N-ACRYLOYL γ-AMINOBUTYRIC ACID AND ITS POLYMERS

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C E R T I F I C A T E

Certified that the work incorporated in the thesis "N-Acryloyl- γ -aminobutyric Acid and its Polymers" by Mr. S.K. Menon was carried out by the candidate under my supervision. Such material, as has been obtained from other sources, has been duly acknowledged in the thesis.

(S. Gundiah)

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(S.K.Menon)

Contents

SYNOPSIS

Synopsis

Water soluble polymers with specific functional groups are coming into prominence in selective separations. Polymers containing $-NH(CH_2)_nCOOH$ group are expected to show selectivity in some of the mineral separations. These can be prepared either by the hydrolysis of polymeric lactams or by preparing the functionalised monomers and subsequent polymerization. The first method has the disadvantage that the unreacted lactams remain in the polymer. The major disadvantage foreseen in the second route is that the monomer has an ampholytic character and polymerization is likely to be severely hindered due to the electrostatic repulsion between the charges on the macroradical and the monomer to be incorporated. Due to large hydrophilicity of these functional group containing polymers, they are mostly insoluble in organic solvents and homogeneous solution polymerization may not be carried in organic solvents. Literature reports are available on preparation of some of these monomers like N-methacryloyl β-alanine and N-methacryloyl glycine. Inspite of anticipated problems in polymerization; N-methacryloyl γ-aminobutyric acid is reported to readily polymerize during preparation and isolation.

N-acryloyl-γ-aminobutyric acid was prepared by Schotten-Bauman reaction by reacting γ-aminobutyric acid with acryloyl chloride at 0 - 5°C in aqueous alkali. The reaction mixture was extracted with chloroform. The monomer was observed to undergo rapid polymerization during concentration of the chloroform extract. A liquid

chromatography method was established for the the estimation of the monomer in the chloroform extract. However, it was observed that the monomer could be isolated in the pure form when the chloroform extract was aged for three days at 0 - 5^{o}C. The structure of the monomer was established by spectral techniques. The monomer resisted attempts to polymerize the same in aqueous solutions. It was suspected that polymerization can be achieved in aqueous solution only over a narrow range of pH. In order to obtain optimum pH conditions, the solution viscosity behaviour of the polymer obtained by the spontaneous polymerization (during the isolation step) was examined as a function of pH. The viscosities were higher at lower values of pH and attained low asymptotic values at higher values of pH. This behaviour was attributed to the minimal contribution of the electrostatic charge on the pendent chain to the electrostatic interactions. Polymerization carried out at a pH values of 12.0 resulted in good rates of polymerization and high molecular weights for the polymer obtained.

In conclusion, methods for the preparation of N-acryloyl ϒ-aminobutyric acid and its estimation by HPLC technique, condition for the polymerization/copolymerization of the monomer in aqueous solution to obtain high molecular weight polymers in good yield have been established in this study.

CHAPTER I
INTRODUCTION

I a. GENERAL INTRODUCTION

Water soluble polymers find varied applications in enhanced oil recovery techniques, treatment of industrial effluents, ore beneficiation etc[1]. Introduction of functional groups (specific to one of the components present in a mixture) in polymers to achieve high selectivity in mineral separations is actively engaging the attention of several research groups. For example, it is reported[2] that polyacrylamide containing hydroxamic acid groups exhibit a ten-fold higher selectivity for iron as compared to aluminium. Methods for synthesizing polymers with functional groups like glycolic, glyoxal bis-hydroxyanil are available in literature[3,4]. The investigation reported here is aimed at preparing polymers with $-NH(CH_2)_nCOOH$ pendent groups. One of the possible methods of preparing these polymers is by the ring opening hydrolysis of polymeric lactams. Thus, alkaline hydrolysis of polyvinylpyrrolidone (PVP) results in polymers having $-NH(CH_2)_3COOH$ pendent groups[5]. Unreacted lactams and $NH(CH_2)_nCOOH$ are simultaneously present in the hydrolysed polymer. Winston et al[2] utilized a novel method for preparing polymers with hydroxamic acid groups of varying chain length. Their interest was to evaluate the effect of the number of carbon atoms (separating the hydroxamic acid groups) on the iron chelating efficiency. Methacryloyl Chloride was reacted with glycine, β-alanine and γ-aminobutyric acid respectively (Schotten-Bauman reaction). The amides formed were esterified and polymerized. The polymers were treated with

methylhydroxylamine and triethylamine to obtain pendent hydroxamic acid groups of differing chain lengths. It may be noted that the amides synthesized by Winston and Kirchner have the desired functional ($-NH(CH_2)_nCOOH$) groups. The polymerization of these amides would yield the desired polymers. Two of the amides synthesized,viz N-methacryloyl glycine and N-methacryloyl-β-alanine could be isolated and their physical properties have been reported. However, the authors could not isolate N-methacryloyl-γ-aminobutyric acid, as it polymerized during isolation. The amides were esterified in situ.

In this study, N-acryloyl-γ-aminobutyric acid was synthesized by analogous Schotten-Bauman reaction. The monomer synthesized was observed to polymerize during its isolation. A HPLC technique was developed for estimating the concentration of the monomer in the reaction mixture. By modification of the postreaction monomer isolation step, the product was isolated in pure form. The structure of the monomer was confirmed by spectral analysis. Inspite of unintended polymerization during its preparation and isolation step, the purified monomer resisted attempts to polymerize/copolymerize the same in aqueous solutions. As the monomer has ampholytic character[6], the propagation step is likely to be greatly retarded due to presence of ionised groups under most experimental conditions. The pH dependence of the solution viscosity of the polymer (obtained during preparation) was examined. It was argued that pH conditions yielding minimum viscosity would favour the propagation step

and hence formation of high molecular weight polymer. Polymerizations carried out under highly alkaline conditions did result in high molecular weight polymers/copolymers in very good yields.

In subsequent sections of this introductory chapter, a brief discussion on monomers containing functional groups and their polymers and the HPLC technique used for determining the rates of monomer formation are briefly discussed.

I b. Monomers containing functional groups and their polymers

The monomers considered here are vinyl monomers. These have functional groups, basically of high polarity and hydrophilicity which confer water solubility characteristics to the polymer obtained from these monomers. These polymers and copolymers find application as flocculants which have high specificity for some of the metal ions.

For the preparation of these functionalised polymers, two routes may be envisaged[7]. In the first method, the functionalised monomer may be prepared using well established methods of organic synthesis and the monomers can be polymerized or copolymerized. Alternatively, the preformed polymer may be modified using suitable reactants and reaction conditions. Both the methods have their advantages and disadvantages. During the preparation of the functionalised monomer addition occurs across the double bond (instead of the intended reaction). Polymerizations during the functionalisation reactions are also encountered. In principle, the functionalised monomer may be isolated in the pure form and polymerized/copolymerized to give the desired product. However, the purification or isolation may prove to be cumbersome. Polymerization, especially if the monomer possesses ionizable groups, may not be easily accomplished. The main advantage of using the functionalised monomer route is that the polymer obtained has known composition. The polymer obtained by the polymer conversion route is contaminated with unreacted groups (originally present in the

polymer used for functionalisation).

The main disadvantages of functionalising a preformed polymer are (i) main chain degradation (ii) presence of unreacted groups originally present in the polymer and (iii) relatively lower concentration of the desired functional groups. The last cited disadvantage is particularly severe if the functionalisation reaction involves more than one step. Unreacted groups from each of the individual reactions are present in the polymer and the concentration of the desired functional group drastically reduces. However, difficulties that may be encountered in the polymerization of functionalised monomers are avoided and polymers used for functionalisation may be selected to meet any of the desired specifications like molecular weight range. Some examples of the two different methods of obtaining functionalised polymers are given below.

The hydrolysis of acrylonitrile to acrylamide and finally to acrylic acid can be carried out in the presence of a polymerization inhibitor[8,9]. The first step of hydrolysis to acrylamide is carried out with sulphuric acid or with copper as catalyst[9]. The second step is a facile hydrolysis in the presence of an alkali. Polyacrylamide can also be hydrolysed under very similar conditions[9]. Thus polyacrylic acid can be obtained by the polymerization of acrylic acid. The acrylic acid can itself be obtained by the hydrolysis of acrylamide. Alternatively, acrylamide may be polymerized and hydrolysed. As in any functionalisation reaction involving

polymers, it is extremely difficult to obtain quantitative conversion of polyacrylamide to polyacrylic acid without main chain degradation[9]. However, acrylic acid resulting from hydrolysis of acrylamide can be isolated in the pure form and polymerized. The resulting polymer has no unwanted amide groups.

For the preparation of acrylohydroxamic acid, in analogy with similar reaction reported[10], ethyl acrylate can be reacted with hydroxylamine hydrochloride

$$CH_2{=}CH(COOC_2H_5) + NH_2OH.HCl \longrightarrow CH_2{=}CH(CONHOH) + C_2H_5OH$$

However, addition across the double bond occurs[10] resulting in the formation of $X\text{-}CH_2\text{-}CH(CONHOH)$

A more convenient and practical method of synthesizing hydroxamic acid group containing polymer is to functionalise preformed polyacrylamide, polyacrylonitrile, polyacrylic acid or polyethylacrylate[7].

It has been noted that N-methacryloyl-Ɣ-aminobutyric acid spontaneously polymerized during isolation[2]. The polymer with the desired $[-NH(CH_2)_3COOH]$ functionality can also be obtained by hydrolysing poly(N hydroxy succinimide ester of N-methacryloyl-ɣ-aminobutyric acid) prepared by the same investigators[2].

Polymers with pendent $-NH(CH_2)_nCOOH$ groups, the subject matter of this investigation, can be obtained by the hydrolysis of polymeric lactams[5,6]. Polymers of N-acryloyl-Ɣ-

aminobutyric acid contain an extra carbonyl group (apart from the desired functionality) in the pendent chain. If the presence of the extra carbonyl group is ignored or the carbonyl group does not significantly interfere to the chelation of the desired metal species, polymers of interest can also be derived by the hydrolysis of polymeric lactams or by the polymerization of N-acryloyl- ϒ-aminobutyric acid and other analogous monomers.

Hydrolysis of polymeric lactams has the advantages and disadvantages of a typical polymer functionalisation reaction. The polymer derived from the polymerization of the functionalised monomer is free from other functional groups like lactam. The reactive species in alkaline hydrolysis is the hydroxyl ion. In case of alkaline hydrolysis of polyacrylamide, it has been speculated[11] that the electrostatic repulsion of charges present on the hydrolysed polymer and the hydroxyl ion results in a random distribution of the hydrolysed groups along the polymeric backbone. NMR evidence appears to support random distribution of hydrolysed group in partially hydrolysed polyacrylamides[12]. Similar arguments are likely to apply to the hydrolysis of polymeric lactams. Indeed, due to the polyampholyte character acquired by the hydrolysed polymers, both acid and alkaline hydrolysis are likely to be retarded. Frank[6] has attributed the resistance of polymeric lactams to hydrolysis to the polyampholitic character of the resulting product. The reported kinetics[5] of hydrolysis of polyvinylpyrrolidone was carried out at elevated temperatures and pressures. These are

likely to have resulted in main chain degradation.

Polymerization of N-acryloyl-ϒ-aminobutyric acid presents interesting challenges. The monomer has ampholytic character and hence possesses either a positive or negative charge under most experimental conditions. It was mentioned earlier that the functionalization reactions, we are interested in, introduces high polarity and hydrophilicity in the monomer. The result is that the functionalised polymer in most of the cases is water soluble and immiscible with organic solvents.

It is known that the aqueous polymerization of vinyl monomers like acrylic acid, methacrylic acid is greatly retarded under conditions favouring monomer ionization[13,14]. When ionization is suppressed (for example, by carrying out the polymerization in organic solvents) these monomers undergo facile radical polymerization[13]. Even in aqueous solutions, normal rates of polymerization and degrees of polymerization are obtained when polymerizations are carried out under suitable conditions of pH and ionic strength of the medium[14]. Monomers like N-acryloyl- ϒ-aminobutyric acid are ampholytes. Monomer is likely to have residual electrostatic charge under most experimental conditions. Hence it would appear that polymerization of these monomers is difficult to achieve especially as i) the polymer is likely to be insoluble in organic solvents and hence homogeneous solution polymerization cannot be carried out in organic solvents and ii) polymerization in aqueous media are likely to be greatly

retarded due to electrostatic charges of one type or the other persisting on the monomer under most experimental conditions.

I c. Liquid Chromatography

Liquid chromatography[15] (LC) and gas chromatography (GC) are two of the analytical techniques which are widely employed for the determination of composition in mixtures. The analytical results obtained by these two techniques are of comparable reliability and accuracy. However, GC analysis requires volatilization of the sample and LC requires sample solubility in a suitable solvent. LC in addition, has the advantage that the separated constituents can be collected, if so desired.

In liquid chromatography, the sample as a solution in the mobile phase, is introduced in the beginning of the column. The separation takes place in the column due to the relative affinity of the constituents to the stationary phase and mobile phase. The constituents migrate at different rates as the mobile phase continuously flows through the column. The eluents at the end of the column contain different constituents at different times. In other words, the different constituents have different retention times. Components of the mixture can thus be separated. LC can be carried out under isocratic conditions (fixed composition for the mobile phase) or under gradient elution conditions (continuous variations of the mobile phase composition and hence its polarity). If elution characteristics for any component in a mixture under set experimental conditions are known, its presence or absence in the sample can be confirmed. The quantity of each constituent present can be

evaluated by the intensity of the detector response. This, in essence, is the principle of detection and quantitative evaluation of the constituents in a mixture by LC.

The method of separation employed in size exclusion chromatography (SEC), more widely used by polymer scientists for the determination of molecular weights and molecular weight distribution in polymers, is as follows: The stationary phase consists of rigid particles having differing porosities. The partitioning of the macromolecules in the mobile phase inside the pores and outside the stationary phase provides the basis of separation of the macromolecules with differing sizes. Large molecules are excluded from some of the pores whereas small molecules can enter most of the pores present in the stationary phase, thus delaying their elution at the end of the column. Calibration of the elution volumes for macromolecules of known molecular weight at set experimental conditions provides the basis of data evaluation in SEC. More recently, low angle laser light scattering (LALLS) detector and viscosity detector are employed[16], so that molecular weights and molecular weight distributions could be determined by SEC without recourse to calibration.

It may be emphasized that the primary requirement for reliable LC analysis are i) reproducible flow rates for mobile phase, ii) suitable stationary phase for efficient separation of the constituents in the mixture, iii) rigid structure for the material used in the stationary phase (to withstand high operative pressures), and iv) sensitive detector for the eluents from the column.

The basic instrumentation for LC consists of a high pressure pump with constant and highly reproducible flow rates, a system to introduce finite volumes of sample solution in the mobile phase onto the stationary phase, efficient and rigid stationary phase capable of withstanding high pressures (of the order of 2500 - 3000 psi) and yielding high resolution in separation, and a sensitive detector. The efficiency of the column for separation is generally denoted in terms of number of theoretical plates per foot of the column.

Quantitative evaluation of the constituents in the mixture by LC requires that the elution characteristics of the individual constituents like the retention time, the detector response per unit quantity of the material are known under the chosen experimental conditions like the column set employed, mobile medium, its flow rate, temperature of analysis and detector chosen, etc. Under similar experimental conditions, a known volume of finite concentrations of pure constituents are injected, the retention times and the detector responses for a unit quantities of the constituents (for each of the component) is obtained. In an unknown sample the retention time would indicate the nature of the constituents and the detector response its quantity as the detector response for unit amount is known.

I d. Present Investigation

N-acryloyl- γ -aminobutyric acid monomer was synthesized by Schotten-Bauman reaction under experimental conditions identical to those employed by Winston et al[2] for the preparation of N-methacryloyl- γ -aminobutyric acid. γ -aminobutyric acid was reacted with acryloyl chloride in aqueous alkali at 0-5°C. At the end of the reaction, the pH of the reaction medium was lowered to 3.5 to 3.0. The mixture was extracted with chloroform. The dried chloroform extract was concentrated on a rotavapour. The monomer was observed to undergo ready polymerization during the isolation step. A HPLC technique was developed for the quantitative estimation of the monomer in the chloroform extract. However, by a slight modification of the isolation step, monomer in the pure form could be isolated. Spectral data confirmed the expected structure. Attempts to polymerize/copolymerize the monomer were not successful. High susceptibility of the monomer for polymerization during its preparation and its resistance to polymerization led us to believe that the ampholytic character of the monomer is hindering polymerization under chosen experimental conditions. The dependence of the solution viscosity of the polymer (obtained during monomer preparation) on pH was examined. It was observed that at low values of pH, solution viscosity was higher and viscosities attained asymptotic values at high values of pH. Polymerization/copolymerization carried out at alkaline condition yielded high rates and high molecular weight polymers/copolymers.

CHAPTER II
EXPERIMENTAL

II a. Preparative Methods

II a i Preparation of N-acryloyl-γ-aminobutyric acid

Principle : γ-aminobutyric acid dissolved in aqueous alkali was reacted with acryloyl chloride. At the end of the reaction, pH was lowered to 3.0. The reaction mixture was extracted with chloroform. The monomer was separated by the addition of n-heptane to the concentrated chloroform extract.

Procedure : The reaction was carried out in a two-necked 100 ml round bottom flask. The flask was mounted in a crystallizing dish. The dish itself was located over a magnetic stirrer. The flask was surrounded by crushed ice. Distilled water (Laboratory distilled water refluxed over alkaline $KMnO_4$ and twice distilled in an all glass assembly) 17 ml, was added to the flask along with 12 g of NaOH (GR grade from M/s Sarabhai chemicals, Baroda, purity > 98 %). The contents were gently stirred. γ-aminobutyric acid (obtained from M/s E Merck, Germany, purity > 98 %) 15.4 g, was added. A 100 ml dropping funnel was attached to the reaction flask. The other opening of the reaction flask was lightly closed with a stopper. A wad of aluminium foil was inserted along with the stopper to allow an air gap. 12.2 ml acryloylchloride (E Merck Germany, purity > 98 %) was transferred to the dropping funnel. Acryloylchloride was slowly added to the reaction flask with continuous stirring. The addition was completed in about half-an-hour. At the end of the addition of acryloylchloride, the dropping funnel was removed and the flask stoppered. The reaction was carried out

for the intended period. (half-an-hour to two hours). At the end of the reaction period, the pH of the solution was lowered to 3.5 - 3.0 by adding 2N HCl. Sodium chloride precipitated was separated by filtering the reaction mixture through a Buckner funnel under reduced pressure. The filtrate was transferred to a 500 ml separating funnel. About 50 - 100 ml chloroform (commercial grade, once distilled) was added. The contents of the separating funnel were shaken vigorously with periodic release of pressure developed. The two layers were allowed to separate and the lower chloroform layer was withdrawn. Additional chloroform was added to the aqueous solution in the separating funnel and the extraction procedure was repeated. The chloroform extraction was carried out 5 - 6 times and the extracts were collected in the same container. It was dried overnight over anhydrous Na_2SO_4. Attempts to concentrate the chloroform extract on a rotavapour and to isolate the monomer by addition of n-heptane (nonsolvent) led to the recovery of a sticky mass (due to polymerization of the monomer during the isolation step). The chloroform extract was preserved at 5 - 10 ^{o}C for about 72 hrs in a refrigerator. Subsequent concentration and addition of n-heptane resulted in the separation of a white crystalline solid. The separated solid was isolated by decantation. It was redissolved in minimum quantity of chloroform and crystallized by adding n-heptane. The recrystallized product was dried between filter paper sheets initially and then in a vacuum oven at ambient temperature for 4 hrs.

II a ii **Polymerization/Copolymerization**

The isolated monomer was polymerized in aqueous solution. Potassium peroxydisulphate was used as the initiator and polymerization was carried out at 65°C. Trace quantities of Na_2SO_3 was used as an oxygen scavenger in the reaction medium. The polymerization was carried out under continuous nitrogen bubbling. The polymer, at the end of polymerization, was isolated by precipitation with acetone as nonsolvent, dissolved in water, purified by reprecipitation and dried in a vacuum oven.

Brine solution (0.5 M) was prepared by dissolving (2.9 ± 0.05)g AR grade sodium chloride (purchased from M/s Reechem pvt. Ltd., Hyderabad) in distilled water in a 100 ml volumetric flask. The distilled water used was purified as discussed in section II a i. Recrystallized monomer (0.300 ± 0.01)g was dissolved in 10 ml, 0.5 M NaCl in a 50 ml conical flask. The flask was placed in a thermostat maintained at 65 ± 0.02 °c and nitrogen was bubbled through the solution for about 20 min. The pH of the solution was adjusted to 3.5 (if necessary by the addition of 0.1 N HCl) or 12.0 by adding 1M NaOH, depending upon the pH condition used for the polymerization. When the contents of the flask reached the reaction temperature, 0.1ml of Na_2SO_3 solution (stock solution concentration 0.02%) was added. Na_2SO_3 is primarily used as a scavenger for residual oxygen in the system and not as a reducing agent in the $K_2S_2O_8$ - Na_2SO_3 redox system. 0.1% stock solution of potassium peroxydisulphate (GR grade; purchased from M/s S.D chemicals , Bombay) was prepared.

Polymerization was initiated by adding 0.1 ml $K_2S_2O_8$ solution through a graduated pipette. When polymerization / copolymerization was attempted at low pH values, (acid range) no polymerization was observed to take place upto 2-3 hrs. The formation of the polymer was checked by adding a drop of the solution being polymerized to acetone. When polymerization was carried out at pH 12.0, the solution became quite viscous in about half-an-hour. Polymerization was continued for 2 hrs. At the end of the reaction period, about 30 ml of distilled water was added and the solution was homogenised over a period for 2-3 hrs. The solution was further diluted by pouring it into 50 ml distilled water contained in a 250 ml beaker. The polymer was coagulated by slowly adding the polymer solution to (500 ml) acetone contained in a 1 liter beaker. The precipitated polymer was allowed to settle. The supernatant layer was decanted. The precipitated polymer was redissolved in water and reprecipitated using acetone. The coagulated polymer was gathered by using a glass rod, transferred to a boat formed of aluminium foil. It was dried in a vacuum oven at 65 ^{o}C for 4 hrs. The yield of the polymer was about 80% when the polymerization was carried out at a pH of 12.0.

Copolymerization experiments were carried out using acrylamide as the comonomer. The acrylamide to monomer ratio selected was 9:1 (molar). Thus in the successful copolymerization experiment carried out , 1.28 g acrylamide and 0.31 g of monomer was dissolved in 10 ml, 0.5 M NaCl. The

pH was adjusted to 12.0 using 1M NaoH. The polymerization was initiated by adding 0.1 ml $K_2S_2O_8$ solution of concentration 0.1%. Trace quantity of Na_2SO_3 was used as oxygen scavenger. The yield of the polymer obtained in 2 hrs was 70 % . The polymer recovery and purification steps were identical to those of the homopolymer contained in the previous paragraph. No copolymerization took place when the copolymerization was carried out at a low pH (acid range).

II b. **Methods of characterization**

II b i Viscosity measurements were carried out with an Ubbelohde suspension - level dilution type viscometer with a efflux time of (153.1 ± 0.1) sec for 0.12 M NaCl at 30°C. Kinetic energy corrections were not applied. All measurements were carried out at 30°C. The stock solution of the polymer was prepared by accurately weighing about 0.06g of the polymer, transferring the polymer to a 25 ml volumetric flask, adding about 5 - 10 ml solvent and allowing the polymer to swell in the solvent overnight. The solution was homogenised the next day by shaking the volumetric flask. Solvent was gradually added with periodic mixing of the contents of the flask by shaking. The solution was diluted to the graduation mark. The NaCl solution was prepared by weighing (3.06 ± 0.005)g dried AR grade NaCl in 500 ml purified water.

Viscosity measurements were carried out by transferring 10 ml of the polymer solution to the viscometer mounted in a thermostat maintained at 30 ± 0.01°C. The efflux times were determined using a stop watch reading upto ± 0.1 sec, till the last two readings agreed within 0.1 sec. The solution was diluted by adding a known volume of the solvent through a pipette. The contents of the viscometer were mixed by gently blowing an air stream through the capillary arm of the viscometer. The efflux times for the diluted solution were also determined similarly. The process was repeated for a minimum five concentration solutions. Relative viscosities were calculated as ratios of the flow times for the solution

and solvent and the intrinsic viscosities were obtained by the linear plots of η_{sp}/C vs C.

II b ii. **HPLC analysis**

HPLC technique was used to determine the concentration of N-acryloyl-γ-aminobutyric acid monomer in the chloroform extract. The analysis became necessary because during the isolation of the monomer from the chloroform extract, its rapid polymerization was observed. It was thought that if the constituents of the chloroform extract could be analysed, polymerization of the monomer in chloroform (along with other monomer present) could be carried out.

A Waters ALC/GPC 150 C was used for analysis. It consists of a high precision pump capable of delivering the mobile phase at a preset flow rate ranging from 0.1 to 9.9 ml per min. Set volume of the sample solution is automatically injected into the mobile phase for the analysis. The column compartment is thermostated and also contains a very sensitive refractive index detector. The detector output is fed to a data reduction system capable of handling both GPC and LC mode of analysis.

μ-Styragel columns of porosity 500°A and 100°A were employed for analysis. Higher porosity columns were considered redundant as the separation was expected on the basis of size exclusion and due to the low molecular size of the reactants and products formed in the reaction. The 100°A column was employed in duplicate along with 500°A column to achieve satisfactory separation. HPLC grade chloroform or tetrahydrofuran (purchased from M/s SD Fine chemicals, Bombay) was employed as the mobile phase. The temperature

employed for the analysis was 40°C. The mobile phase was circulated at a flow rate of 0.5 ml/min for a minimum period of 2-3 hrs for thermal equilibrium before analysis and to obtain stable base line. The mobile phase flow rate employed for analysis was 1.0 ml/min.

Approximately 1 to 1.5% solutions of acrylic acid, (purchased from BDH, UK and distilled under reduced pressure), acryloyl chloride, (E Merck, Germany) and γ-aminobutyric acid were prepared by weighing them accurately in a 10 ml volumetric flask and diluting with the mobile phase to the graduation mark. About 0.25 ml chloroform extract was transferred to a 10 ml volumetric flask accurately weighed and diluted to the graduation mark with the mobile phase. 50 or 100 µl of these solutions were injected to the chromatograph and the retention times and the areas under the chromatograms were recorded. The response factor per milligram of the sample injected was calculated.

The total chloroform extract was weighed and about 0.25g of the extract was dissolved in 10 ml of the mobile phase. 100 µl of the solution was injected into the chromatograph and the chromatogram scanned. The experimental condition such as column set employed, mobile phase, its flow rate etc. were maintained identical to those used for determining the response factors. The constituents were identified by the retention times and the amount present was calculated from the response factors earlier determined. From the known injection volume, the amount of acryloyl chloride

and acrylic acid present in the total chloroform extract was calculated, the amount of these chemicals present in the injected volume being known. The amount of acryloyl chloride reacted and thus the conversion to the monomer was calculated as the difference between the acryloyl chloride employed in the reaction minus acryloyl chloride and acrylic acid present in the chloroform extract.

II b iii. **Elemental analyses** for carbon and hydrogen were carried out by micro combustion method. Micro Kjeldahl method was used for nitrogen analysis. Oxygen content was deduced as the difference between hundred and nitrogen, carbon and hydrogen content.

II b iv. **Melting point** was recorded using a Thermonix melting point apparatus supplied by M/s Campbell Electronics, Bombay. The readings were determined in duplicate and the two readings agreed within $\pm$ 0.2^{o}C.

II b v **Infra-red spectra** were recorded using a Pye-Unicam SP3-300 infra-red spectrophotometer. The spectra for the monomer and polymer were recorded in nujol.

II b vi **NMR spectra** were recorded using a Bruker MSL 300 NMR spectrometer. The ^{1}H NMR spectra (for the monomer) were recorded in $CDCl_3$ solutions using tetramethyl silane as internal standard. 5 mm O.D. NMR tubes were used. The ^{13}C NMR spectra (for the polymers and copolymers) were recorded by dissolving the samples in minimum amount of deionised water in a 10 mm O.D. NMR tube and using a capillary containing D_2O for field frequency locking. Chemical shifts were identified with reference to 67.4 ppm for dioxane.

CHAPTER III
RESULTS AND DISCUSSION

III a. Monomer Synthesis and Isolation

N-acryloyl- γ-aminobutyric acid, the monomer synthesized, has the desired $-NH(CH_2)_3COOH$ functional group. Due to the presence of the functional group, the polymers derived from the monomer are likely to exhibit interesting selectivities in mineral separation. They have also intrinsic biological activity and potential applications as drug carriers[17].

The monomer was synthesized by Schotten-Bauman reaction under analogous reaction conditions to those employed by Winston and Kirchner[2] for the preparation of N-methacryloyl- γ-amino-butyric acid. γ-Aminobutyric acid was dissolved in aqueous alkali and reacted with acryloyl chloride at 0 - 5 ^{o}C. Acryloyl chloride is susceptible for hydrolysis under the experimental conditions. The lower temperature employed for the reaction minimizes hydrolysis. At the end of the reaction period, the aqueous solution containing unreacted γ-aminobutyric acid, acryloyl chloride, acrylic acid (formed by the hydrolysis of acryloyl chloride) and the monomer was extracted with chloroform. Repeated extraction was carried out so that the total monomer formed may be recovered. It has already been stated that during the concentration of the chloroform extract a sticky material (polymer formed) was found to separate from the chloroform layer. The solid separated had a variable melting temperature and repeated attempts at recrystallization did not result in a solid with a sharp melting point. Hence, HPLC technique was developed for estimating the concentration of the monomer in

the chloroform concentrate so as to carry out the polymerization/copolymerization without monomer isolation. It was observed that the concentration of the monomer in the chloroform extract remained unaltered over a period of 72 to 96 hrs. As the polymer separated out from the chloroform layer during the concentration step, the polymer is insoluble in chloroform. If the polymerization had occurred during the monomer preparation, the polymer would have remained in the aqueous phase. As the concentration of the monomer remained unaltered in the chloroform extract, the spontaneous polymerization observed must be taking place during concentration step only. The higher temperature of 45-50^{o}C (attained by the solution during concentration) may have aided polymerization. After HPLC analysis, attempts were made to isolate the monomer from the aged solutions. Monomer could be isolated with minimum effort. The reasons for spontaneous polymerization when the chloroform extract is immediately concentrated and easy isolation from the aged solutions are not clear. It is speculated that source of free radical is present in the aqueous solution. It is extracted into chloroform and brings about polymerization during concentration. The contaminant gets deactivated (perhaps by bimolecular anhilation) when stored at low temperatures. It was repeatedly confirmed that the monomer could be isolated when the chloroform extract was aged for 72 hrs.

III b **Liquid Chromatography analysis**

In view of the difficulties encountered in monomer isolation, it was considered desirable to develop an analytical method for the in situ analysis of the monomer. We preferred to analyse the chloroform extract rather than aqueous solution due to i) the highly alkaline nature of the reaction medium and ii) the limited solubility of γ-aminobutyric acid in organic solvent as compared to aqueous solution.

Analysis was carried out using a Waters ALC/GPC 150 C chromatograph. μ-styragel columns of porosity 100°A - (two nos) and 500°A - (one no) were employed as stationary phase. When chloroform was employed as the mobile phase the constituents were not separated. Tetrahydrofuran yielded satisfactory separation.

The chromatogram obtained for the two hours reaction sample (chloroform extract) is shown in Fig 1. From the retention times obtained by injecting pure components under identical analytical conditions, the following peaks are identified. The peak with a retention time (RT) of 27.99 min. is due to chloroform and the one with RT 27.25 min. is due to acrylic acid. The late eluting peak with a RT of 29.3 min. is ascribed to stabiliser in the mobile phase. The early eluting peaks at 22.35 and 23.14 min. are unidentified. The peak with a RT 24.42 min. is due to the monomer.

It is interesting to note that the peak due to acryloyl chloride (expected to elute at 28.60 min) is not observed in Fig.1. However, when the reaction was carried out

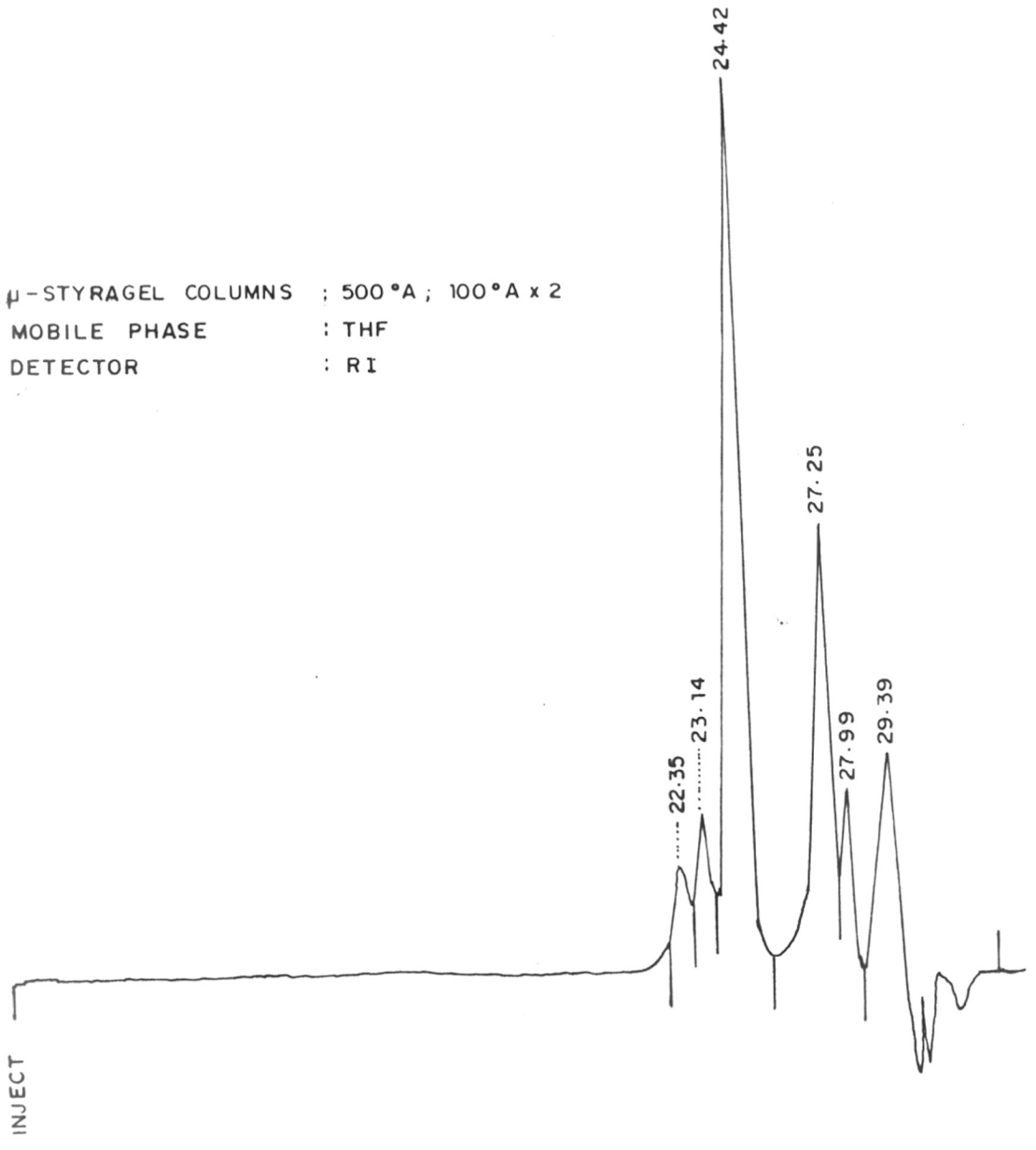


FIG. 1. LC OF CHLOROFORM EXTRACT

(REACTION MIXTURE, 2hr)

for half-an-hour, the presence of both acrylic acid and acryloyl chloride could be clearly seen in the chromatogram. It is also noted that γ-aminobutyric acid is not detected in the chromatogram (Fig.1). This is due to its very low solubility in chloroform. It is highly soluble in water. It may be stated that the isolated monomer when subsequently injected also yielded a RT 24.40 min.

The amount of monomer formed was calculated based on acryloyl chloride employed in the reaction. The amount of acryloyl chloride reacted to form the monomer was calculated as the difference between acryloyl chloride employed and acryloyl chloride present either as acryloyl chloride and as acrylic acid in the reaction mixture (chloroform extract). These calculations were carried out on the basis of response factors obtained by injecting pure components under identical conditions of analysis. Tab.1 gives the values of the response factors obtained for the different components.

The monomer conversion obtained in 1 / 2 hr reaction time (reaction time counted from the end of the addition of acryloyl chloride) was about 8 % and the conversion after two hours was 18 %. It has been noted that for the two hours reaction sample, the chromatogram (Fig. 1) does not indicate the presence of acryloyl chloride. Due to the simultaneous hydrolysis of acryloyl chloride (undesirable byproduct, acrylic acid), the maximum conversion of acryloyl chloride to monomer obtained is less than 20 %. It has already been stated that the monomer concentration in the chloroform

Table I

LC Analysis of N-Acryloyl-γaminobutyric acid

Stationary phase : μ-styragel 500 A^{o} x 1
100 A^{o} x 2
Mobile phase : THF
Flow rate : 1 ml/min.
Detector : RI
Temperature : 40°C

Sample injected	Solution concn. (g/dl)	Inj. vol. (μl)	Retention time (min)	Area under LC curve	Response factor (amount/ area)
Acrylic acid	1.419	50	27.26	256988480	2.761×10^{-12}
Acryloyl chloride	1.1685	50	28.60	188770980	3.095×10^{-12}
γ-amino butyric acid	0.080	100	27.53	161180482	4.963×10^{-13}
Chloro-form	0.233	50	27.98	30108849	3.869×10^{-12}

extract remained unchanged for a period of 3 - 4 days.

Thus satisfactory separation of the constituents is obtained when THF was used as the mobile phase and μ-Styragel as the stationary phase. From the retention time listed Tab. 1 and the sensitivity of separation to the mobile phase used (satisfactory separation obtained with THF as mobile phase; no separation with chloroform as mobile phase), it would appear that the separation mechanism involved is liquid chromatography and not size exclusion. The reaction kinetics for the formation of the monomer can be evaluated by extending the liquid chromatographic technique.

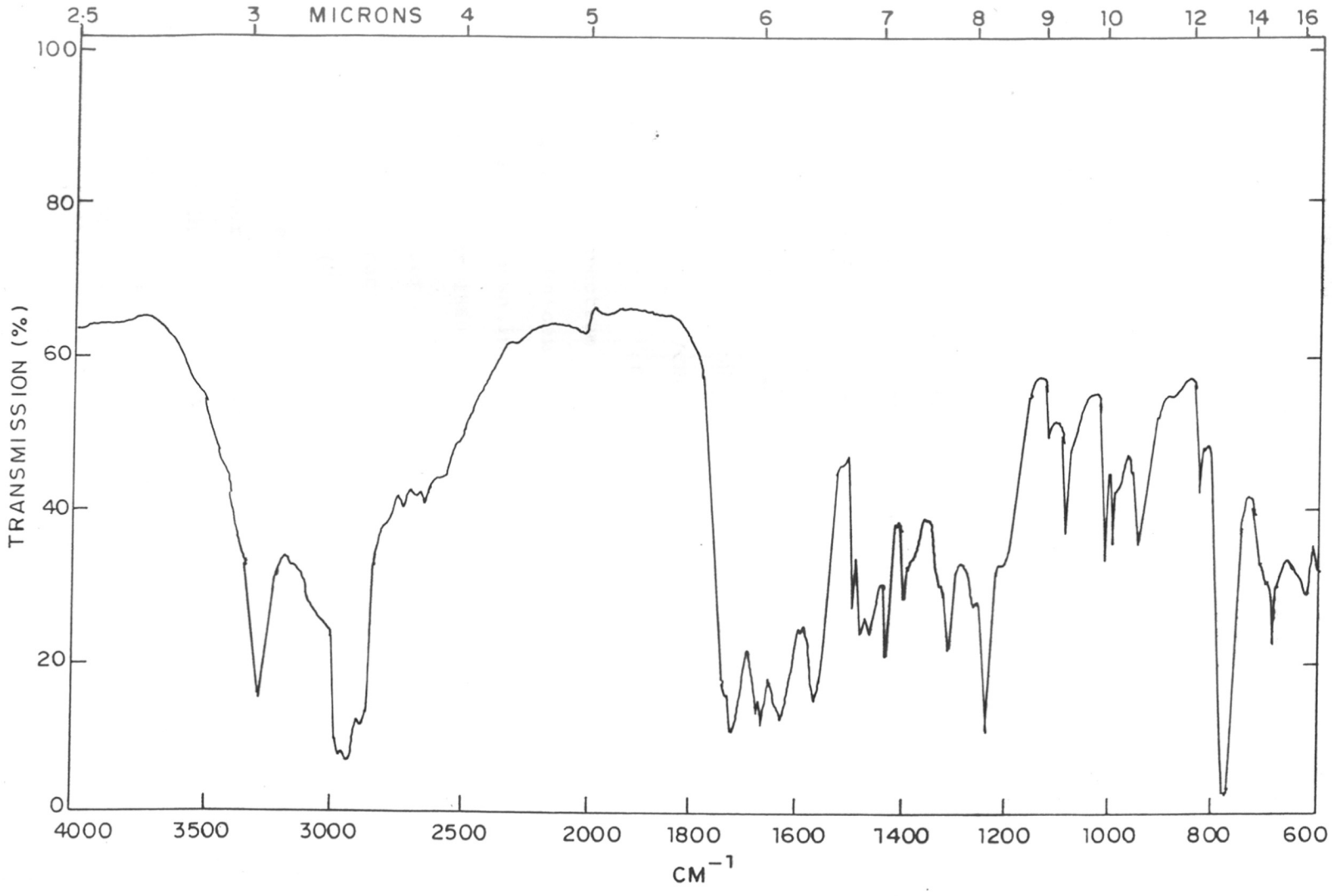


FIG. 2. IR SPECTRUM OF N-ACRYLOYL γ-AMINOBUTYRIC ACID (MONOMER) IN NUJOL

III c. N- Acryloyl- γ -aminobutyric acid

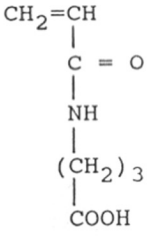
The isolated product from the chloroform extract was a white crystalline solid. The melting point determined using Thermonix Melting Point apparatus was 98°C.

Elemental analysis :

	Carbon	Hydrogen	Nitrogen	Oxygen
Calculated (%)	53.90	7.01	8.92	30.60
Found (%)	52.67	6.84	9.00	31.49

The calculated and experimentally obtained values are in very good agreement.

The monomer has the following structure



IR spectrum of the monomer was scanned in nujol using a Pye Unicam SP3-300 spectrometer. The spectrum obtained is shown in Fig.2. The peak observed at 3290 cm^{-1} due to -NH, at 1560 cm^{-1} due to -NH (amide), amide C = O at 1620 cm^{-1}, acid C = O at 1720 cm^{-1} are in agreement with expected structure of the monomer. The presence of unsaturation (C = C) is shown by the absorbance at 1660 cm^{-1}.

The NMR spectra were scanned with a Bruker MSL 300 spectometer. The spectrum was scanned for the monomer solution in $CDCl_3$ (Fig.3). The spectrum obtained after D_2O exchange is shown in Fig.4. The vinyl protons at 6.3, 6.1, and 5.65 ppm show a typical AMX pattern, total 12 signals, 4

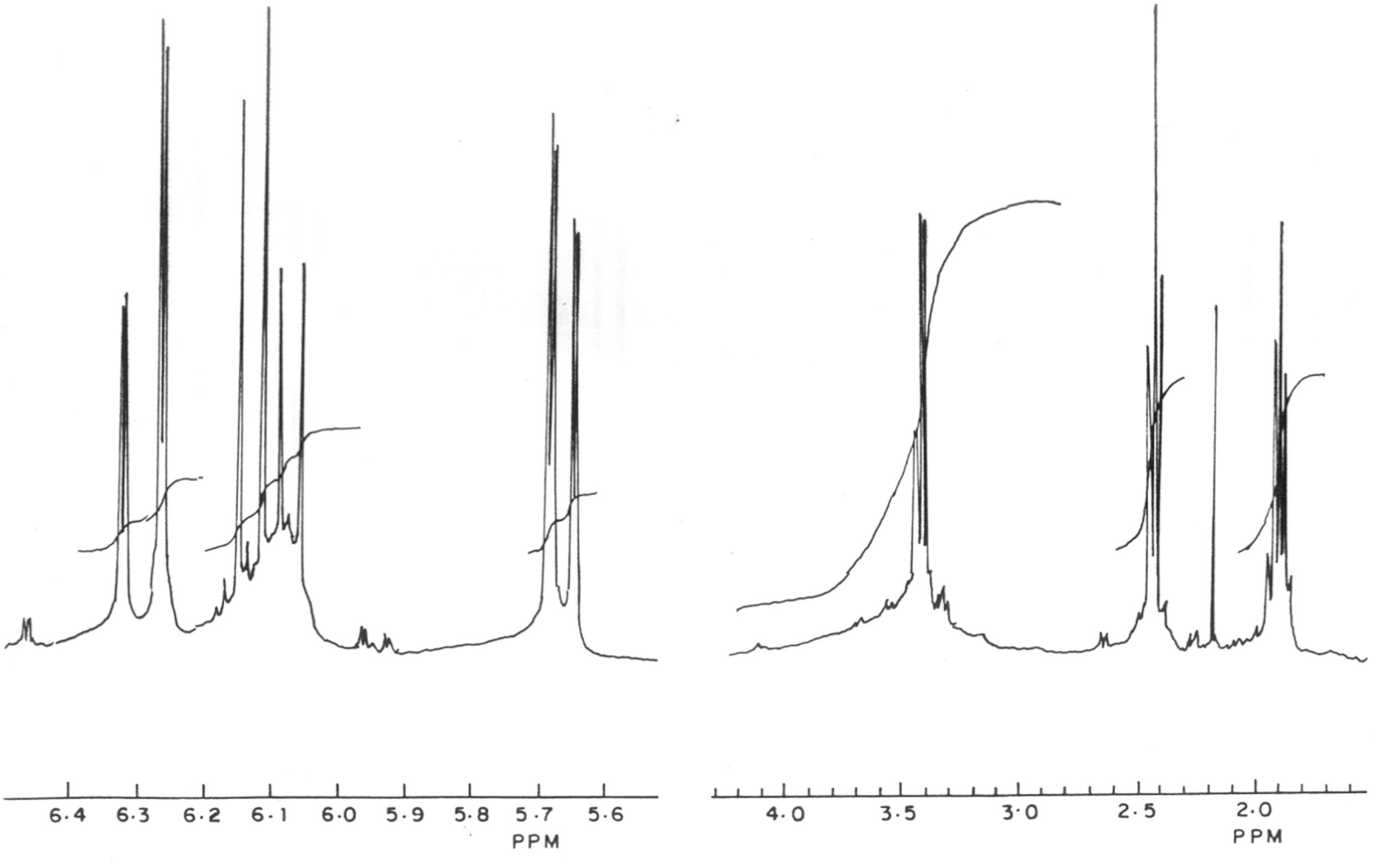


FIG. 3. ^{1}H NMR SPECTRUM OF N-ACRYLOYL γ-AMINOBUTYRIC ACID (MONOMER) IN $CDCl_3$

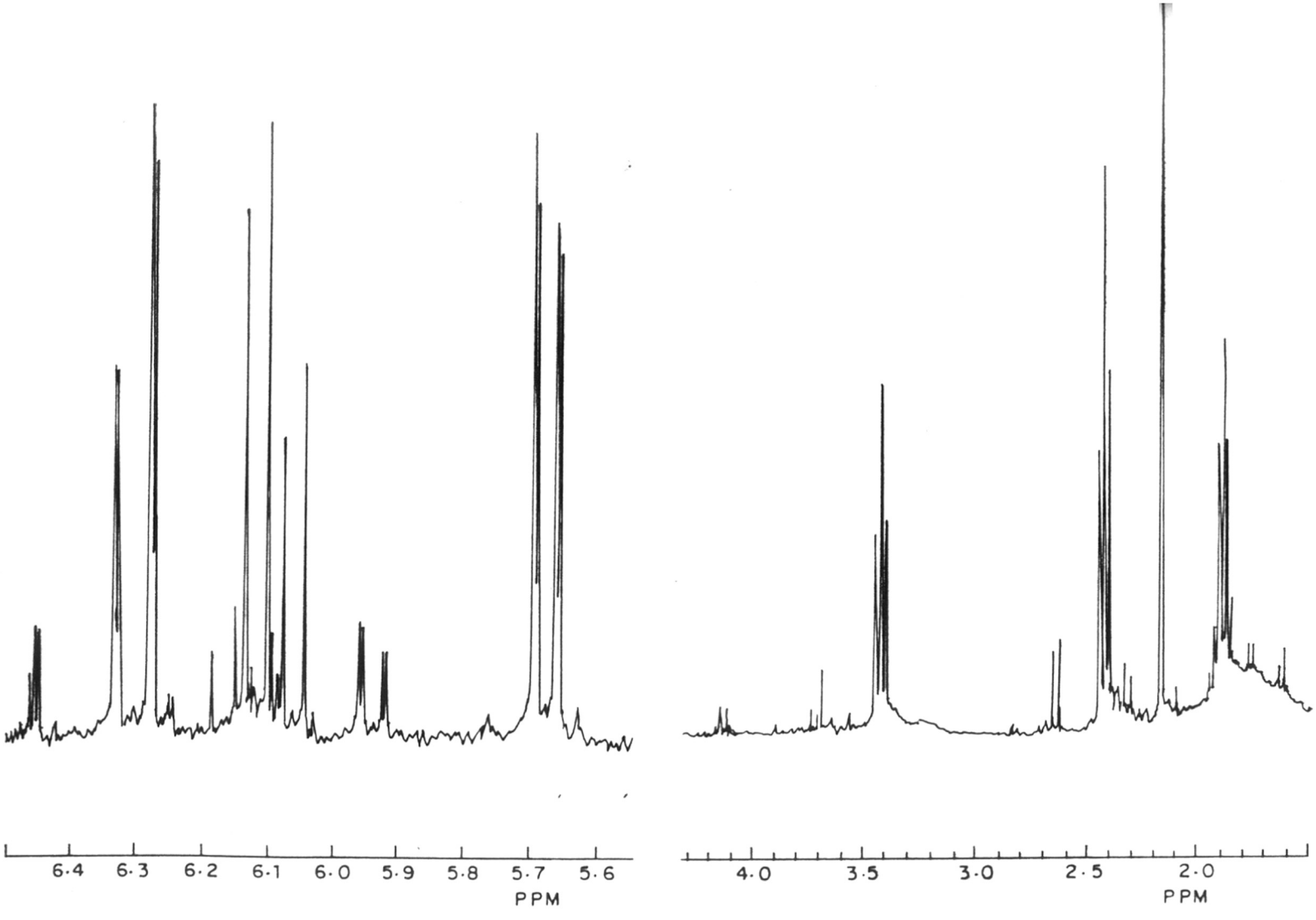


FIG. 4. ^{1}H NMR SPECTRUM OF N-ACRYLOYL γ-AMINOBUTYRIC ACID (MONOMER) IN $CDCl_3$ AFTER D_2O EXCHANGE

for each proton. The signal at 6.1 ppm is due to the proton on the substituted carbon and those at 6.3 and 5.65 ppm due to the two protons on the other vinyl carbon. The multiplicity of the signals obtained is in agreement with the above assignment. The quartet at 3.5 ppm is due to the methyl group alpha to the amide NH. The quintet at 1.9 ppm is due to the protons beta to the amide NH. The triplet at 2.5 ppm is due to the methylene protons attached to the carbonyl. By comparing the original spectrum (Fig.3) with the spectrum obtained after D_2O exchange (Fig.4), it is seen that the original quartet at 3.5 ppm is reduced to triplet after D_2O exchange due to the presence of the amide NH group. The signal at 6.1 ppm is twice in intensity to those at 6.3 and 5.65 ppm respectively indicating the contribution from two protons. One of the protons is already accounted to be the proton on the substituted vinyl carbon. The other proton, by elimination, should be the proton of the carboxylic acid. On deuterium exchange, the intensity of the peak at 6.1 ppm is indeed observed to reduce. This confirms the assignment of 6.1 ppm for the proton in the carboxylic acid group. The spectrum obtained thus clearly indicates that the NMR spectrum is in agreement with the expected structure of N-acryloyl-γ-aminobutyric acid.

III d. **Polymerization of N-acryloyl-γ-aminobutyric acid**

The monomer has an amino acid side chain. Amide NH can acquire a hydrogen ion. Similarly the carboxylic acid can dissociate. The monomer thus has an ampholytic character. Difficulties in polymerizing ionizable monomers in aqueous solutions have been recognized[13,14] and these have been briefly discussed in Sec.I b. Several of these monomers, due to their hydrophilic nature, are soluble in a limited number of organic solvents. Even if the monomers are freely miscible with some organic solvent, the polymers obtained, in most of the cases, are insoluble in organic solvents. N-acryloyl glycine and N-acryloyl-6-amino caproic acid have been polymerized in 1,4 dioxane by precipitation polymerization technique[18]. Hence homogenous solution polymerization can be carried out only in aqueous solutions. However, due to the presence of electrostatic charges the propagation reaction is severely hindered. Suppression of ionization and facile polymerization are practical in the case of many ionizable monomers[13,14]. For example, acrylic acid can be conveniently polymerized at low pH values in aqueous solutions. In the case of the monomer synthesized here, either a positive or negative charge is likely to be present when the monomer is dissolved in water due to its ampholytic character. The spontaneous polymerization of the monomer during its preparation and isolation, it may be noted, is contrary to the above reasoning. We have already seen (Sec. III a) that polymerization took place only during concentration of the

chloroform extract. Acryloyl chloride and acrylic acid content in the chloroform extract and monomer recovered accounted to over 95 % of the acryloyl chloride employed in the reaction. Hence, polymerization during the monomer preparation and polymer remaining in the aqueous layer are overruled.

Attempts to polymerize / copolymerize the synthesized monomer in aqueous solutions were not successful. These experiments were carried out using potassium peroxydisulfate as initiator at a pH of 3.5. Acrylamide was used as the comonomer.

Viscosity behaviour of the aqueous solution of polymer formed during monomer isolation was examined as a function of pH. Due to insufficient quantity of the polymer, solution of only one concentration was investigated. Initial pH of the aqueous solution was brought to 2.0 - 2.5 using 2 N HCl. The solution was transferred to an Ubbelohde viscometer mounted in a thermostat. After determining the flow times, aliquots of 2 N NaOH were added to the solution through a graduated pipette. After allowing sufficient time for thermal equilibrium, the solutions were mixed and the flow times were determined. The solution was transferred to a beaker and the pH of the solution was determined. The process was repeated for 7 - 8 values of pH. It is assumed that the effect of dilution (due to the addition of 2 N NaOH) on the viscosity of the solution can be ignored. The results obtained are shown in Fig.5. It is observed that viscosities are high at low values of pH and attain lower asymptotic values at higher

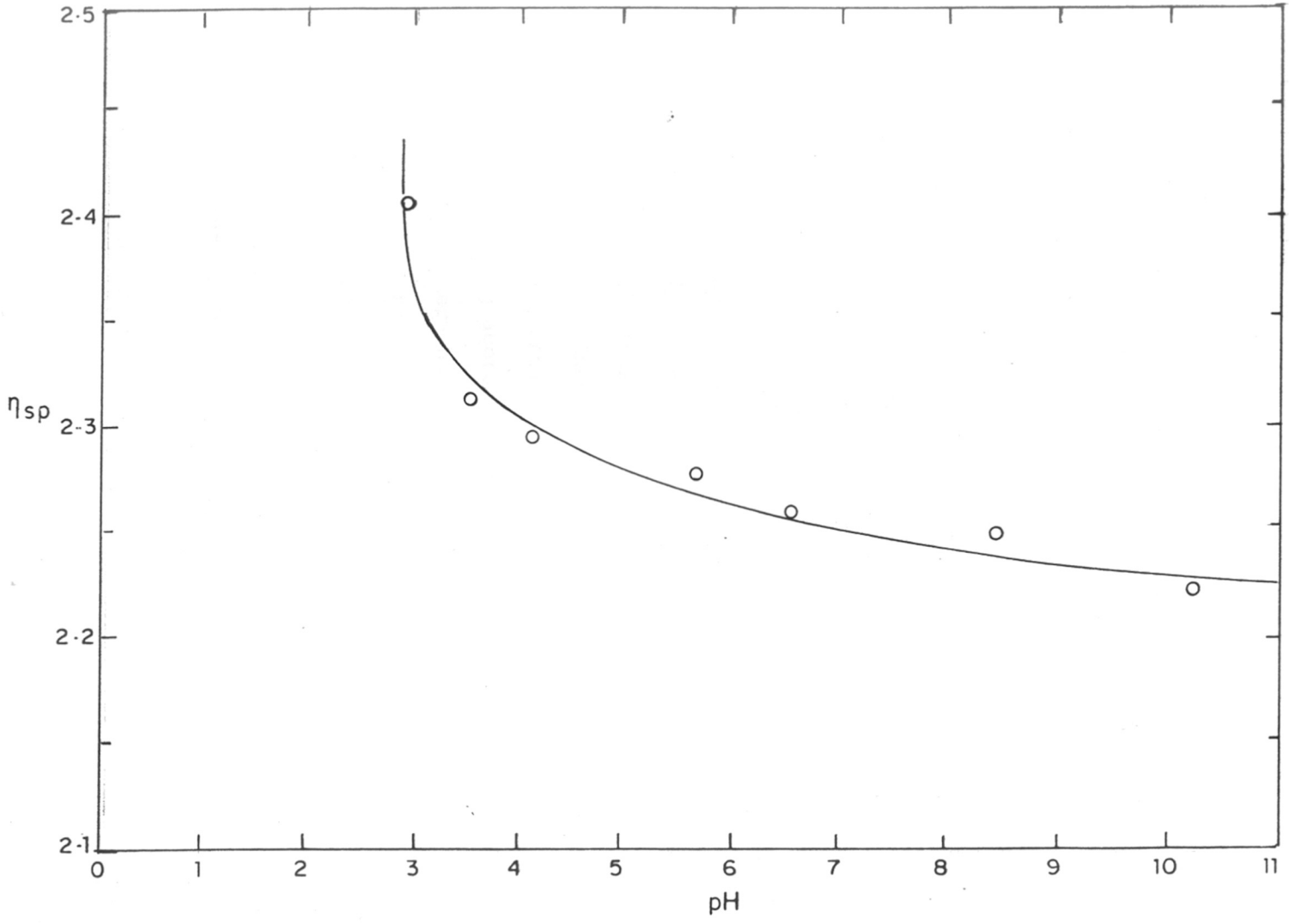


FIG. 5. PLOT OF η_{sp} vs pH FOR SOLUTION OF POLY (N-ACRYLOYL γ-AMINOBUTYRIC ACID) (POLYMER OBTAINED DURING ISOLATION)

values of pH. The result is unexpected. It demonstrates that when the electrostatic charge is far removed from the main chain, the charge does not contribute to the net electrostatic interactions of the charges along the chain. Hence it is expected that the propagation rate constant is not greatly retarded in aqueous polymerization of the monomer when the electrostatic charge is farther away from the site of addition. We are not aware of any such report in literature. It may be noted that the reported[18] polymerization of N-acryloyl glycine and N-acryloyl-6-amino caproic acid has been carried out in 1,4 dioxane.

Polymerizations were carried out at alkaline conditions of pH to verify whether the conclusions drawn above represent reality in polymerization. Experimental details are given in Sec. II a ii. Potassium peroxydisulphate was used as initiator. 0.5 M NaCl was used as solvent and polymerization was carried out at 65^{o}C at a pH of 12.0. The reaction was discontinued after 2 hrs and the polymer was coagulated using acetone. About 80 % conversion was obtained and the polymer had a intrinsic viscosity of 0.67 dl/g in 0.12 M NaCl at 30^{o}C.

Copolymerization of the monomer with acrylamide was similarly carried out using 0.5 M NaCl as solvent, potassium peroxydisulphate as initiator. The experiment was also carried out at 65^{o}C at pH of 12.0. The monomer to acrylamide molar ratio was 1 : 9. The polymer was isolated after 2 hrs of reaction using acetone as precipitant. The monomer

conversion to polymer was 70% The polymer had a intrinsic viscosity of 5.9 dl / g in 0.12 M NaCl at 30°C.

The lower value of intrinsic viscosity for the homopolymer and higher intrinsic viscosity for the copolymer obtained under similar conditions of polymerization shows that the effect of the electrostatic charge on polymerization persists even under these highly alkaline conditions. Polymerization is completely inhibited when carried out at lower values of pH. Good rates of polymerization and molecular weights are obtained when polymerizations are carried out in alkaline ranges of pH. Hence the unexpected results obtained in the pH dependence of the viscosity of the polymer shown in Fig.5, is confirmed by polymerization experiments.

Recently, the potentiometric behaviour of poly(N-methacryloyl glycine) and poly(N-methacryloyl-β-alanine) has been reported[19]. It may be noted that Winston and Kirchner have also reported[2] the preparation and isolation of the corresponding monomers of these polymers. Winston and Kirchner, however, did not report the preparation of poly(N-methacryloyl glycine) and poly(N-methacryloyl- β -alanine). Instead they have esterified the monomers and polymerized them. It may be noted that Barbucci et al[18] have carried out the polymerization of N-acryloyl glycine and N-acryloyl-6 caproic acid in 1,4 dioxane using azobisisobutyronitrile (AIBN) as initiator. The authors have noted that it resulted in precipitation polymerization i.e. separation of polymer from the reaction mixture as polymerization proceeds.

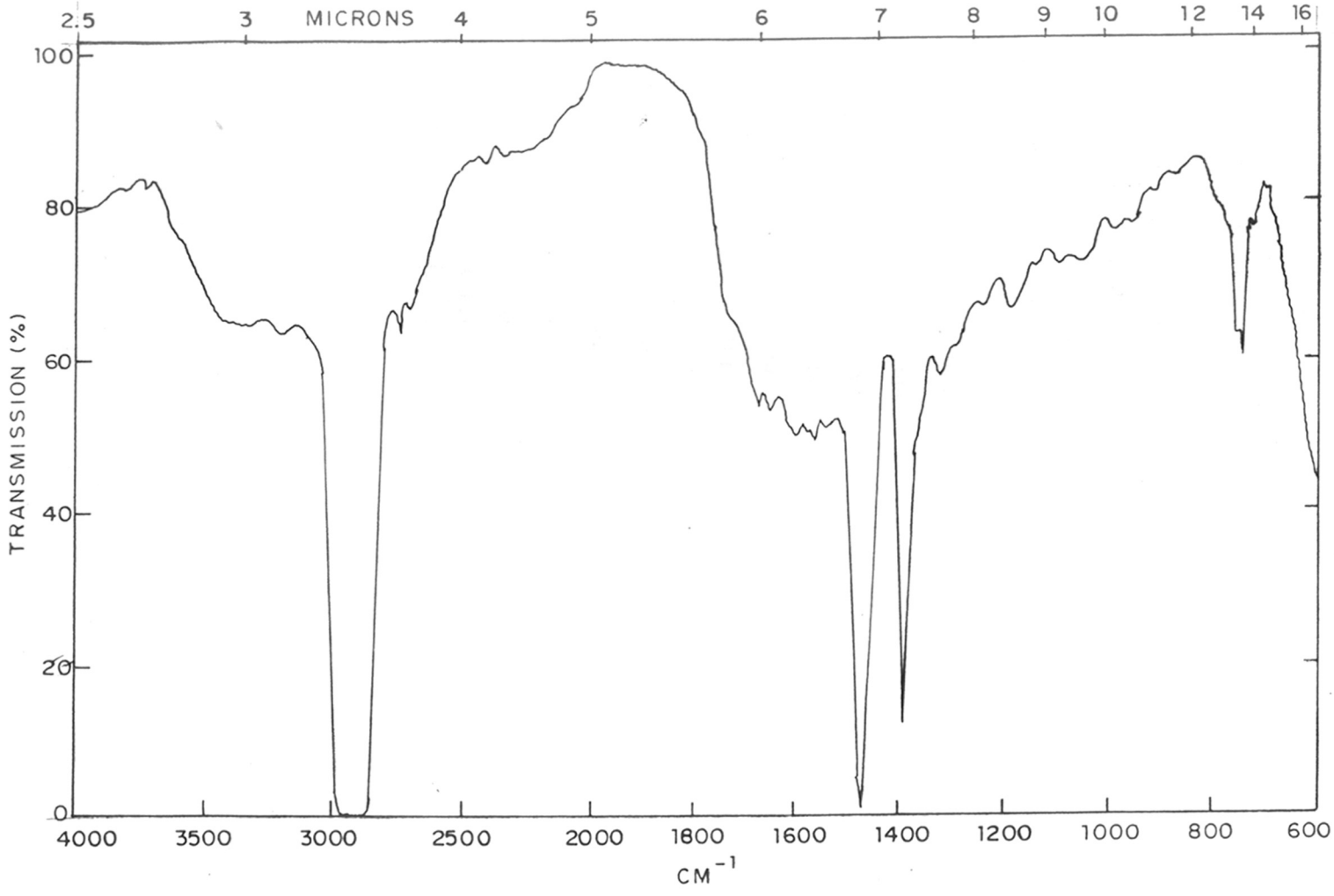


FIG. 6. IR SPECTRUM OF POLY (N-ACRYLOYL δ- AMINOBUTYRIC ACID) IN NUJOL

Fig.6 shows the IR spectrum of the homopolymer in nujol. As compared to Fig.2, the spectrum in Fig.6 is not resolved. A broad minima at 3200 - 3300 cm^{-1} confirms the presence of NH. The presence of NH (amide) is shown by sharp peak at 1500 cm^{-1}. The presence of amide C = O and acid C = O are shown as broad minima between 1500 - 1700 cm^{-1}. It may be noted that the band at 1660 cm^{-1} due to unsaturation present in Fig.2 is absent in Fig.6.

The ^{13}C NMR spectrum recorded for the copolymer solution in water is shown in Fig.7. The main chain carbon atoms, both from acrylamide and N-acryloyl γ-aminobutyric acid, give signals around 34 - 36 ppm due to unsubstituted C (main chain). These signals are also present in the spectrum of homopolymers, i.e polyacrylamide and poly(N-acryloyl γ-aminobutyric acid) (Figs not included). The amide carbonyl from acrylamide gives signal at 180 ppm and due to the monomer at 176.8 ppm. The signals present at 42.7, 26.0 and 35.4 ppm (also present in the in the spectrum of the homopolymer) are ascribed to the three methylene groups in the pendent chain. Their presence unequivocally confirms the incorporation of N-acryloyl γ-aminobutyric acid into the copolymer chain. It is not the purpose of this investigation to evaluate the copolymerization composition and reactivity ratios. Moreover, copolymerization has been carried to high conversions (70 %). The copolymer composition estimated by the peak intensity at 176.8 ppm (amide carbonyl of the monomer) to total amide carbonyl intensity at 176.8 and 180

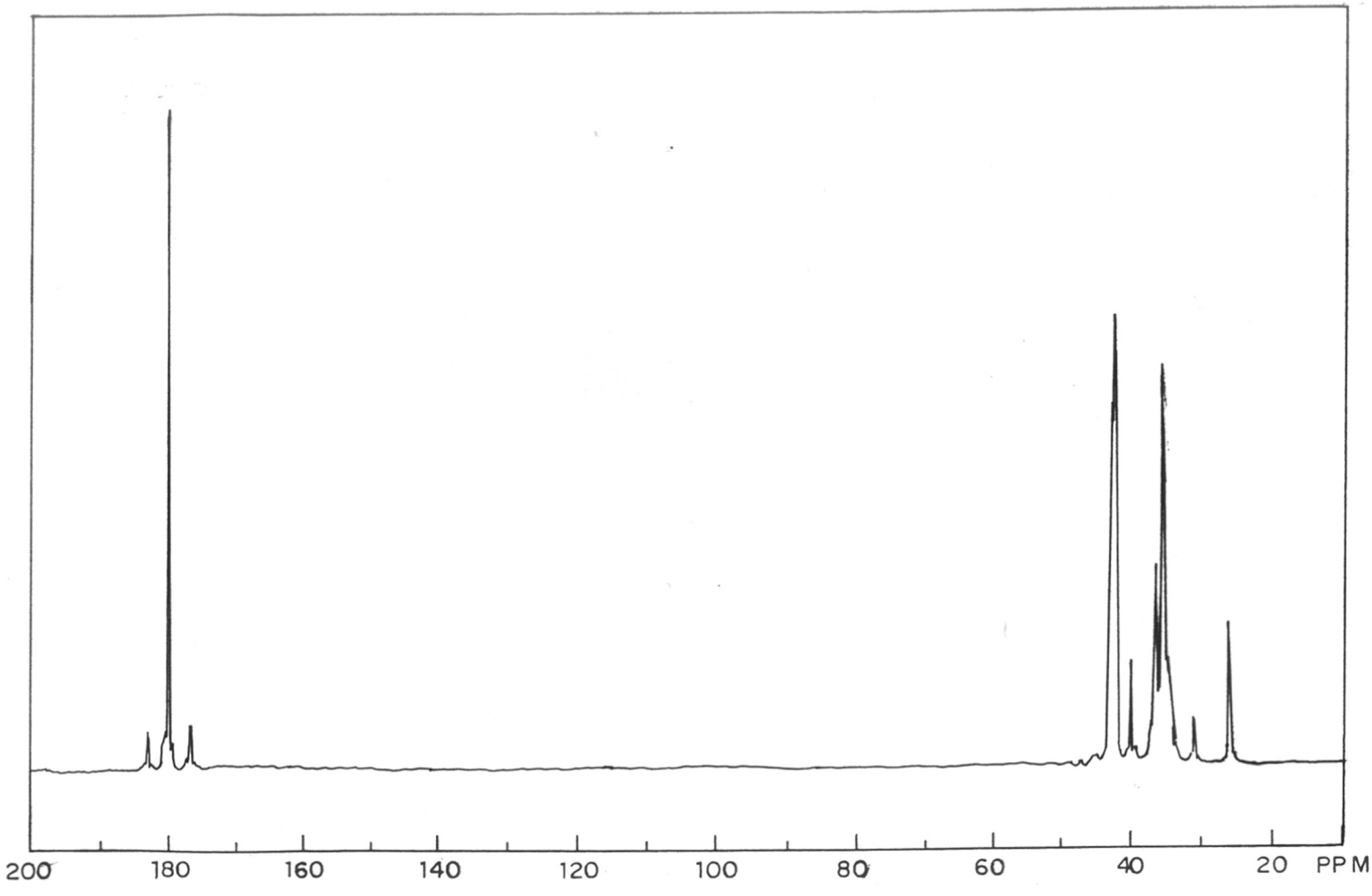


FIG. 7. ^{13}C NMR SPECTRUM OF COPOLYMER POLY(ACRYLAMIDE-CO-N-ACRYLOYL γ-AMINOBUTYRIC ACID) IN WATER

ppm shows that the copolymer has 8 mole percent N-acryloyl γ-aminobutyric acid in the copolymer. The monomer feed molar ratio in the copolymerization employed was 10 % . It shows that the monomer is sluggish to polymerization as compared to acrylamide.

III e. **Conclusion**

i) N-acryloyl γ-aminobutyric acid was synthesized by reacting acryloyl chloride with γ-aminobutyric acid in aqueous alkali. The monomer undergoes spontaneous polymerization during isolation. By suitable modification of the isolation procedure (aging the solution), pure monomer has been isolated.

ii) Inspite of low temperature employed for the reaction, the conversion of acryloyl chloride to monomer is limited to 18 to 20 percent. Most of the acryloyl chloride is hydrolysed under the experimental conditions.

iii) Elemental analysis, IR and NMR confirm the structure of the monomer.

iv) Homogenous polymerization of the monomer in organic solvents is not feasible due to its hydrophilic nature. The monomer resists polymerization / copolymerization in aqueous solutions at low pH. Polymerization /copolymerization could be conviently carried out in aqueous solution at high pH. The behaviour is ascribed to low contribution of the carboxylate ion of the pendent group to total electrostatic interaction of the charges along the polymer due to its remoteness from the backbone.

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