 83701 Hz

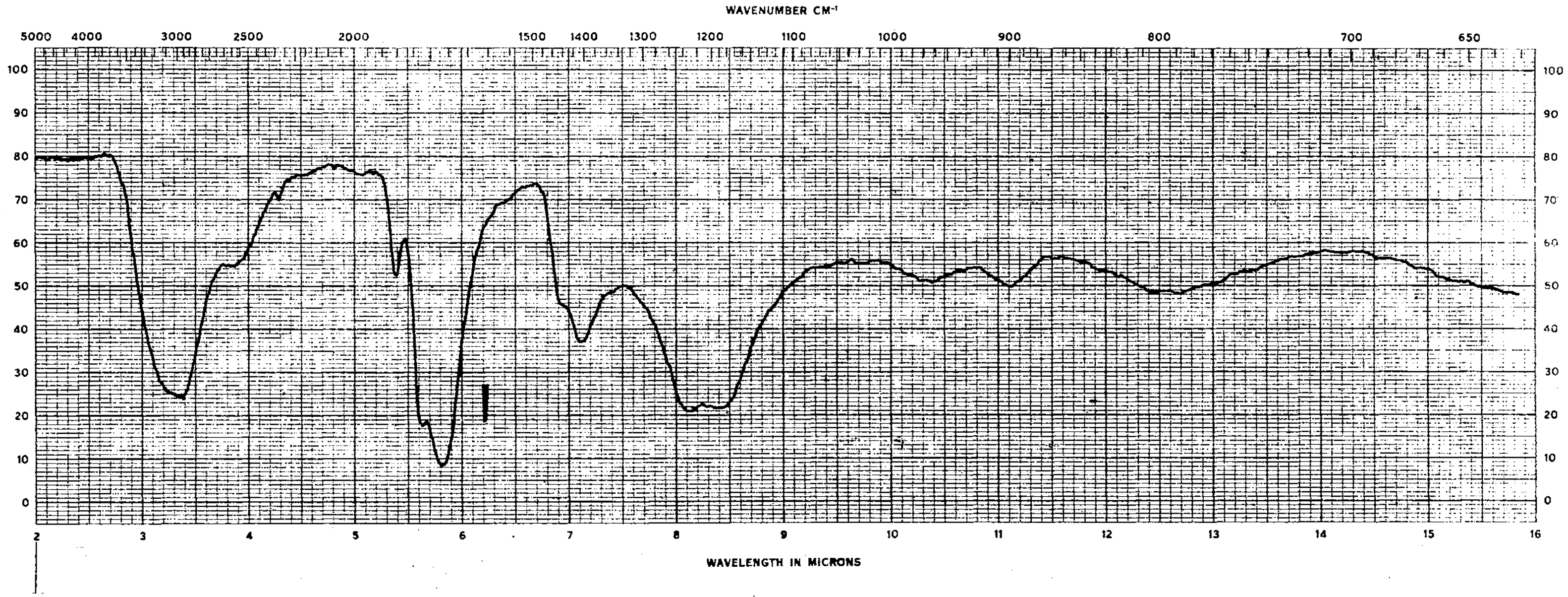


Figure 6. Infrared Spectrum of Itaconic Acid-Acrylic Acid Copolymer (IX)--Film on Sodium Chloride Plates

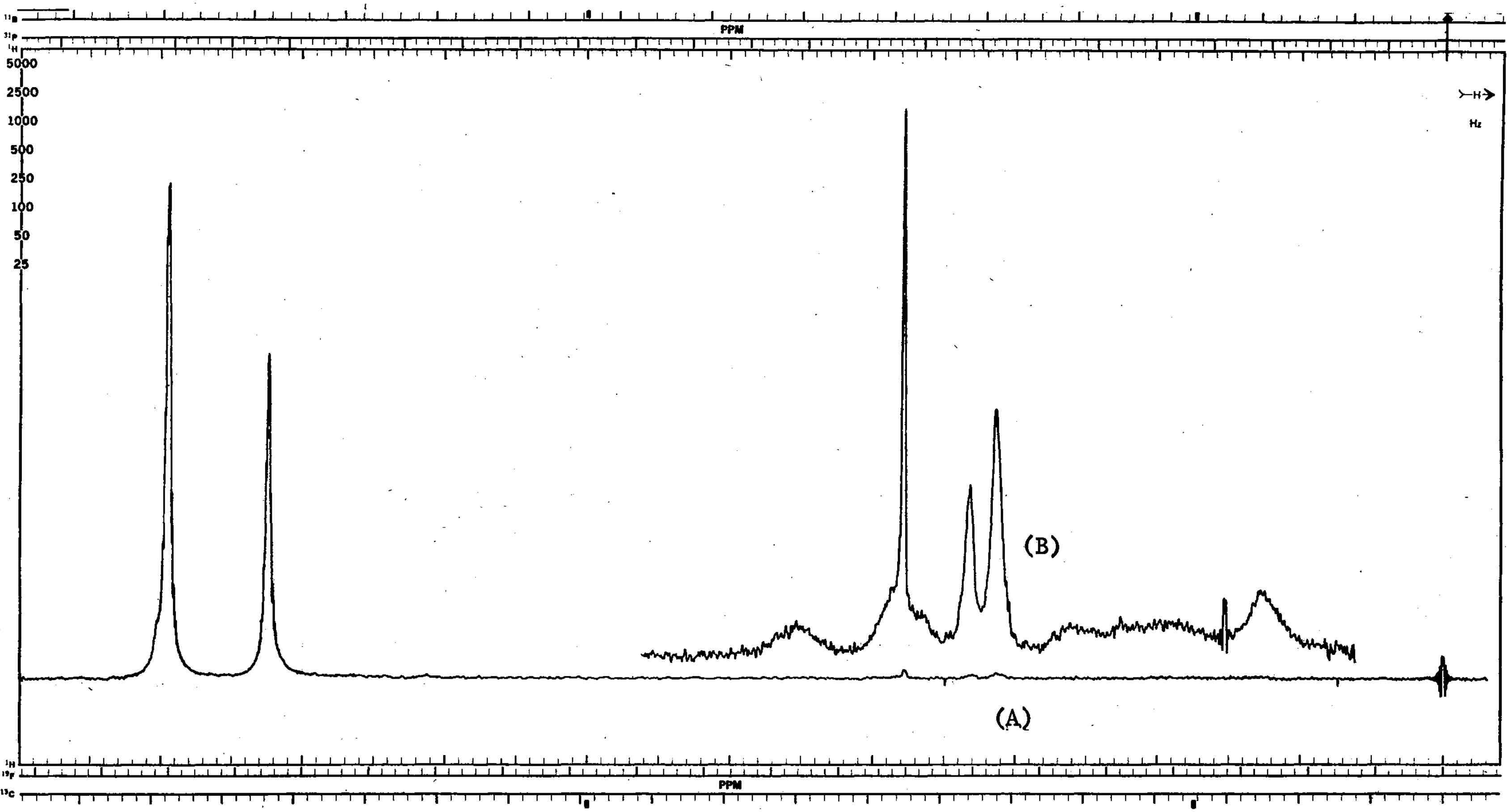


Figure 7. Proton Magnetic Resonance Spectrum of Acrylic Acid-N,N-Dimethylaminoethyl Methacrylate Copolymer -- 100 MHz

R.F. . . (A) 46, (B) 63 dB S.W. . . 1000 Hz S.A. . . (A) 4, (B) 25
F.B. . . (A) 2, (B) 1 Hz S.T. . . 500 sec S.O. . . . . 83701 Hz

The two sharp singlets at δ 8.22 (τ 1.78) and δ 8.98 (τ1.02)[112] and the singlet at δ 3.79 (τ 6.21) are the solvent absorptions. All these three peaks were confirmed by comparing to the spectrum of a solvent sample.

The composition of Polymer XI (acrylic acid-isooctyl vinyl ether copolymer) was calculated from titration data in the same manner as described for the AA-IBVE copolymers. The equivalent weight was found to be 95.1; the isooctyl vinyl ether to acrylic acid ratio in monomers used was 0.46 to 1.0; and the ratio of monomer units in the polymer was 0.15 to 1.0.

The NMR spectra of poly(methyl acrylate) prepared by two different methods are shown in Figure 8. Polymer XII was prepared by polymerizing methyl acrylate (Figure 8 (A)), and Polymer XIII was prepared by methylating poly(acrylic acid) (I) (Figure 8 (B)). These two polymers have exactly the same IR absorptions. The two NMR spectra agree very well in the general absorption patterns and in proton integrations. A minor difference is noted in that a sharp singlet appears in Figure 8 (A) at δ 2.12 (τ 7.88), which is absent in Figure 8 (B). This singlet is attributed to higher isotacticity which was produced through predominately trans opening of the double bond[113-117] Methyl acrylate, with a larger side chain, methoxycarbonyl, as compared to the carboxyl group in acrylic acid, would be subject to more stereoregular polymerization.

Potentiometric Titrations

The titration curves were all constructed by plotting pH vs α (degree of ionization). The α was calculated as the ratio of titrant added at a particular pH to that required for the complete neutralization

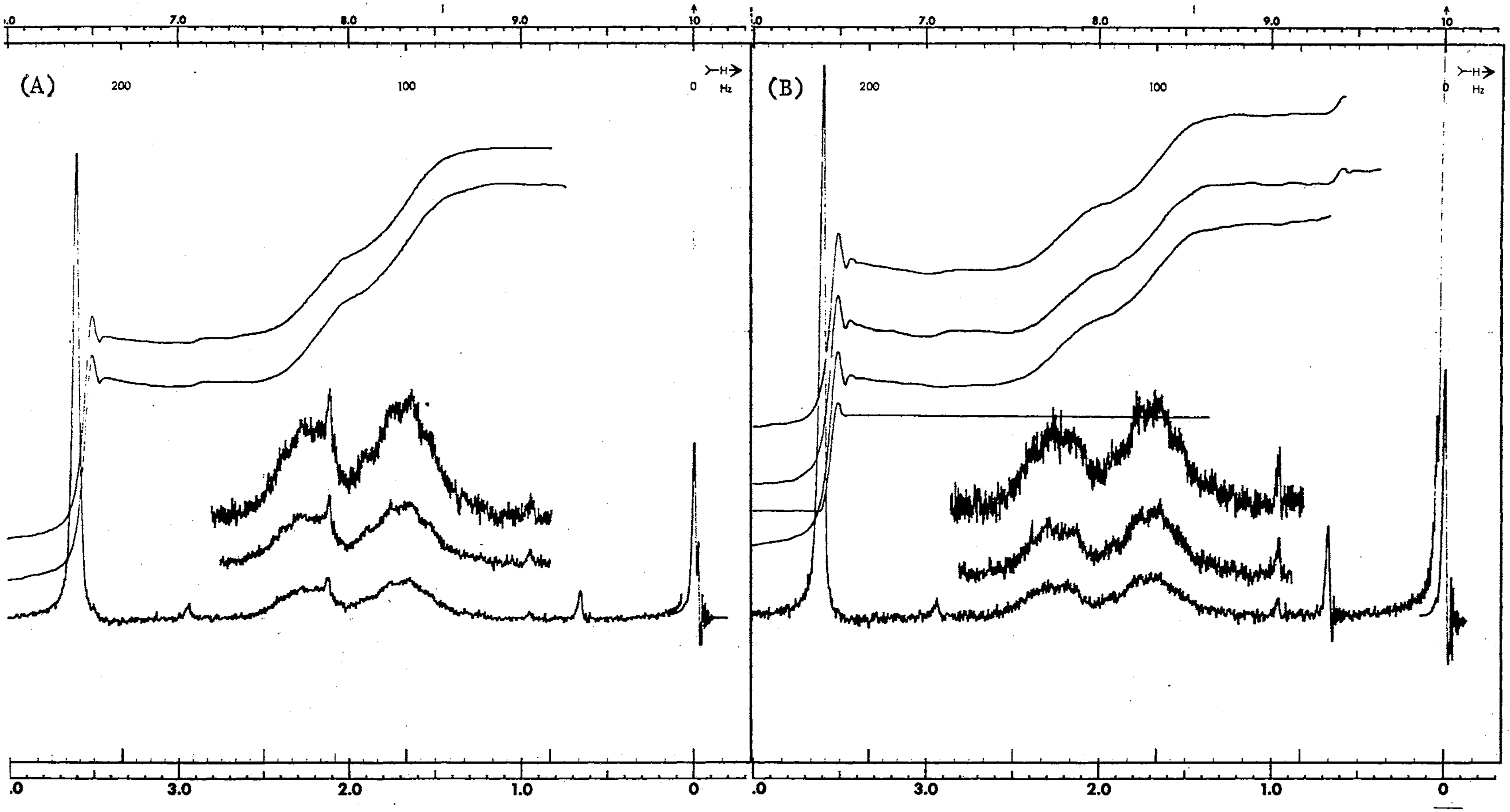


Figure 8. Proton Magnetic Resonance Spectra of Poly(methyl acrylate) in $CDCl_3$: (A) Polymer XII, Polymerization of Methyl Acrylate, (B) Polymer XIII, Methyl Ester of Polymer I

R.F. . . 0.05 mG S.W. . . 500 Hz S.A. . . (A) 5, (B) 10 F.B. . . 4.0 Hz
S.T. . . 250 sec S.O. . . 008 Hz I.A. . . . 80

(estimated from the end point). Also, the plots of pH vs $\log(1-\alpha)/\alpha$ of α ranges from 0.7 to 0.3 are attached. These plots were done in such a way that each curve was offset by one pH unit to give a better comparison of the shapes of the curves.

Figure 9 shows the titration curves of Polymers I, II, III, IV, and XI for which the polymers were simply dissolved in water and titrated with standard sodium hydroxide solution at 25° (method 1). Figure 10 shows the titration curves of Polymers I, II, XIV, and XV for which the polymers were dissolved in standard sodium hydroxide solution, then were acidified with hydrochloric acid, and titrated from the acid side with standard sodium hydroxide at 25° (method 2). This method of solution preparation for potentiometric titration was adopted mainly because of the commercial polyacrylate samples (XIV and XV) were in the form of sodium salts*. Polymers I and II were used for comparison purpose. Figure 11 shows the titration curves of Polymers I, III, IV, V, and VI, in which the polymers were dissolved in standard sodium hydroxide solution, and then titrated from the basic side with standard hydrochloric acid solution at 25° (method 3). This method was adopted for potentiometric titration mainly because of solubility difficulties. Polymers V and VI were found to be water-insoluble, but dissolved readily in base. Polymers I, II, III were studied mainly for comparison purpose.

All these titration results follow the expanded Henderson-Hasselbalch equation:

---

*These two commercial samples were tested for metal ions with Varian Techtron Model AA-5 Atomic Absorption Spectrophotometer (manufactured under licensed by Commonwealth Scientific and Industrial Research Organization (CSIRO), Sydney, Australia) and found to contain only metal. Courtesy to Dr. Gordon Wallace.

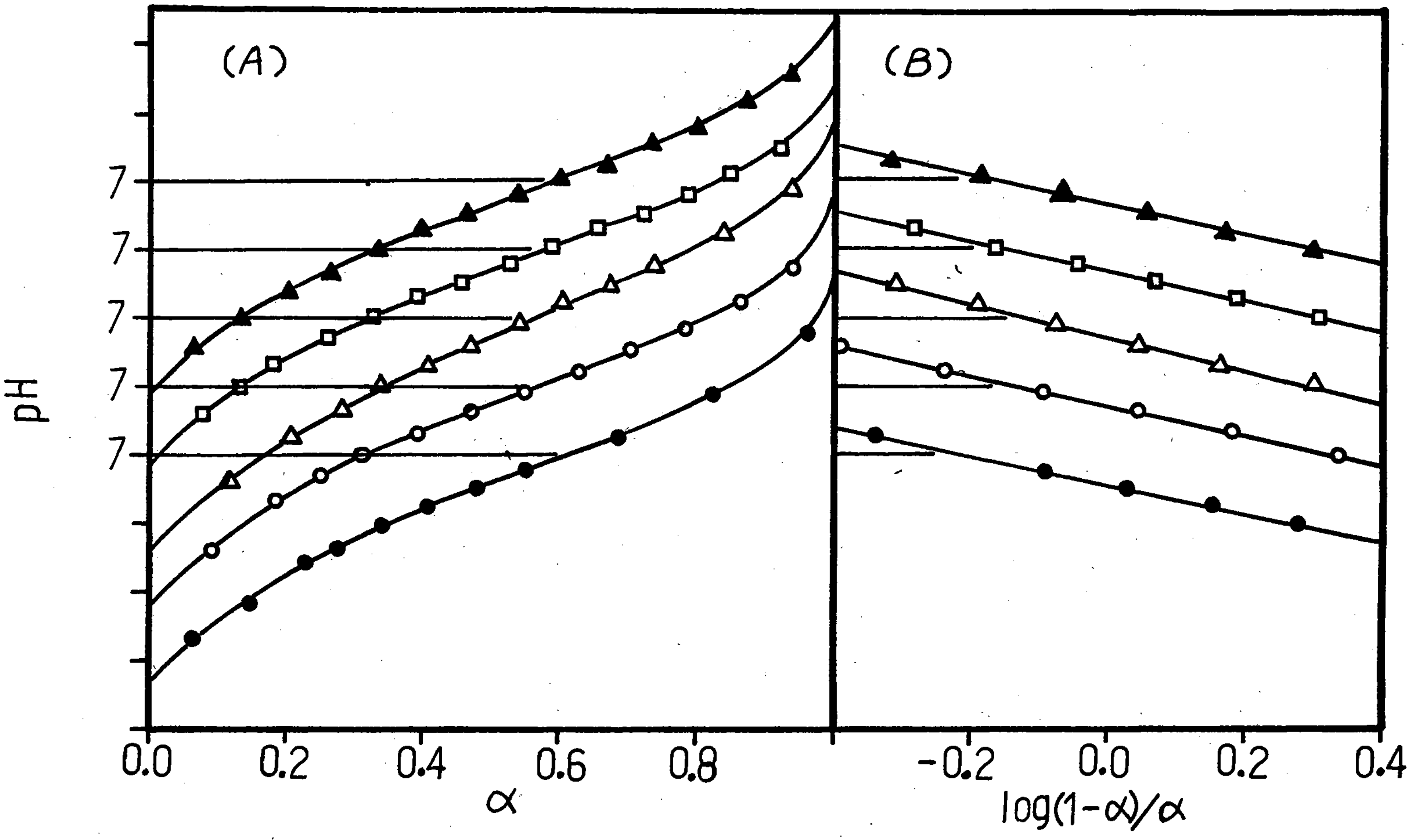


Figure 9. pH vs α (A) and pH vs log(1-α)/α (B), of Polymers I (●), II (○), III (△), IV(□), and XI (▲)

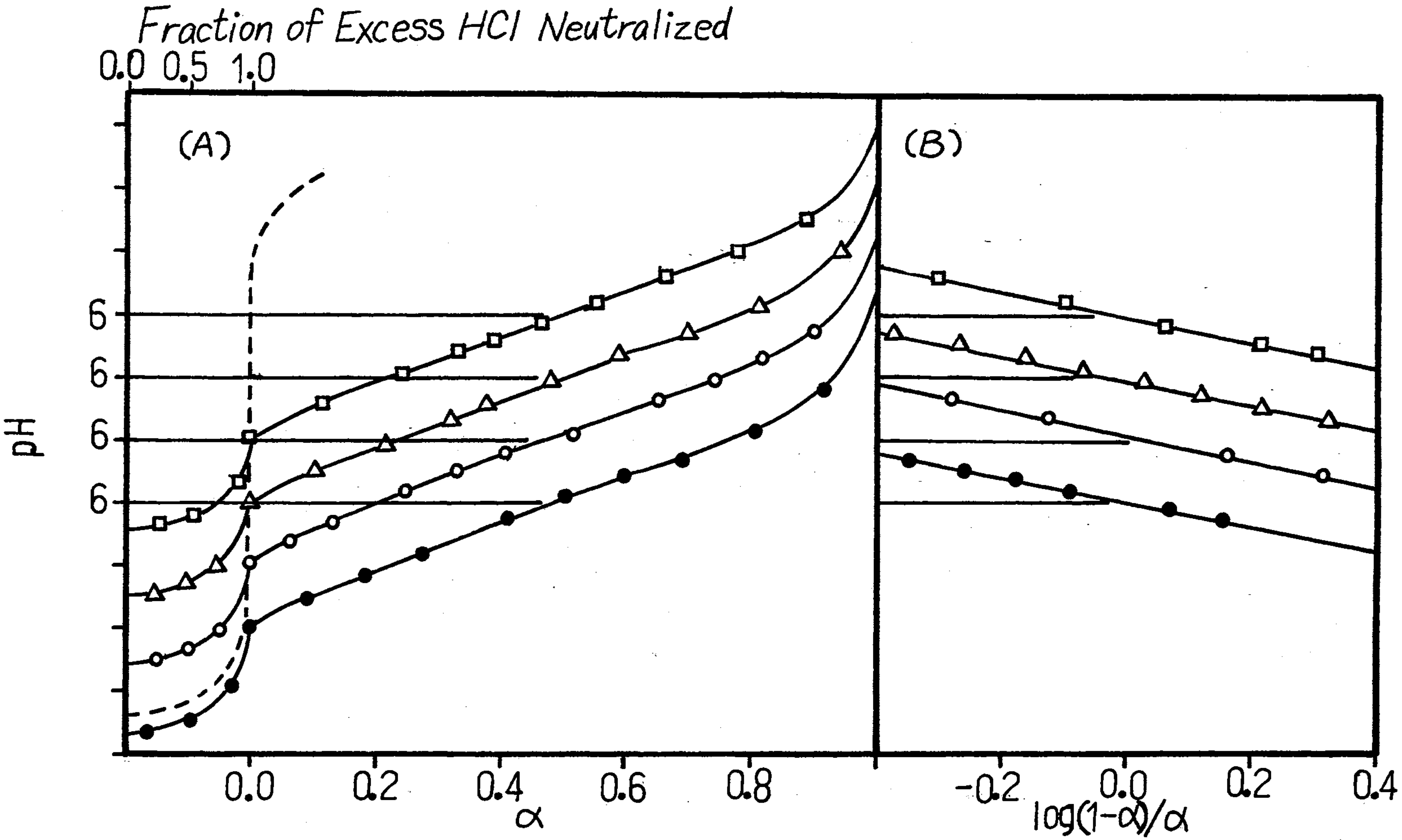


Figure 10. pH vs α (A) and pH vs log(1-α)/α (B), of Polymers I (●), II(O), XIV (Δ), XV (□), and HCl (---)

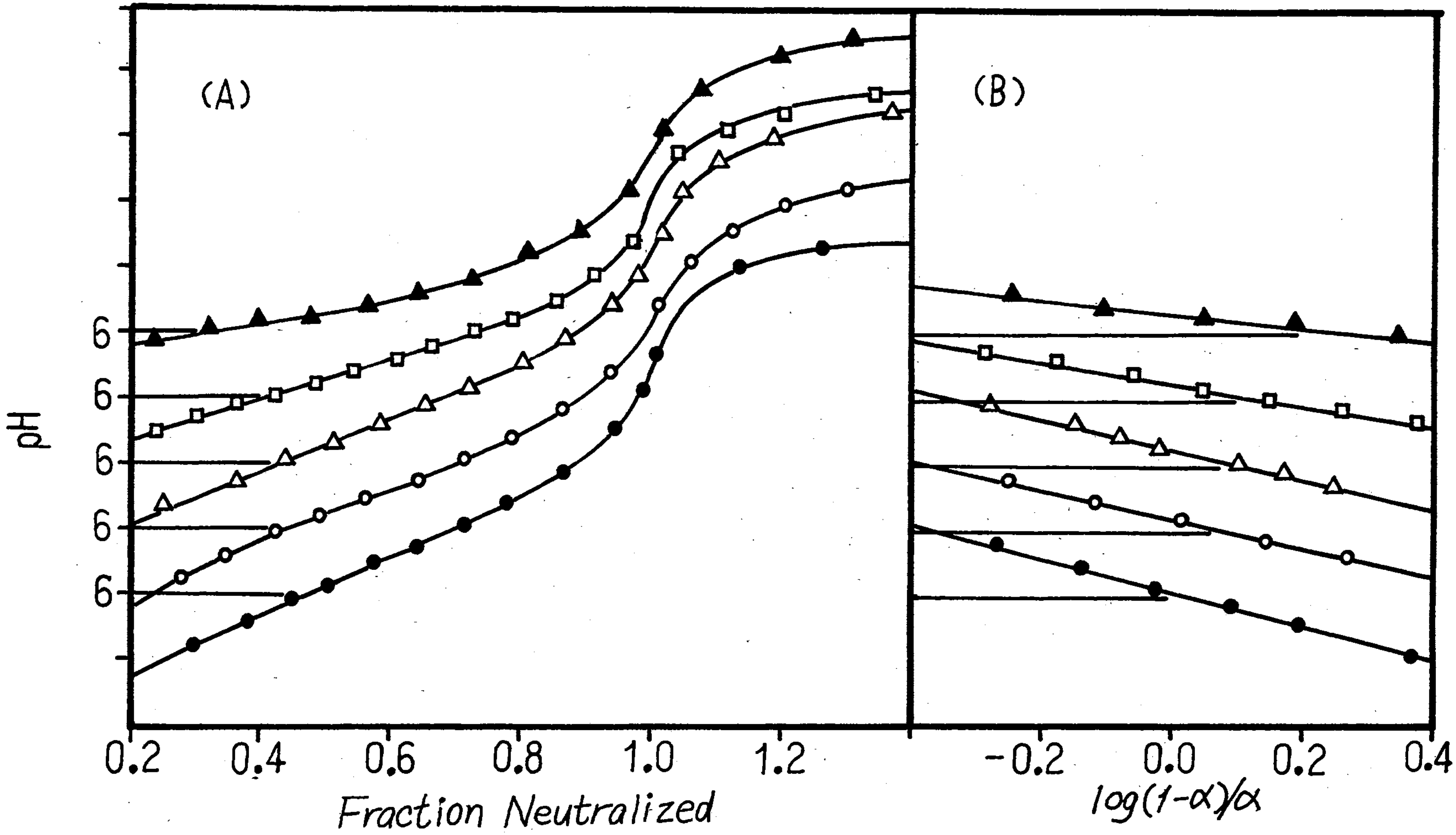


Figure 11. pH vs Fraction Neutralized (A) and pH vs $\log(1-\alpha)/\alpha$ (B), of Polymers I (●), III (O), IV (Δ), V (□) and VI (▲)

$$pH = p\underline{K}_{av} - \underline{n}\log(1-\alpha)/\alpha$$

The parameters in the above equation ($p\underline{K}_{av}$ and $\underline{n}$) for all polymers studied are summarized in Table VIII.

The $p\underline{K}_{av}$ value of Polymer I matches well that reported by Gregor and Frederick[83]. Polymers I, II, III, XIV and XV have about the same $p\underline{K}_{av}$ and $\underline{n}$. Polymers I, XIV and XV are essentially the same kind of polymers. Polymers II, III, and XI have very low vinyl ether content. The resemblance of these latter polymers to poly(acrylic acid) is to be expected. The results shown in the column for method 3 shows a trend of gradual decrease in the acidity of polymers as the vinyl ether content increase (reference to Table V). Also the $\underline{n}$ values of these polymers show a general trend of decreasing in value from Polymer I to VI. As the $\underline{n}$ is a measure of the degree of the additional interactions between -COOH groups[29-36], the further separation of carboxylic groups by the vinyl ethers tends to minimize this additional interaction.

The presence of added salt causes an expected effect on the $p\underline{K}_{av}$ of the polyacids (compare $p\underline{K}_{av}$ of I by the three different methods): the $p\underline{K}_{av}$ decreases as the concentration of NaCl increases. The concentrations of NaCl present in solution in methods 2 and 3 did not remain at constant level. The range of the salt concentration is also tabulated in Table VIII and the qualitative change of $p\underline{K}_{av}$ can be observed.

The results of the potentiometric titration of Polymer VIII are plotted in Figure 12. The equivalent weight of this polymer as calculated from its elemental analysis and confirmed by NMR study is 71.3. The total equivalents of acid in the sample for potentiometric titration was calculated from this equivalent weight. The α was estimated by

TABLE VIII

HENDERSON-HASSELBALCH PARAMETERS FOR TITRATIONS OF POLYMERS I-VI, XI, XIV, AND XV

Method of Solution Preparation	Method 1		Method 2		Method 3	
Concn. of NaCl, N	--------		0.013-0.026		0.00-0.013	
Polymer	pK_{av}	n	pK_{av}	n	pK_{av}	n
I	6.58	2.13	6.02	2.00	6.11	2.29
II	6.69	2.21	6.06	1.98	----	----
III	6.70	2.43	----	----	6.17	2.14
IV	6.67	2.13	----	----	6.24	2.19
V	----	----	----	----	6.28	1.75
VI	----	----	----	----	6.27	1.00
XI	6.63	2.15	----	----	----	----
XIV	----	----	5.94	1.95	----	----
XV	----	----	5.96	1.93	----	----

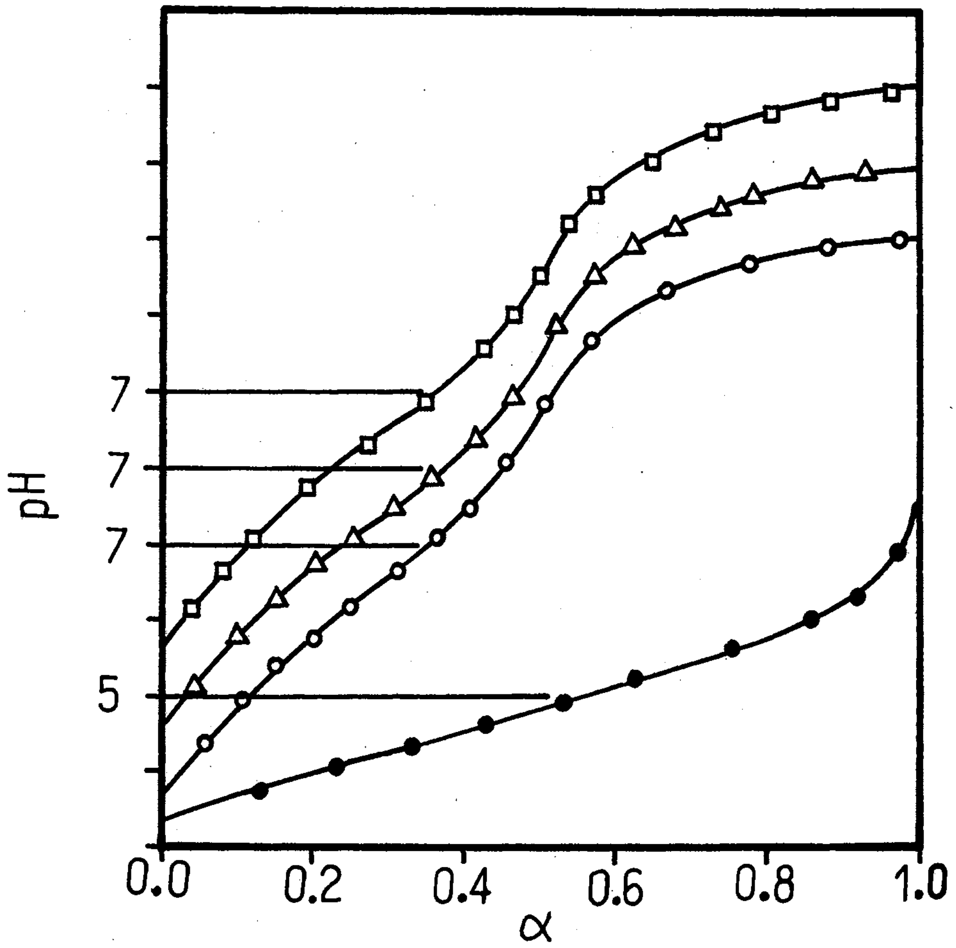


Figure 12. pH vs α. Polymer VIII Titrated with Sodium Hydroxide (O), Potassium Hydroxide (Δ), and Potassium Hydroxide after Three Hours of Heating (□); Itaconic Acid Monomer Titrated With Sodium Hydroxide (●)

dividing the added amount of base by the amount of base required for complete neutralization. The titration curve constructed by plotting pH vs α thus calculated, shows an end-point break at half neutralization.

Gregor and Frederick[83] observed that different cations have different binding ability toward polyanions, and when different alkali metal bases were used for titration, the titration curve of the polyacid may vary its shape due to this difference in binding. Polymer VIII was titrated with sodium hydroxide and with potassium hydroxide in the hope that if a change in the shape of the titration curve did occur, this might lead to an indication of another endpoint break at the theoretical equivalent weight. However, the same titration curve was observed in both cases.

Despite its low carbon content, Polymer VIII was tested for the possibility of anhydride formation by heating its water solution for three hours at 70° under nitrogen and then titrating with potassium hydroxide. Still, the same titration curve was obtained. This leads to the conclusion that the poly(itaconic acid) has two end points in its titration curve and the second end point appears at a fairly high pH which can not be seen in the titration with water as solvent.

The titration curve of an acid containing two different types of carboxyl groups will ordinarily show two end point breaks corresponding to each of the two different acid groups[92]. However, cases are known for which the titration curve shows only one end point break corresponding to one type of the acid group, the second end point being smeared out at high pH values[44,118,119].

Usually, when two different acid groups are present in an acid it possesses different p$\underline{K}$'s, which would be represented by two end point breaks about four pH units apart[44]. The end point break appearing in Figure 12 is at pH 8.5. The second end point would then be at around pH 12, which is pretty well in the solvent (water) buffer region and should not be seen.

From the NMR analysis of Polymer VIII (Figure 1 and 2), it is found that poly(itaconic acid) was formed predominately through head-to-head propagation. This arrangement places carboxyl groups at close proximity to each other. The interaction and H bonding are expected to be very strong. This could be the reason why the second type carboxyl group tends to dissociate at such a high pH.

The titration results of Polymer IX are plotted in Figure 13. The equivalent weight of 69.7 used for the calculation of α was estimated from its composition. The plot of pH vs α with either sodium hydroxide or potassium hydroxide as the titrant, showed an end point break at α about 67%. After the water solution was heated at 70° under nitrogen for three hours, the same titration curve was obtained, which indicated no anhydride linkages present in this copolymer.

Polymer IX was found to contain a 1 to 1 ratio of itaconic acid and acrylic acid units. This will give three different carboxyl groups per repeating unit of the polymer. It has already been noted that of the two carboxyl groups in each repeating unit of Polymer VIII only one end point break is shown in the titration curve. On this basis, for each repeating unit in Polymer IX, two carboxyl groups out of the three should give end point break in the titration curve (one from the itaconic acid and the other from acrylic acid). The net result will be a titer corresponding to two-thirds of the total carboxyl groups present. This is exactly what was observed.

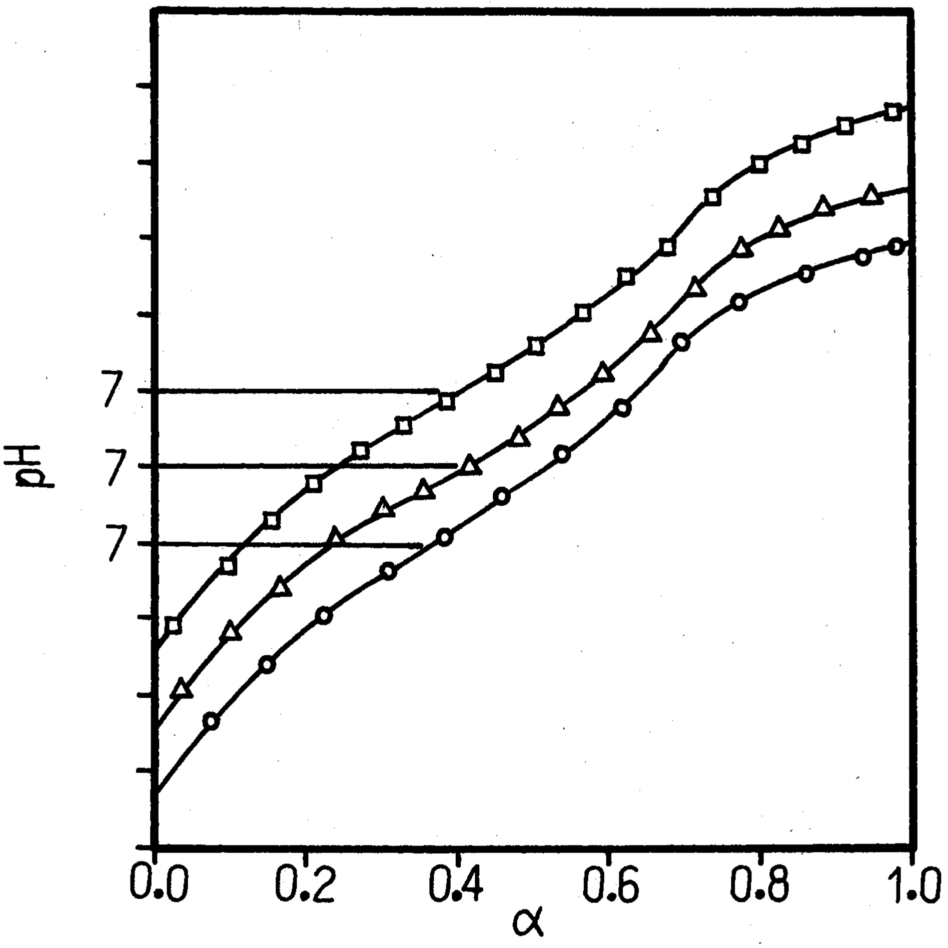


Figure 13. pH vs α of Polymer IX Titrated With Sodium Hydroxide (O), Potassium Hydroxide (Δ), and Potassium Hydroxide After Three Hours of Heating (□)

Viscosity Measurements

The viscosities of the polymer solutions were calculated according to the following equations[120]:

$$\eta_{rel} = \eta_1/\eta_0 = \rho_1 \underline{t}_1/\rho_0 \underline{t}_0$$

where η_1 is the viscosity of polymer solution,

η_0 is the viscosity of solvent,

ρ_1 is the density of polymer solution,

$\underline{t}_1$ is the efflux time of polymer solution,

ρ_0 is the density of solvent,

$\underline{t}_0$ is the efflux time of solvent.

$$\eta_{sp} = \eta_{rel} - 1$$

$$\eta_{red} = \eta_{sp}/\underline{c}$$

where $\underline{c}$ is the concentration of polymer solution in g/100 ml.

In most cases investigated, the polymer solutions employed for the viscosity measurements had concentrations much less than 1 g/100 ml. The density correction in these dilute solution is insignificant and can be neglected. The η_{rel} can be approximated as t_1/t_0[62,63]. Also, the viscometer was so chosen that the solvent efflux time was longer than 100 seconds. The kinetic correction can then be neglected also[51].

The molecular weight of Polymer I in dioxane at 30° determined from the Mark-Houwink equation ($[\eta] = \underline{K}\cdot M^{\underline{a}}$) with constant $\underline{K} = 8.5 \times 10^{-4}$; $\underline{a} = 0.5$[121] is 2.5×10^5 (Figure 14). The molecular weights of Polymers

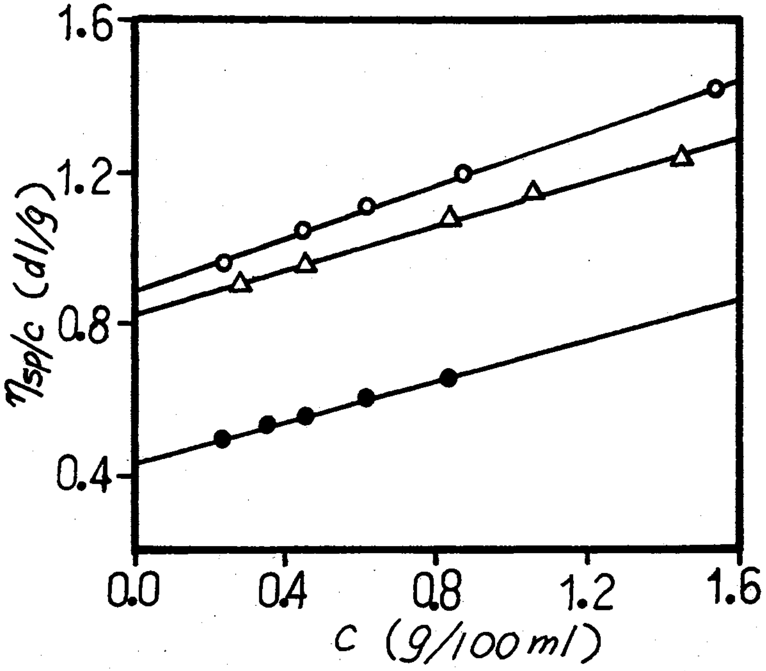


Figure 14. Viscosity of Polymer I (●) in Dioxane at 30°, XII (Δ) and XIII (O) in Acetone at 20°

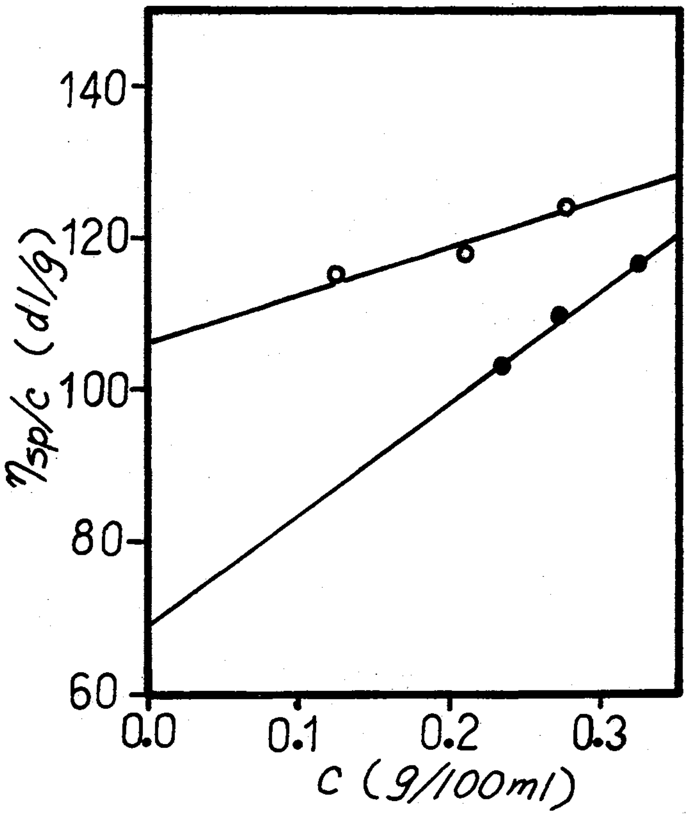


Figure 15. Viscosity of Polymers XIV (●) and XV (O) in Water at pH 7.0 and 25°

XII and XIII in acetone at 20° determined with $\underline{K} = 5.6 \times 10^{-3}$, and $a = 0.75$[122] with $[\eta]$ in ml/g is 3.8×10^5 for XII and 4.0×10^5 for XIII (see Figure 14).

The number average molecular weights of Polymers XII and XIII determined with the Coleman 15 Molecular Weight Apparatus by measuring the vapor pressure difference of solvent and solutions are 2.33×10^4 for XII and 2.15×10^4 for XIII. The difference between the number-average molecular weight (M_n) and the weight-average molecular weight (M_w) for XII is $M_n/M_w = 0.06$, and for XIII is $M_n/M_w = 0.05$. These fairly large differences between M_n and M_w indicate a large dispersion of molecular weights of these two polymers[123].

The viscosity of polyelectrolytes in water solution in the presence of added salts has been found to have the same behavior as in a non-ionizing solvent[124]. Polymers XIV and XV, owing to the presence of sodium ion, show a normal viscosity plot for regular polymers (Figure 15). The molecular weights of these two polymers estimated with the same constants for dioxane solvent are in the neighborhood of 7.0×10^9 for XIV and 1.6×10^{10} for XV.

The viscosities of other polyanions in water solutions at 25° were found to fit the Fuoss equation best

$$\eta_{sp}/\underline{c} = \underline{A}/(1 + \underline{B}\sqrt{\underline{c}} + \underline{D},$$

In all cases studied, the D value found can be neglected (i.e., $\underline{D} = 0.0$). A plot of $\underline{c}/\eta_{sp}$ vs $\sqrt{\underline{c}}$ gave a straight line, with the slope equal to $\underline{B}/\underline{A}$ and the intercept equal to $1/\underline{A}$. The $\underline{A}$ and $\underline{B}$ values thus determined are tabulated in Table IX for those polymers studied in water at a defined pH.

TABLE IX

PARAMETERS OF THE FUOSS VISCOSITY EQUATION FOR POLYMERS I-VI, VIII, IX, AND XI IN WATER AT 25°

Polymer	pH	α	A	B	Molecular Weight x 10^{-4}	Figure
I	3.8	0.0	--	--	---	16
II	3.8	0.0	--	--	---	17
VIII	3.7	0.0	--	--	---	17
IX	3.7	0.0	--	--	---	17
I	7.0	0.62	200	8	25	16
II	7.0	0.58	200	10	25	18
III	7.0	0.56	31	11	8.5	18
IV	7.0	0.58	200	16	25	18
V	7.0	0.73	200	30	25	18
VI	7.0	0.77	55	12	12	18
VIII	7.0	0.35	33	10	8.7	17
IX	7.0	0.36	10	7.3	4.4	17
XI	7.0	0.59	12.5	1.1	4.9	19
I	10.2	1.0	12.5	11.5	---	16

The variable A is the limiting value of the reduced viscosity (η_{red}) at infinite dilution[56]. It was found to be a function of the molecular weights of polyelectrolytes[54]. Liberti and Stivala found that A for heparin is proportional to $M^{1.59}$ (M is the molecular weight)[60]. Strauss and Smith found that A is proportional to $M^{1.87}$ for polyphosphate[58]. The order of proportionality for the molecular weights of the acrylic-acid-containing polymers is not known. However, an order of close to 2 is to be expected[56].

For the purpose of a simple qualitative comparison, a value of 1.7 is assigned as the order of the molecular weights of acrylic acid polymers which will make them proportional to A. Using the data measured for Polymer I at pH 7.0 and 25°, the proportionality constant relating A and $M^{1.7}$ is 1.3×10^{-7}. The molecular weights of other polymers listed in Table IX were calculated from $A = 1.3 \times 10^{-7} M^{1.7}$. These molecular weights are listed in the Table IX.

It should be pointed out here that this method of estimating the molecular weights of polyelectrolytes is approximate. Confidence in the results is based on the established fact of a linear relationship between A and molecular weight raised to some power close to 2.0. The molecular weights calculated for those copolymers containing small percentages of vinyl ethers should be reasonably correct.

The value of A for Polymer III is relatively small compared to the values of A for other polymers of this series. The difference can not result from the difference in composition but must be due to the lower molecular weight of III as compared to that of other polymers.

Polymer X (acrylic acid-N,N-dimethylaminoethyl methacrylate copolymer) shows a very different viscosity behavior from that of the other

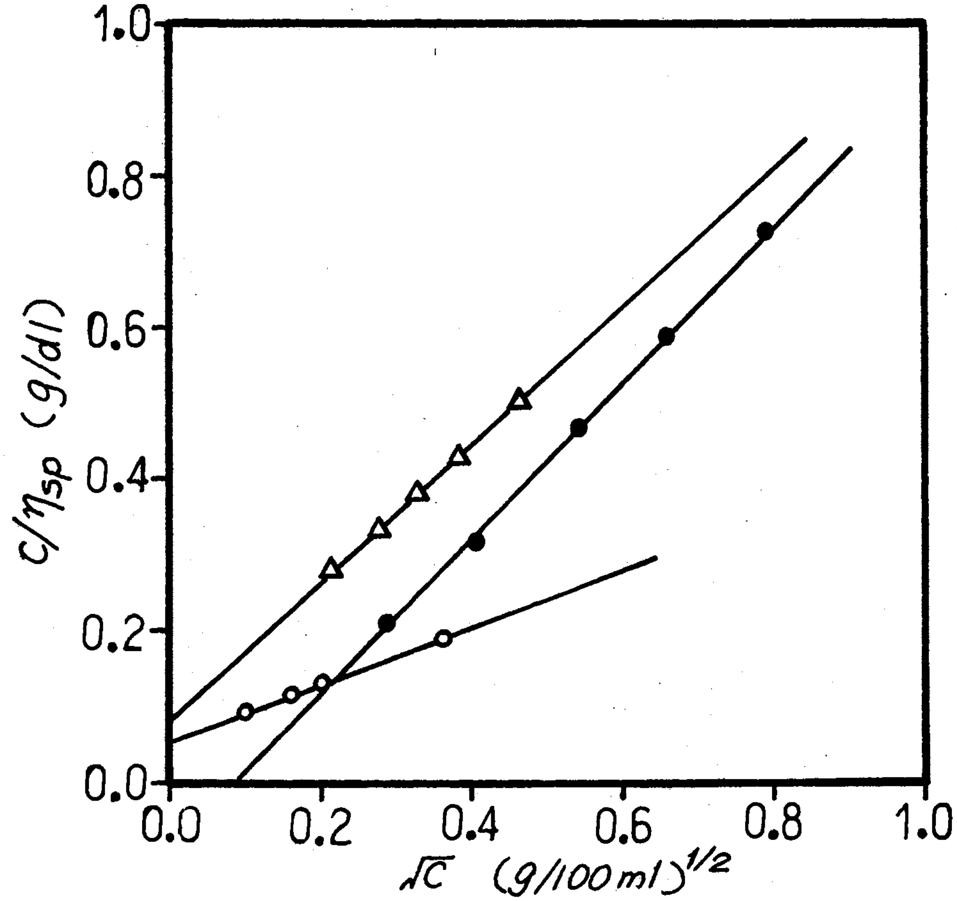


Figure 16. Viscosity of Poly(acrylic acid) (I) in Water (●), in Water at pH 7.0 (O), and in Water at pH 10.2 (Δ) at 25°

polymers (Figure 19), i.e., the plot of $\underline{c}/\eta_{sp}$ vs $\sqrt{\underline{c}}$ is a curve instead of a straight line. Polymer X differs from other polymers owing to its amphoteric nature. The viscosities observed for other polyampholytes, such as methacrylic acid-2-vinylpyridine and methacrylic acid-N,N-diethylaminoethyl methacrylate copolymers, also show a curvature in their viscosity plot[125,126] at concentrations around 0.2 g/100 ml. This phenomenon is due to the coiling of this type of macromolecule at high dilution which results from the intramolecular attraction between the positive and negative centers with the molecule. The molecular weight of Polymer X should be on the order of $10^4 - 10^5$ as compared with that of Polymer I.

Turbidity Measurements

The concentrations, $[M^{+2}]$, of bivalent metal ions (calcium and magnesium) at the precipitation point determined from turbidity measurement are plotted against [-COOH] of the polyanions at the same point in Figures 20-26. In all cases, a straight line is obtained. The free metal ion concentration (unbound) in the solution was estimated from the intercept of this straight line at [-COOH] = 0.0. The slope of this line relates to the equivalents of metal ions bound to each -COOH group. The equation relating the total metal ion concentration, the free metal ion concentration, and the concentration of the -COOH group can be expressed as:

$$[M^{+2}]_{total} = [M^{+2}]_{free} + \beta[-COOH]_{total}$$

Since in most cases, the polyacids studied were not completely ionized, the equivalents of the M^{+2} bound to the polyacid (β') were cal-

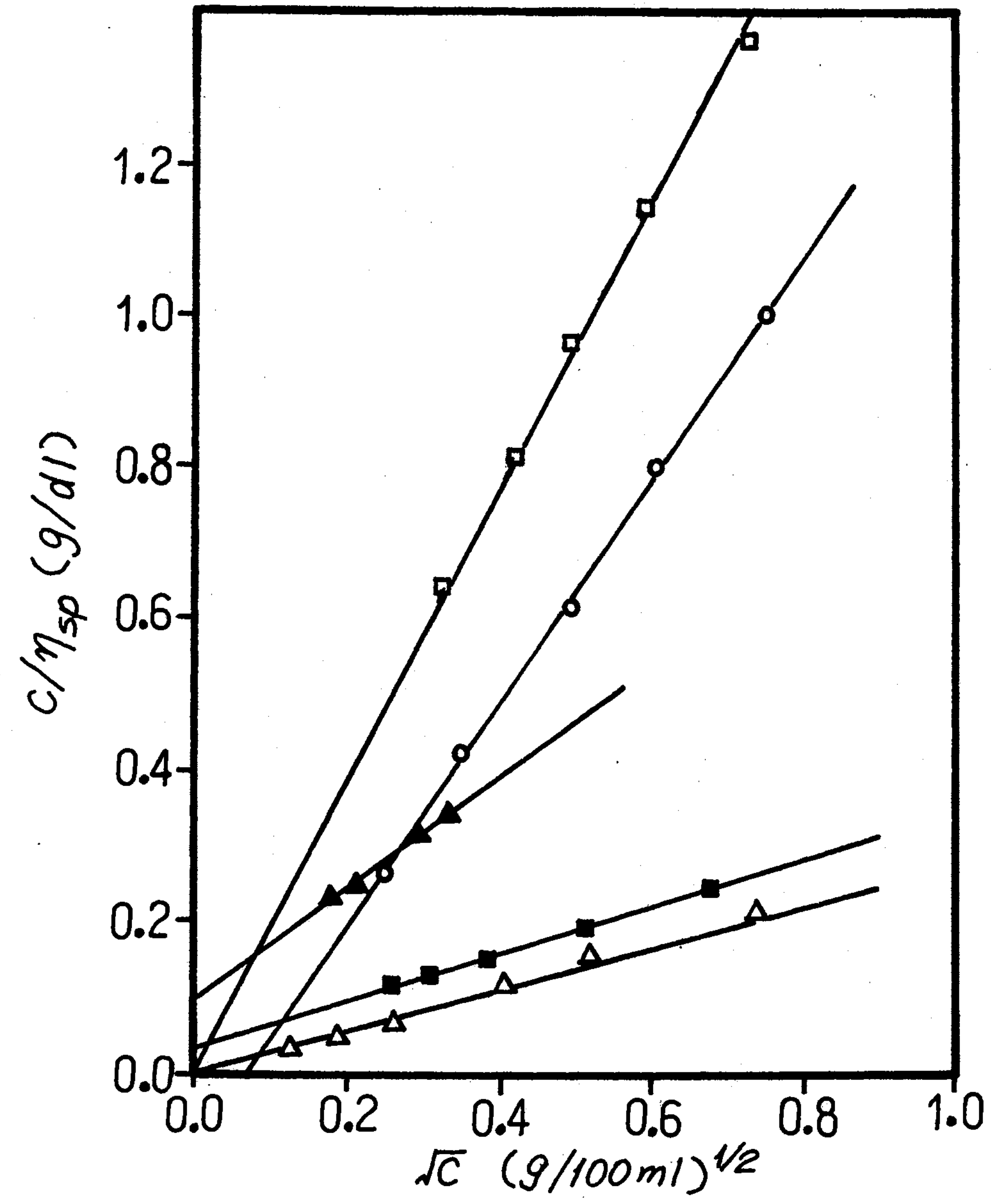


Figure 17. Viscosity of Polymers II (O), VIII (□), IX (Δ) in Water at 25°, and VIII (■), IX (▲) in Water at pH 7.0 and 25°

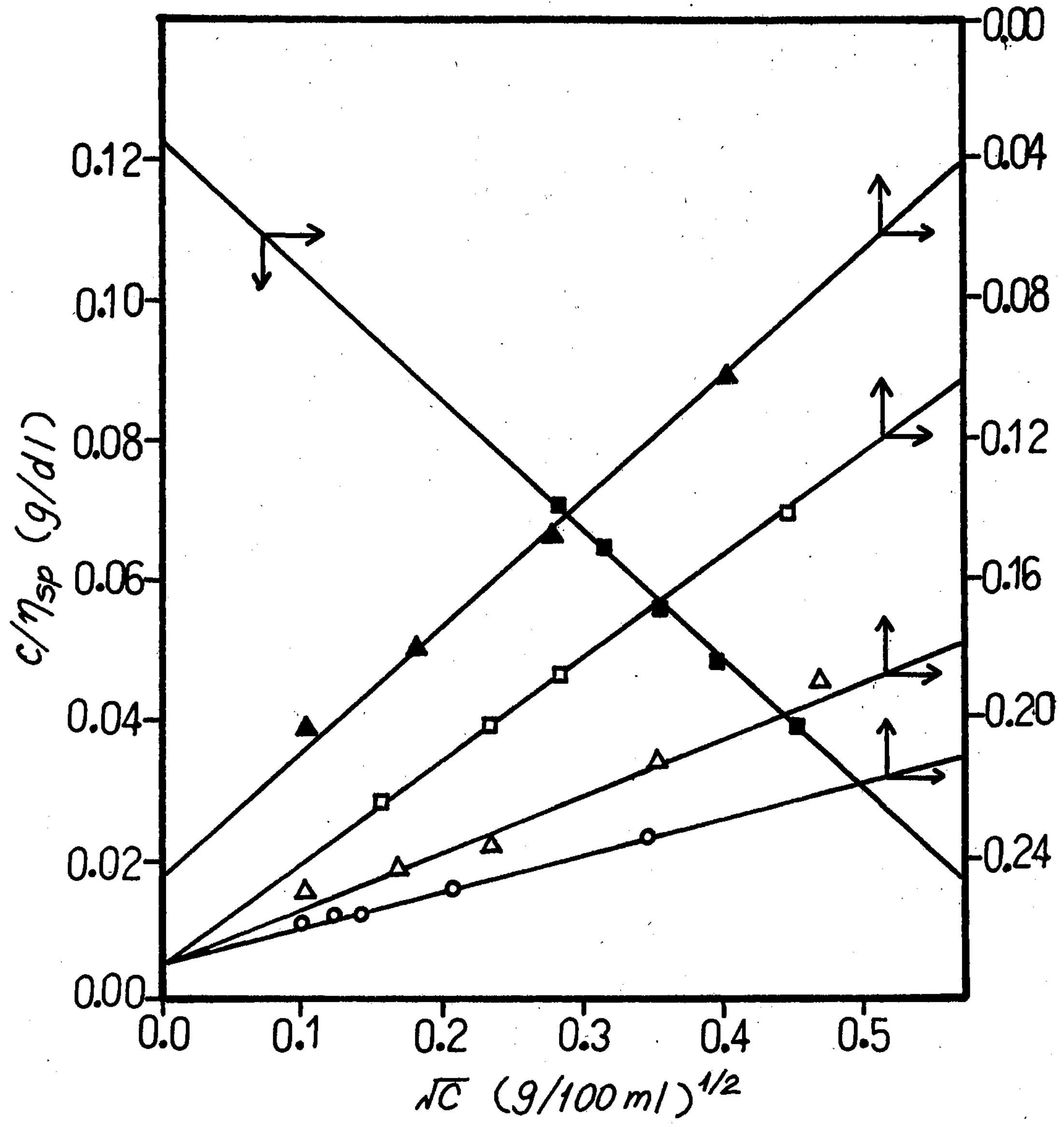


Figure 18. Viscosity of Polymers II (O), III (■), IV (Δ), V (□), and VI (▲) in Water at pH 7.0 and 25°

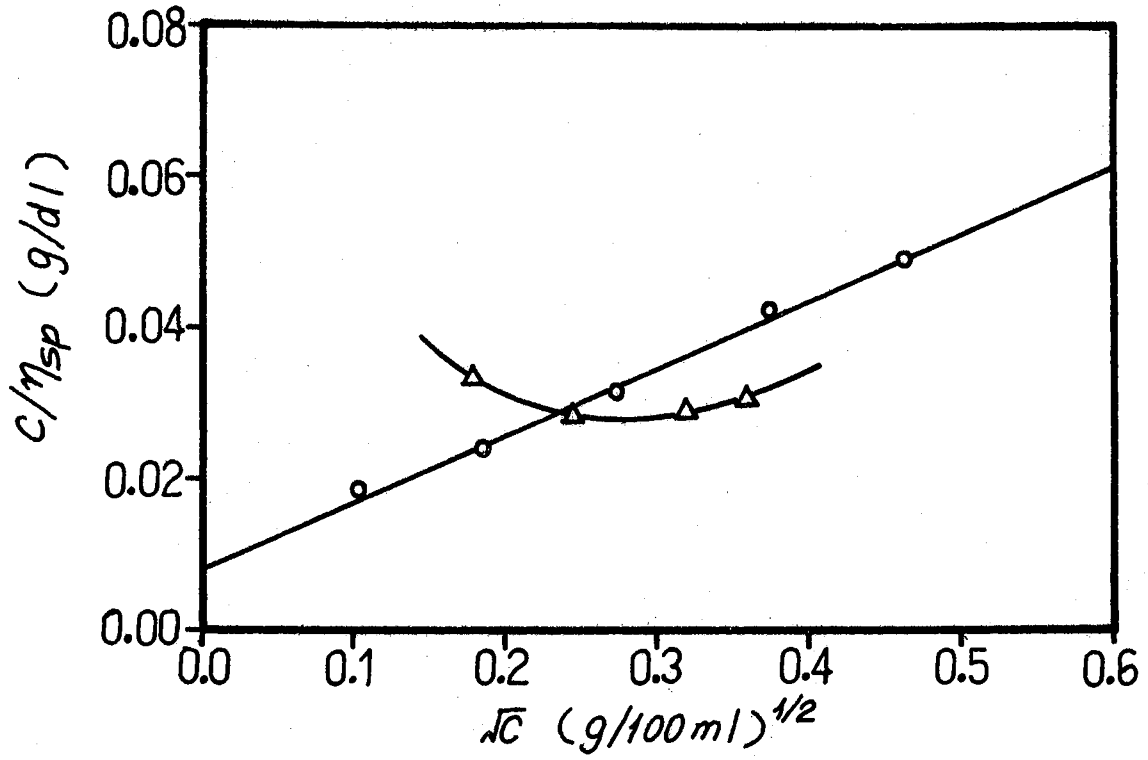


Figure 19. Viscosity of Polymers X (Δ) and XI (O) in Water at pH 7.0 and 25°

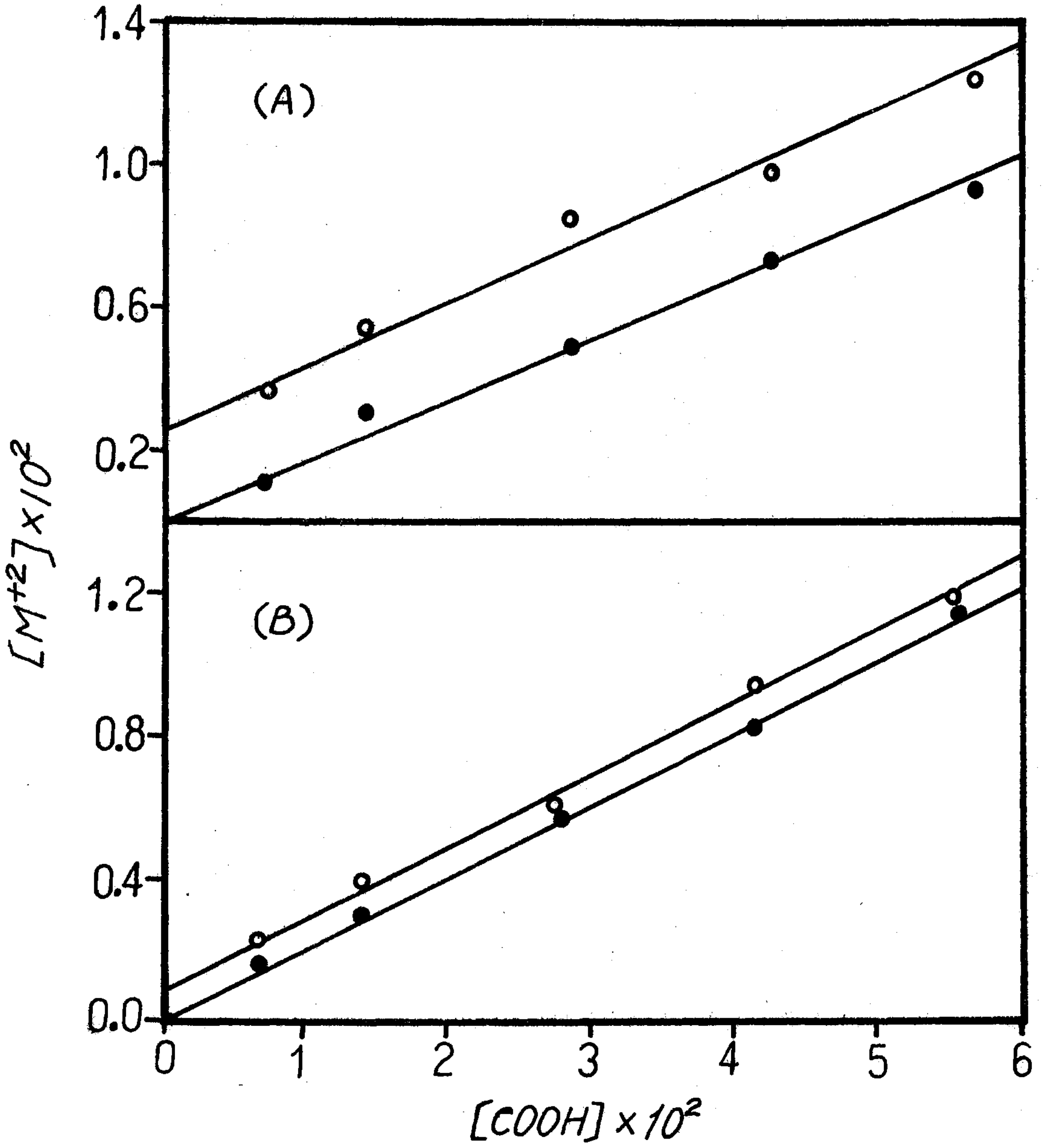


Figure 20. Plots of N of Calcium Ion (●) and Magnesium Ion (O) vs N of -COOH in Polymer I at 25° with pH 7.0 (A), pH 10.2 (B) in Water

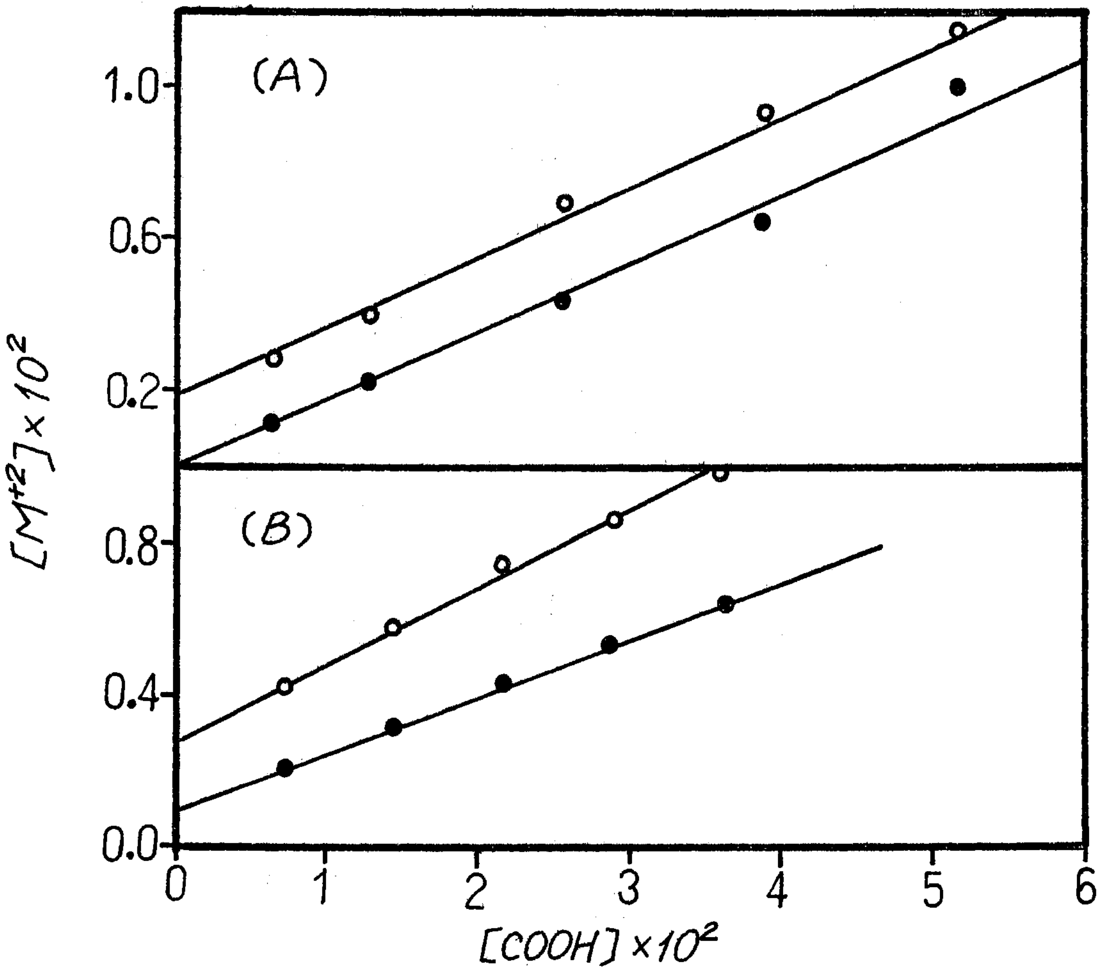


Figure 21. $[Ca^{+2}]$ (●) and $[Mg^{+2}]$ (O) vs [-COOH] of Polymer II (A) and Polymer III (B) at 25° and pH 7.0

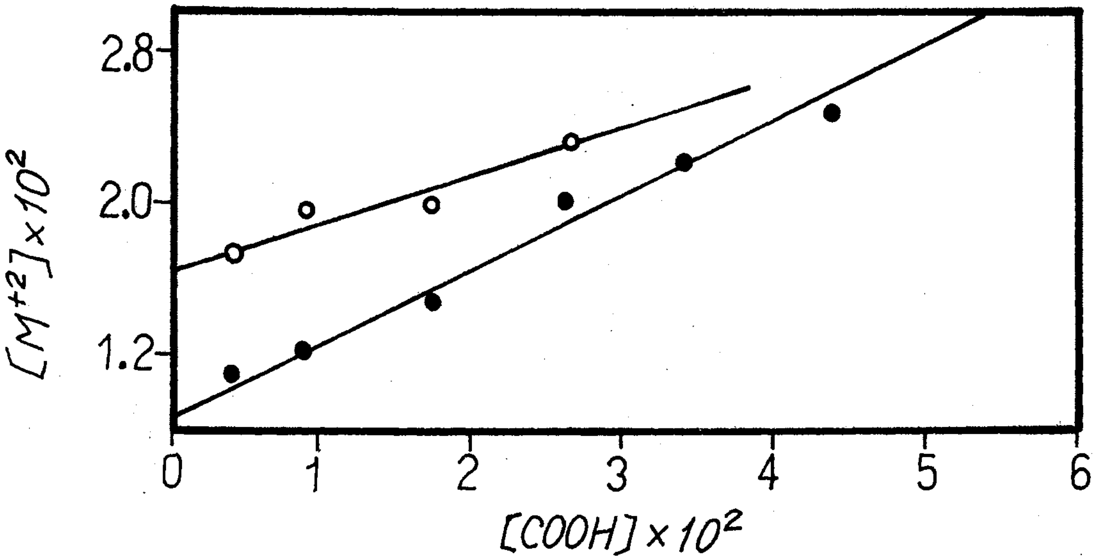


Figure 22. $[Ca^{+2}]$ (●) and $[Mg^{+2}]$ (O) vs [-COOH] of Polymer IV at 25° and pH 7.0

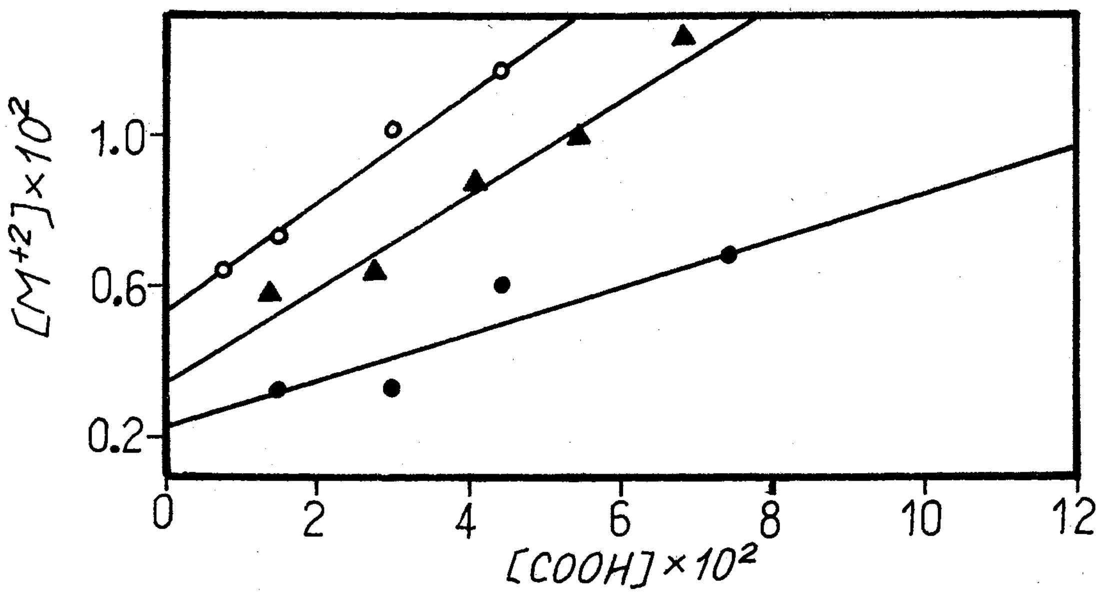


Figure 23. $[Ca^{+2}]$ (▲) vs [-COOH] of Polymer VIII, and $[Ca^{+2}]$ (●) and $[Mg^{+2}]$ (O) vs [-COOH] of Polymer IX at 25° and pH 7.0

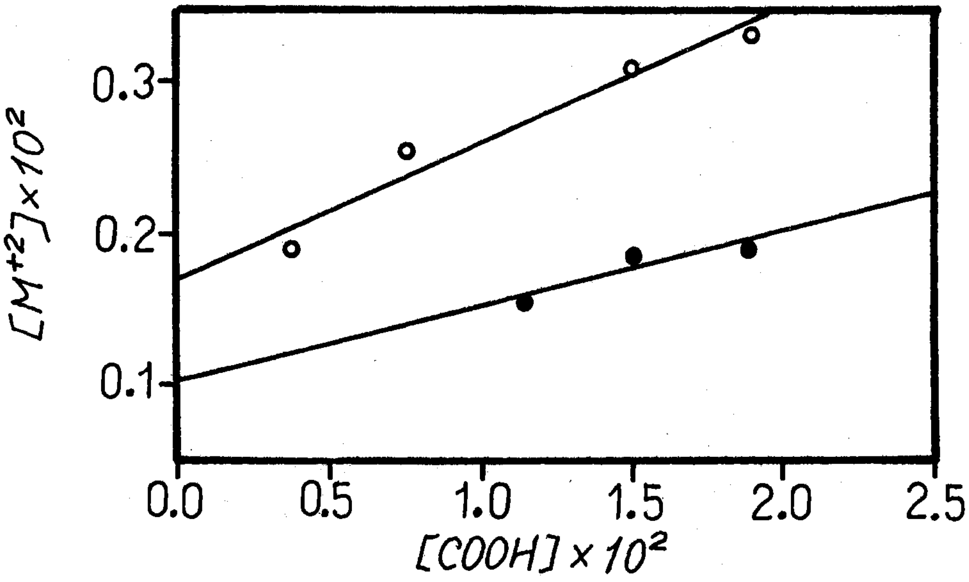


Figure 24. $[Ca^{+2}]$ (●) and $[Mg^{+2}]$ (O) vs [-COOH] of Polymer X at 25° and pH 7.0

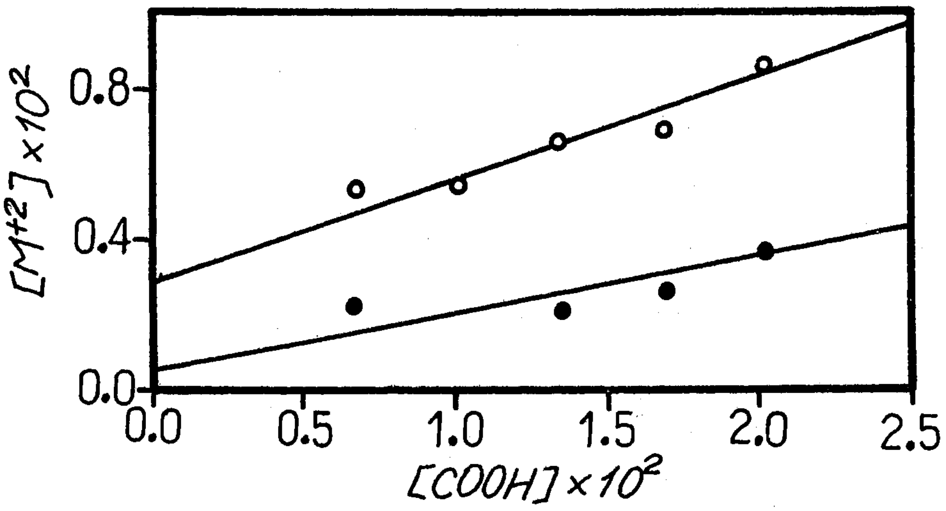


Figure 25. $[Ca^{+2}]$ (●) and $[Mg^{+2}]$ (O) vs [-COOH] of Polymer XI at 25° and pH 7.0

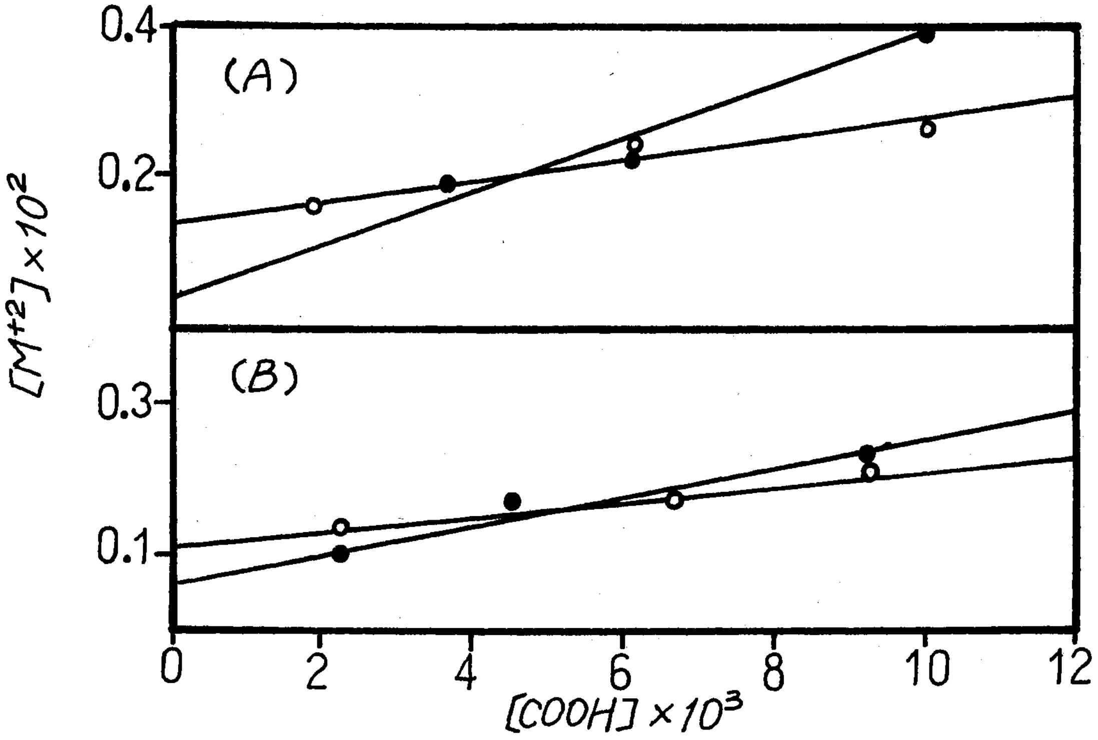


Figure 26. $[Ca^{+2}]$ (●) and $[Mg^{+2}]$ (O) vs [-COOH] of Polymer XIV (A) and Polymer XV (B) at 25° and pH 7.0

culated by the method proposed by Michaeli[86]:

$$\beta' = \beta/\alpha$$

where β is the slope obtained from the $[M^{+2}]_t$ vs $[-COOH]_t$ plot, and α is the degree of ionization. The pH, α, $[M^{+2}]_f$, β, and β' are tabulated in Table X, for all the polymers studied.

The divalent metal ions and the polyacids can undergo the following two modes of binding:

Mode 1:

$$2\ (-COO^-) + M^{+2} \rightleftarrows (-COO^-\ M^{+2}) + (-COO^-)$$

Mode 2:

$$2\ (-COO^-) + M^{+2} \rightleftarrows (-COO^-)_2M^{+2}$$

The exact nature of binding is difficult to predict. However, the experimental result should indicate which mode of binding between the polyelectrolyte and the metal ions. Ikegami and Imai[87,93] suggested that at high values of α the binding tends to follow mode 1, while at low α mode 2 will probably predominate. From our results, it is apparent that mode 2 predominates (i.e., usually two carboxyl groups bind to one metal ion). Wall and Drenan[85] in 1951 also reported that the results of precipitating polyacrylic acid by some divalent metal ions indicated this mode of binding. In all cases examined, it is believed that when over 60% of the ionized carboxyl groups became bound to metal ions, the precipitation occurred. This agrees reasonably well with the value of 80% reported for poly(methacrylic acid)[86].

Polymer I does not give a precipitate at $\alpha = 0$, even after the

TABLE X

PRECIPITATION OF POLYMERS I-IV, VIII, IX, XI, XIV, AND XV BY CALCIUM AND MAGNESIUM IONS IN WATER AT 25°

M^{+2}	Polymer	pH	α	$[M^{+2}] \times 10^2$	β	β'	Figure
Ca^{+2}	I	7.0	0.62	0.0	0.18	0.30	20 (A)
	I	10.2	1.0	0.0	0.20	0.20	20 (B)
	II	7.0	0.58	0.0	0.18	0.31	21 (A)
	III	7.0	0.56	0.09	0.16	0.30	21 (B)
	IV	7.0	0.58	0.88	0.38	0.66	22
	VIII	7.0	0.35	0.35	0.25	0.71	23
	IX	7.0	0.37	0.23	0.13	0.35	23
	X	7.0	0.77	0.11	0.054	0.072	24
	XI	7.0	0.59	0.06	0.15	0.26	25
	XIV	7.0	0.79	0.04	0.35	0.44	26 (A)
	XV	7.0	0.77	0.07	0.19	0.25	26 (B)
Mg^{+2}	I	7.0	0.62	0.25	0.18	0.30	20 (A)
	I	10.2	1.0	0.08	0.20	0.20	20 (B)
	II	7.0	0.58	0.18	0.18	0.31	21 (A)
	III	7.0	0.56	0.28	0.20	0.36	21 (B)
	IV	7.0	0.58	1.66	0.25	0.43	22
	VIII	7.0	0.35	(no ppt.)			
	IX	7.0	0.37	0.55	0.28	0.76	23
	X	7.0	0.77	0.17	0.093	0.12	24
	XI	7.0	0.59	0.29	0.32	0.54	25
	XIV	7.0	0.79	0.13	0.16	0.20	26 (A)
	XV	7.0	0.77	0.11	0.10	0.13	26 (B)

addition of 20 equivalents of metal ions. This indicates that the precipitation is not merely a salting-out effect. An ionized carboxyl group and metal ion bonding must actually be involved for the precipitation to occur. A lower degree of metal binding in Polymer I at higher α value could be due to the competitive binding of sodium ion[94], since the sodium ions were present at a much greater quantity in this solution of high α.

The qualitative comparison of the binding ability of the polyacids studied revealed that as the content of IBVE increases (Polymers I through VI), a change in the nature of binding can be observed. Polymers I, II, and III do not differ very much in their nature of metal binding as shown by their consistent β' values. In Polymer IV, the content of IBVE is becoming substantial, which means that the acrylic acid groups are more separated in the polymer and the β' value increases markedly. This comparatively large value of β' indicates that some portion of this binding is due to mode 1. With Polymers V and VI, no precipitation was observed even when three equivalents of the metal ions were added. This would be true if either the binding is very weak or the bound salt is rather water-soluble. Also it can be seen from Table X that a high $[M^{+2}]_f$ is usually accompanied by a high β' value. A higher value of $[M^{+2}]_f$ refers to a less effective binding between the polymer and the metal ion. This is believed to be more favorable in the case of mode 1.

Poly(acrylic acid) (I), by the present system of measurement, seems to bind metal ions by mode 2. When the acrylic acid has copolymerized with another comonomer, the further separation of acrylic acid will tend to shift some of the binding to mode 1. Poly(itaconic acid) (VIII), owing to its more flexible carboxylated side chains, is found to favor binding by mode 1. In this study [M^{+2}] was smaller for Ca^{+2} than for Mg^{+2}; this agrees with results of others.[1]

Antitumor Studies

The results of single-dose treatment of mice inoculated with ascitic Sarcoma 180 are tabulated in Table XI and those for triple-dose in Table XII.

The dose was expressed conventionally, as milligrams of sample injected per weight of the animal in kilograms. The survivors were the number of mice living six days after the inoculation of tumor cells compared to the number of mice tested. The difference in weight was obtained by subtracting the average gain in weight of the treated animals nine days after the inoculation from the average gain in weight of the control animals. The average survival time (T) of the treated animals is the average survival times of the third and fourth mice to die (of the original six); the average survival time (C) of the control animals is calculated in the same way. A value of T/C greater than 125 is considered to indicate activity toward Sarcoma 180.

The antitumor activities of some polymers studied are listed in Table XIII together with their molecular weights and the total moles of ionized carboxyl groups (TCOO) per injection. The TCOO was calculated from the formula weight of the polymer and its degree of ionization at pH 7.

Poly(acrylic acid) (Polymer I) shows activity toward Sarcoma 180 with good reproducibility, as can be seen from the results of triple-dose treatment. For six runs of Polymer I at a dose of about 11 mg/kg,

TABLE XI

SINGLE-DOSE TREATMENTS OF MICE INOCULATED WITH ASCITIC SARCOMA 180

Polymer	Dose, mg/kg	S^a	W.D.^{b}, g	T, days	C, days	T/C, %
I	11	6/6	-5.8	23.5	15.0	157
	12	6/6	-6.5	16.5	15.0	110
II	4.3	5/6	-0.4	11.5	9.5	121
	3.8	6/6	-6.2	26.0	18.0	144
	3.6	6/6	-5.5	23.5	15.0	157
	2.9	6/6	-1.4	10.5	9.5	111
III	33	6/6	-0.3	14.0	13.0	108
	36	6/6	-2.1	11.5	12.5	92
IV	9.5	6/6	-4.7	11.5	12.5	92
	6.7	6/6	-2.3	11.5	13.0	88
V	6.0	6/6	-1.2	14.0	12.5	112
	5.1	6/6	-4.2	10.0	9.5	105
VI	4.1	4/6	-1.8	10.0	12.5	80
	3.3	6/6	-2.4	12.0	13.0	92
VIII	190	6/6	1.4	11.0	13.5	81
	150	6/6	1.6	12.5	13.5	93
IX	155	6/6	-4.1	11.0	9.5	116
	133	6/6	8.9	27.5	15.0	183
	113	6/6	-3.1	11.0	9.5	116
X	82	6/6	-0.3	10.5	12.5	84
	52	6/6	-0.1	15.0	13.5	111
XV	3.3	5/6	-4.6	17.0	18.0	94
	2.4	6/6	-0.7	13.0	13.0	100

[a] Number of mice surviving six days after the test was started/number of mice tested.

[b] Weight difference.

TABLE XII

TRIPLE-DOSE TREATMENTS OF MICE INOCULATED WITH ASCITIC SARCOMA 180

Polymer	Dose, mg/kg	S[a]	W.D.[b], g	T, days	C, days	T/C, %
I	16	6/6	+1.5	14.5	14.0	103
	12	6/6	+2.5	15.5	9.5	163
	12	6/6	-3.6	15.5	10.5	147
	12	6/6	-3.6	16.5	15.0	110
	11	6/6	-5.8	23.5	15.0	157
	11	6/6	-2.1	14.5	14.0	103
	9.3	6/6	-2.5	17.5	14.0	125
II	4.4	6/6	+0.8	15.0	10.5	143
	4.1	6/6	-1.2	17.5	14.5	125
III	31	6/6	+0.1	15.0	13.0	115
	18.5	6/6	-6.4	19.5	13.0	150
IV	6.4	6/6	-4.4	12.5	13.0	96
	3.8	6/6	-9.9	14.0	13.0	108
V	2.7	6/6	-4.5	12.5	13.0	96
	1.9	6/6	-8.7	13.0	13.0	100
VI	1.9	6/6	+0.4	13.0	13.0	100
	1.5	5/5	-1.6	18.0	15.0	120
VIII	195	6/6	+1.3	26.0	16.0	162
	206	5/6	0.0	15.0	9.5	158
IX	98	6/6	-2.1	18.5	16.0	116
	59	6/6	-2.8	14.5	14.0	103
X	92	6/6	-2.6	18.0	16.0	112
	200	5/5	-1.2	22.0	15.0	147
XV	1.7	6/8	-1.6	14.5	13.0	112

[a] Number of mice surviving six days after the test was started/number of mice tested.

[b] Weight difference.

TABLE XIII

ANTITUMOR ACTIVITIES AND MOLECULAR PARAMETERS OF SOME POLYMERS STUDIED

Polymer	MW[a] x 10^{-4}	Single-Dose Treatment		Triple-Dose Treatment	
		T/C, %	(TCOO)[b] x 10^5, moles	T/C, %	(TCOO)[b] x 10^5, moles
I	25	157	9.6	103	14
		110	10.4	163	10
				147	10
				110	10
				157	9.6
				103	9.6
				125	8.1
II	25	121	3.2	143	3.3
		144	2.9	125	3.1
		157	2.7		
		111	2.2		
III	8.5	108	24.1	115	23
		92	26.4	150	14
IV	25	92	5.3	96	3.6
		88	3.8	108	3.2
V	25	112	3.8	96	1.7
		105	3.3	100	1.2
VI	12	80	2.2	100	1.0
		92	1.7	120	0.80
VIII	8.7	81	93	162	96
		93	74	158	101
IX	4.4	116	78	116	49
		183	67	108	30
		116	57		

[a] Molecular weight of the polymer.

[b] Total moles of ionized carboxyl groups per injection.

an average activity of T/C = 135 was obtained.

The activity of AA-IBVE copolymers (II-VI) decreases as the content of IBVE increases (Table V). This is in accord with the observation that the hydrophobic constituents decrease the activity (or in other words, increases the toxicity)[12].

The safe dose (the amount of the injection which causes no toxicity) of poly(acrylic acid) and AA-IBVE copolymers is shown to be related to the total moles of ionized carboxyl groups and the molecular weight (Table XIII). Polymer III, VIII, and IX have comparatively low molecular weights. The mice can take a much larger dosage of them.

Polymer XI (acrylic acid isooctyl vinyl ether copolymer) does not show a significant antitumor activity. These results are not included in the Tables.

When the results obtained from single-dose and from triple-dose treatments are compared, it is found that those polymers showing significant difference in activities also have a different kind of metal binding (Table X).

Polyelectrolytes can absorb viruses to certain degrees and can be used to remove viruses from water[127]. Tunis and Regelson also have shown that polyelectrolytes lose their activity in tumor inhibition if they are premixed with magnesium ions before being used in treating animals with Sarcoma 180 cells[128]. This indicates that there is some kind of interaction between the polyelectrolyte and the tumor cells. These interactions, whether having direct effect in retarding the tumor cells replication or in enhancing the interferon production or in blocking the receptor sites on the cell surface[8], can not be ignored as far as the antitumor activity is concerned. Though the bindings between the polyelectrolytes and the tumor cells and those between the polyelectrolytes and metal ions may be different in nature, their competitive effect, as revealed by Tunis and Regelson[128], shows that they must have some basic similarities in nature.

In the metal binding studies of the polymers investigated, those compounds having larger values of $[M^{+2}]_f$ and β' have less effective binding and form more soluble salts. A notable difference between the single-dose and triple-dose treatments is found in Polymer VIII, where an enhanced activity is found in triple-dose treatment. For those polymers with effective binding abilities, antitumor activities were found to have a consistent value, whether in single dose or triple doses. An extended introduction of those polymers with less effective metal binding ability seems to increase the activity. This same effect can also be observed, though it is less apparent, in Polymers IV to VI.

Polymer X (acrylic acid-N,N-dimethylaminoethyl methacrylate copolymer) is shown to be less toxic than any other acrylic acid copolymer studied. The comparison of its antitumor activity to that of the others is limited because of the difference in the chemical structure of X.

Polymer XIV and XV have shown activity toward the Walker carcinosarcoma 256 according to the screening results from Cancer Chemotherapy National Service Center, National Cancer Institute, Bethesda, Maryland. Polymer XIV has activity with dosage range from 0.5 to 50 mg/kg toward Walker carcinosarcoma 256 (subcutaneous), and Polymer XV has activity with dosage range from 2 to 160 mg/kg toward Walker carcinosarcoma 256 (intraperitoneal).

Summary

Since the first cancer chemotherapeutic study was carried out with polyanions in 1910[8], hundreds of different polyanions, naturally occurring or synthetic, have been tested for their activities toward many different tumor or virus systems. For each individual study, with a narrow spectrum of polyanions and some specific tumor systems, new discoveries are made for further speculation. It is very unlikely, though, that any study of this kind can throw light to clear all the mysteries hidden behind the alley. Conclusions, should they be drawn after each of these studies, without being supplemented with sufficient knowledge of the molecular parameters of tumor cells and the site and mechanism of action, would probably be erroneous.

No attempt is made to conclude the results of this study by relating the chemical properties of the polymers studied and their antitumor activities. However, some indications which suggest a possible interrelation between the two properties are summarized below.

(1) Hydrophobic constituents do have positive effect on the toxic level of polymers.

(2) Molecular weight and charge density are directly related to the antitumor activities of the polymers investigated. Unfortunately, these effects are suppressed by the toxicity introduced by the hydrophobic nature of IBVE. Therefore, the exact measure of these two properties to the antitumor activity can not be estimated.

(3) Different modes of metal binding may have an effect on the multiple-dose treatment. It also affects, to some degree, the toxic level of the dose.

More polymers should be prepared with designed properties, if the above observations were to be extended. A series of copolymers prepared from an ionizable anionic monomer and another non-toxic monomer with varied charge densities and molecular weights could be used to study the effect of charge densitites and molecular weights on the antitumor activities. A series of polymers with anionic groups separated by different distances in the polymeric chain, or on the attached side chains, could be used to study the effect of different metal binding ability to their antitumor activities.

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