Polycondensation of Acid anhydrides with Asparaginyl Imide Diamine and Curing with Styrene as Smart Polymers for Sustained Release Drug Delivery System

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Abstract
Polymers can provide new and effective ways of administering drugs and enhance therapeutic efficacy. Polymers also present a means for controlled delivery of several drugs in the form of polymeric drug carriers or polymeric prodrugs. Our study involved synthesize of new condensed polymers from reacting of unsaturated acid anhydride such as maleic or methylnadic anhydrides, with prepared N-asparaginyl imide diamine, which prepared from reacting of asparaginyl chloride with ammonia. The new condensed polymers were curing with styrene and the cross-linked polymers were obtained with high conversion percent. The physical properties were measured and all the prepared monomers and polymers were characterized by FT-IR and UV spectroscopy. The intrinsic viscosity was calculated and swelling index was measured for the prepared cross-linked polymers. The inserted asparagine as amino acid could enhance the biodegradability properties, and these prepared bioactive polymers can be used as biomaterial in different applications, which could be acted as smart polymers due to pH sensitivity.

Keywords
Polycondensation; Acid anhydride; Asparagine; Polymers; Sustained Release; Drug Delivery

توفر البوليمرات وسائل جديدة وفعالة لاعطاء الادوية وتعزيز الفعالية العلاجية .البوليمرات تقدم ايضا واسطة لاسلوب التحرر المراقب للادوية كبوليمرات حاملة او كمنتجات للدواء. حضر في هذا البحث بعض البوليمرات التكاثفية الجديدة من تفاعل الحوامض اللامائية مثل حامض الماليئيك أو مثيل نادك اللامائي من N-أسبارجين أيمايد داي أمين المحضر من تفاعل الأسبارجنيل كلورايد مع الأمونيا. إن نسبة التحويل المئوية للبوليمر التكاثفي المحضر كا نت عالية . قيست الصفات الفيزياوية وشخصت المونومرات والبوليمرات المحضرة بواسطة مطياف الأشعة فوق البنفسجية والأشعة تحت الحمراء وقيست اللزوجة الجوهرية وأجري التحليل الحراري للبوليمرات المحضرة. إن إدخال الأسباراجين كحامض أميني في السلسلة البوليمرية ساعدت على صفة التحلل البايولوجي . وإن البوليمر المحضر الفعال بايولوجيا يمكن إستعماله لتطبيقات مختلفة، والذي يمكن إستعماله كبوليمر ذكي نتيجة تحسسه للدالة الحامضية في أوساط مختلفة.

Introduction

In the last two decades, the development of polymers, which change their structures and properties in response to environmental stimuli such as pH, temperature and light, has attracted a great deal attention [1-3]. Such polymers have been called "Smart Polymers", "Intelligent Polymers", "Stimulus-Sensitive Polymers" or "Responsive Polymers". They have been used in many applications ranging from bioactive delivery to separation [4]. New complex polymers used as drug delivery systems allow to reduce side effects and toxicity of drugs, and to improve patient compliance. Polymers also provide the means for controlled delivery of drugs in the form of polymeric drug carriers or polymeric pro-drugs.[5] Polymeric drug delivery systems allow for a variety of routes for drug administration (oral, parenteral, transdermal, nasal, ocular, etc.)[6] Asparagine is a nonessential amino acid, which means that it is manufactured from other amino acids in the liver. [7] Asparagine, formed from another amino acid, aspartic acid, and it is needed to maintain balance in the central nervous system; it is an essential component of those proteins that are concerned with signaling, neuronal development and transmission across nerve endings [7]. As it is transformed back into aspartic acid, asparagine releases energy that brain and nervous system used for metabolism .Shortage of asparagine can lead to confusion, headaches, depression, irritability, or, in intense cases, psychosis [8]. Also it promotes the process by which one amino acid is transformed into another in the liver. Asparagine is found in dairy, beef, poultry and eggs. [9] It has the ability to be modified by several structural group and joined with medications to be released as targeted or pH-induced drug delivery mechanism [10] the synthesis of a new family of biodegradable α-amino acid polymer with pendant benzyl ether groups and hydroxyl functional groups was reported [11].An example is the strategy employs the ring opening reaction of O-benzyl-serine-N-carboxy anhydride with di p-toluenesulfonic acid salt, of bis -1-valine butane-1,4-diester, followed by solution Polycondensation reaction with di-p-nitrophenyl sebacate in N,N-dimethyl acetamide, catalytic hydrogenation benzyl ether protected resorbable poly (ester anhydride) networks based on caprolactam, L-lactide and D,L-lactide oligomers . The ring opening polymerization of the monomer yielded hydroxyl oligomers, which were end-functionalized with succinic anhydride and reacted with methacrylic anhydride to yield dimethacrylated oligomeric containing anhydride bonds. The cross linking of the oligomers were carried out thermally with dibenzoyl peroxide leading to polymer network with high glass-transition temperature and high water absorption and complete mass loss in 4 days [12]. Hydroxyl-group functional polylactone were prepared and converted to acid terminated poly esters in a reaction with a series of alkenylsuccumic anhydrides containing 8, 12 or 18 carbons in their alkenyl chains moiety in their poly ester blocks. The hydrolysis behavior was found to depend on the thermal properties of the polymer precursors [13]. Many new condensed polymers were synthesized from reacting sulfonated amino acids derivatives such as histidine and D-alanine with benzidine as biomaterial polymers [14]. New poly (ester amide) of 1, 4-butane-diol acid, and caprolactam were prepared and appears to be completely biodegradable and shows both good performance and good processing behavior [15]. Poly maleimide is a type of polymer with high reactivity and excellent thermal stability [16]. Generally, polymaleimide is synthesized by homopolymerization via free radical or anionic polymerization [17, 18]. The other is thermolysis or hydrolysis. In this study, our primary aim was to synthesize and validate new condensed polymers of the amino acid asparagine with unsaturated acid anhydride such as maleic or methyl-nadic anhydrides then studying the release profile of asparagine from these two polymers at acidic and alkaline media.

Experimental

Instrumentation:

Melting points were measured using Gallen Kamp M. F. B-600 melting point apparatus. Infrared spectrophotometer measurements were performed using pye-unican SP3-100 U.V-Visible double beam scanning spectrophotometer-260 at room temperature. Differential scanning calorimetry (DSC) and Thermo gravimetric analysis (TGA) were recorded using (PL-STA 1500, Rheometric Scientific co. UK). The inherent viscosities were measured at 25^{o}C, DMSO. Swelling percent of the polymers was determined by using 0.2g of polymer soaked in solution of pH 1.1 for 5 days Viscosity measured by Ostwald viscometer. All chemicals were purchased from Fluka and BDH; all the available chemical reagents were used without further purification.

Controlled Release Study

100 mg of the prepared asparagine polymer was kept in cylinder containing 50:50 ml of buffer: dioxane and in water bath at 37^{o}C without stirring. A 2 ml sample from the release medium was periodically withdrawn and analyzed by U.V at 300nm in order to determine the amount of the released asparagine.[19] The same volume replaced with equal volume of fresh dissolution media .Calibration curve was constructed with a software built in the computerized U.V spectrophotometer, the amount 0.1mg of the released asparagine was determined directly from the software for many days, using the calibration curve in different pH values at 37^{o}C as shown in fig. (3) and fig. (4).

Swelling percent ($\Delta_{m)}$ of the prepared polymers was studied and calculated according to the following equation

$\Delta_m = m_1 - m_0 / m_0 \times 100$

When m_0 is the weight of a dry polymer , m_1 is the weight of swelled polymer in pH 1.1. The softening point of the cross-linked polymer was > 300^{o}C, which was measured by using melting point apparatus.

Modification of Asparagine to its Acid chloride [M_1]

To a round bottomed two necks flask equipped with a magnetic stirrer, condenser and separatory funnel, it was charged with (5g, 0.01mole) of asparagine with 15ml of 1:10 of DMF: Dioxane. Thionyl chloride (0.01mole) was added drop wise for about 15 minutes, a yellow product was isolated and washed with diethyl ether for several times and dried at 50^{o}C, the yield was 75%.

Synthesis of Imide Asparagine Diamine [M_2]

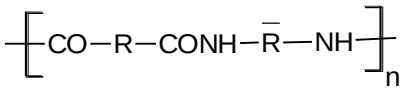
(5g, 0.01mole) asparaginyl chloride [M_1] was dissolved in 10ml of dioxane with few drops of DMF (Dimethylformamide); the mixture was placed in a round bottomed flask with continued stirring. 0.5 molar ratio of ammonia was added drop wise at room temperature. The imide diamine product [M_2] was filtered and purified from ether, dried at 50^{o}C, the yield was 75%.

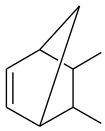
Melting point was equal to 135^{o}C.

Polycondensation Polymerization [M_2] with Acid anhydrides [M_3-M_4]

In a round bottomed two necks flask equipped with a thermometer and reflux condenser was charged with (2.5g, 0.001mole) of prepared product [M_2] and dissolved by adding 15ml of dioxane with few drop of DMF. Acid anhydride (0.001 mole) that is maleic anhydride (first experiment) and methyl nadic anhydride (second experiment) was added gradually, and then the mixture was stirred and refluxed. The stirring was continued for 1hr. the colored condensed polymers [M_3 & M_4] was collected from the first and second experiments respectively, filtered and washed with ether and dried at 50^{o}C, the conversion % was (75-80) %.

Table (1):- Physical Properties of Condensed Polymers [M_3 & M_4]

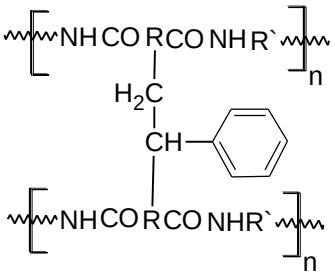


Poly No.	R	$\bar{R}$	Color	$[\eta]_{in}$ d/g	Softening point ^oC	Conversion %
M_3			Yellow	0.71	163.3-177.3	75
M_4			Brown	0.78	250-260	80

Cross linking of Prepared Polymers [M_3 & M_4] to [M_5 & M_6] by Using Styrene

The condensed polymers [M_3 & M_4] were cross-linked with styrene monomer by free radical polymerization using 1:1 molar ratio, in the presence of mixture solvents of DMF: Dioxane with 1:10 volume, the dibenzoyl peroxide was used as initiator with 0.05% concentration. The mixture was heated about 1 hr. at 90^{o}C. The cross-linked polymer was poured into petri dish, and then the solvent was evaporated through air-drier at room temperature for 24 hrs. Followed by a vacuum drying cycle at 50^{o}C , washed with ether for several times. Table (2) shows the physical properties of cross-linked polymers [M_5, M_6]

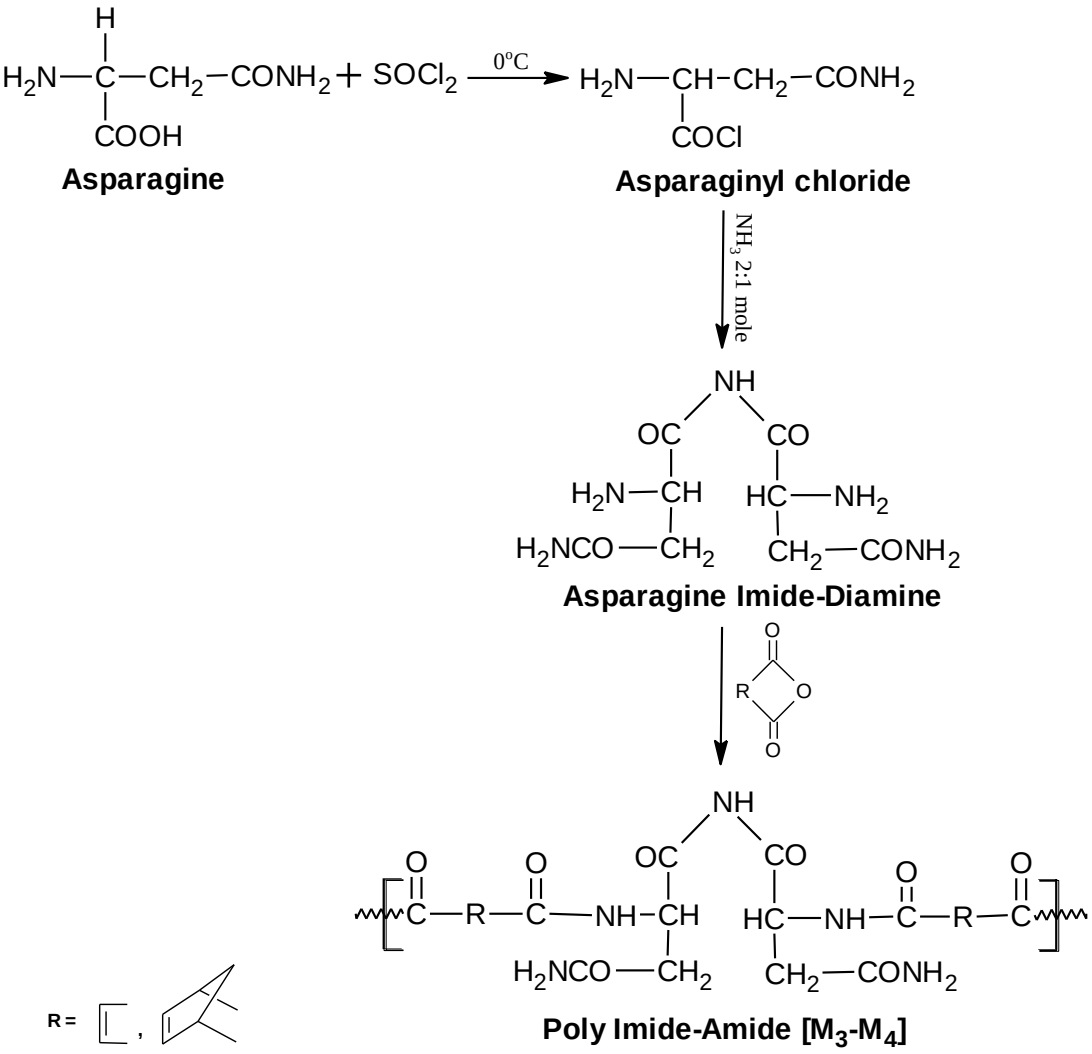
Table (2):- Physical Properties of the Prepared Cross linked Polymers [M_5, M_6]



Poly No.	Color	Conversion %	Softening Point ^oC	Swelling% in pH 1.1
M_5	Deep Yellow	90	>300	15
M_6	Brown	92	>300	25

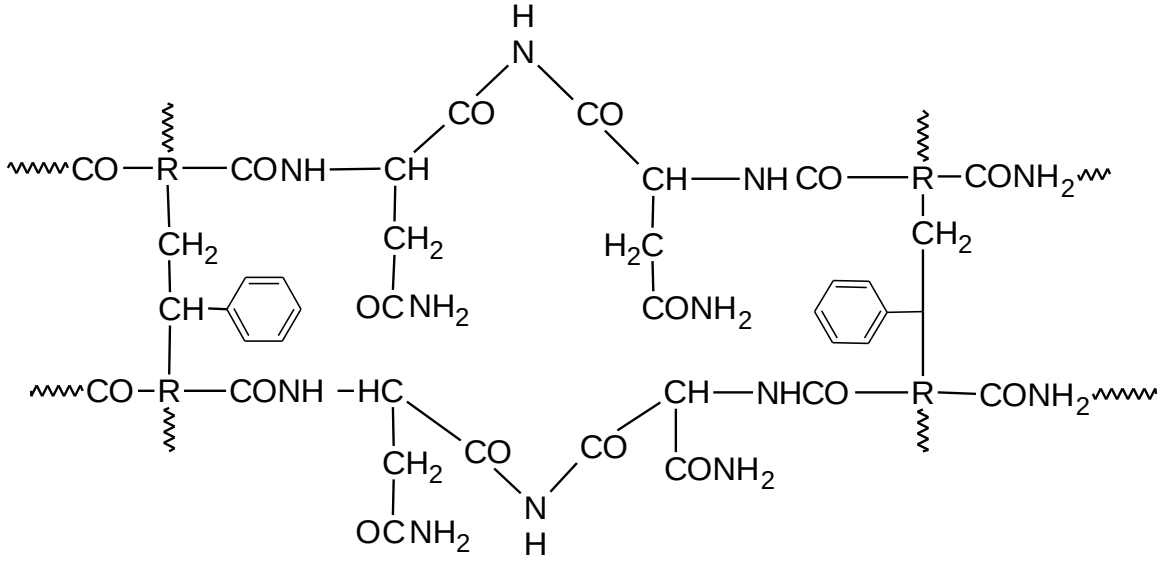
Results and Discussion

In this study new bioactive polymers were prepared through condensed polymerization of unsaturated acid anhydride such as maleic or methyl-nadic anhydrides, with prepared asparagine imide-diamine, which prepared as shown in the following equation:



scheme (1)

Then cross-linked polymers M_5 and M_6 were synthesized from M_3 and M_4 with styrene monomer by free radical polymerization as described below:



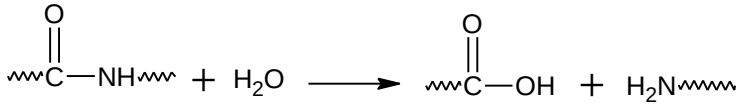
Crosslinked Polymers [M_5-M_6]

scheme (2)

Fig. (1) shows the FTIR spectra of prepared asparagine imide-diamine with maleic anhydride polymer [M_4], the υC=O absorption revealed at 1730 cm^{-1} for imide and at 1665cm^{-1} for amide. The υ(–NH_2) stretches absorption were showed at 3450-3350cm^{-1} and υ(-NH) at 3252cm^{-1}, and around 1220-1240cm^{-1} because of C-N absorption. Fig. (2) of prepared polymer M_3 shows absorption for υC=O amide at 1750cm^{-1} and υ-NH imide at 3115cm^{-1} with remained υ-NH_2 of υ$CONH_2$ amide at 3452-3385cm^{-1} with peak at 2955cm^{-1} assigned to aliphatic C-H stretching, also the FTIR of M_3 and M_4 showed peaks at 1600cm^{-1} due to C=C stretching of unsaturated acid anhydride. The physical properties of prepared asparagine imide-amide polymers were studied such as intrinsic viscosity, which was measured at 30°C with Ostwald viscometer.

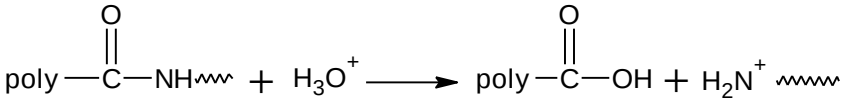
Asparagine release profile

Figure (3) and figure (4) showed the effect of pH values on the release rate of asparagine from polymers [M_3] and [M_4] and profiles of mole fraction of asparagine ratio to total moles presented in the sample versus time at pH values 10 and 1.1 at 37°C. The only nucleophilic acyl substitution reaction that amide undergoes was hydrolysis. Amides are fairly stable in water but the amide bond is cleaved on heating in the presence of basic, normally this cleavage produces an amine and carboxylic acid.



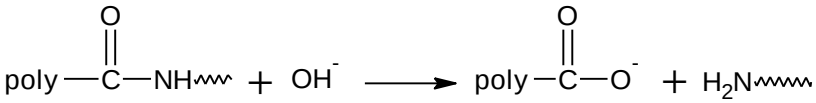
Carboxylic Acid **Amine**

The release of asparagine at suitable condition gradually with outside effect, this hydrolysis of amide group, which was shown in the following mechanism:



Amide **Hydronium Ion** **Carboxylic Acid** **Ammonium Ion**

In basic media the carboxylic acid is deprotonated giving a carboxylate ion:



Amide **Hydroxide Ion** **Carboxylate Ion** **Amine**

Swelling percentage of cross-linked polymers M_5 or M_6 equal to 10 and 25% respectively .The process were achieved at pH 1.1 solutions for five days as shown at fig. (5). Fig. (6) Shows the thermal analysis of polymer [M_3], which indicated the thermal stability in the range of melting temperature equal to 163.37-177.36 ^oC. The intrinsic viscosities of the prepared polymers [M_3] and [M_4] ranged between (0.71, 0.78) dL/g respectively.

Conclusion

The core goal of this study was attained by the achievement of synthesize of polymers based with a smart bioactive asparagine. The drug release profiles displayed a pH-dependent behavior. Asparagine release rate increased significantly as the solution pH increased from acidic pH to the alkaline one. The effects of pH values at 37^oC on the hydrolysis of amide bond which acts as base higher than in acidic medium and this was shown in the release profile figures. Therefore ,we successes in the synthesize of a novel asparagine-polymers that have the ability of controlled release of the amino acid which open an era of administering more complex polymers or other drugs that could be coupled with the amino acid and/or the polymers as a smart line of application in drug design .

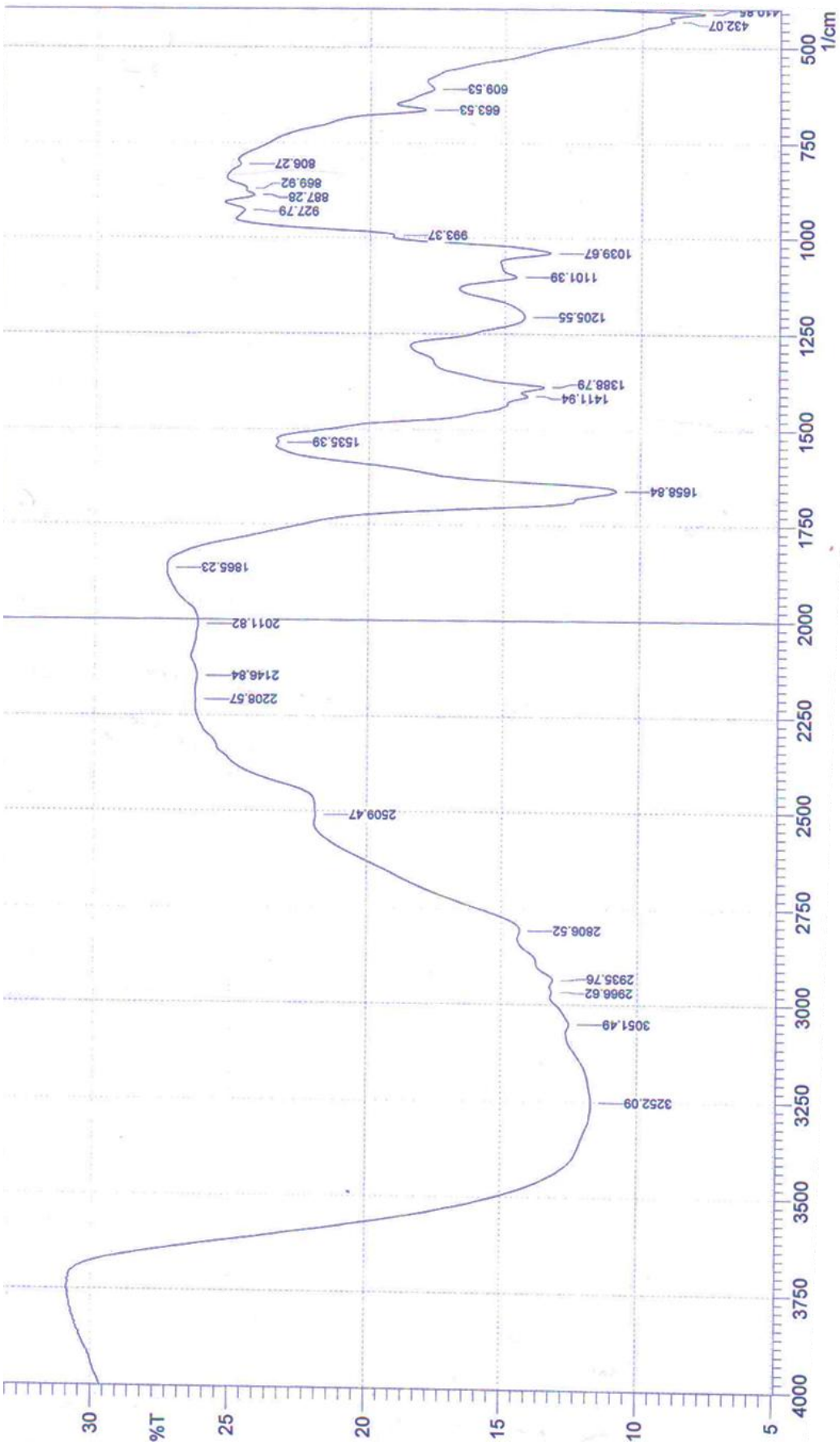


Fig. (1) : FTIR Spectra of asparagine Imide-Diamine with Maleic anhydride Polymer [M_4]

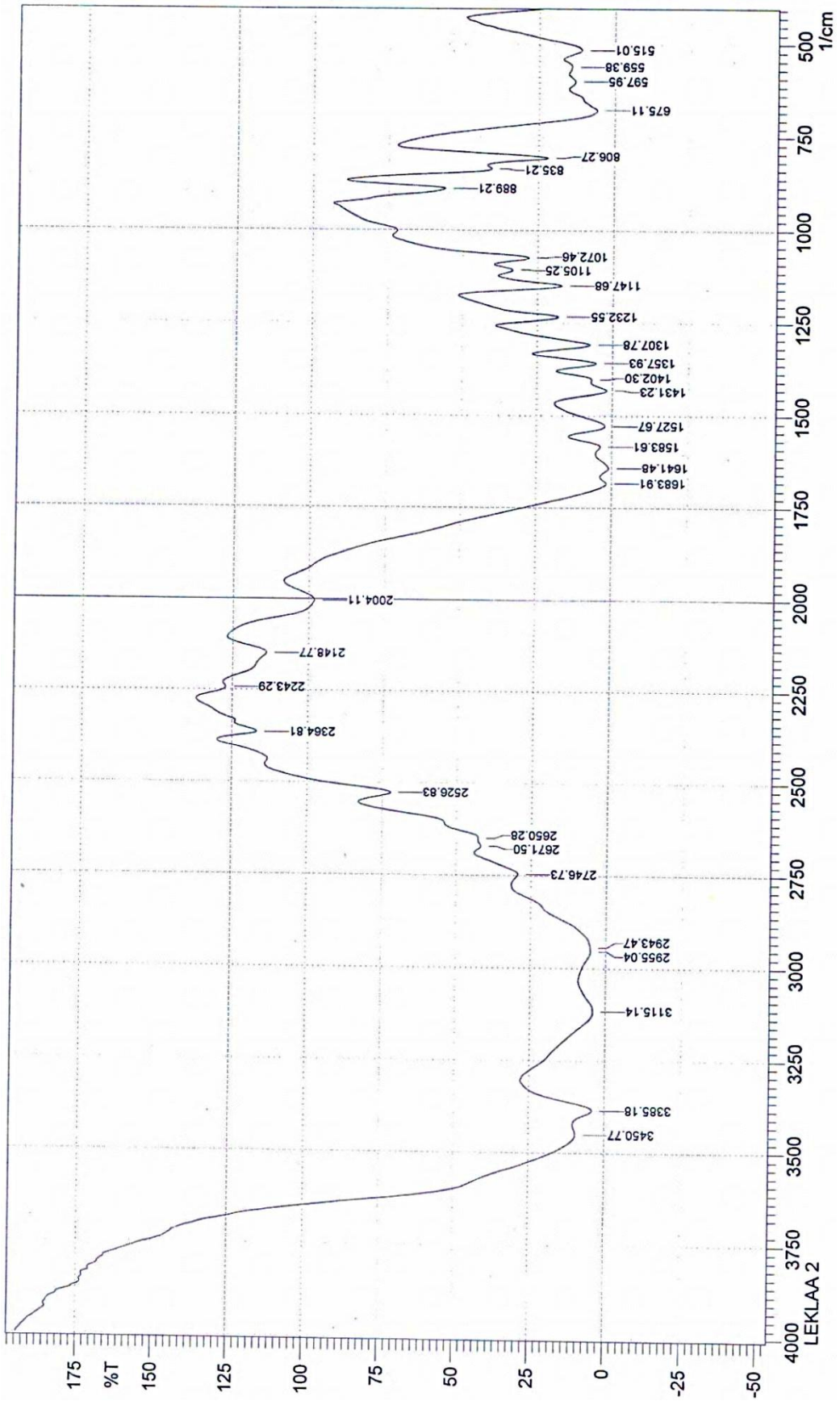


Fig. (2) : FTIR Spectra of Prepared Polymer [M_3]

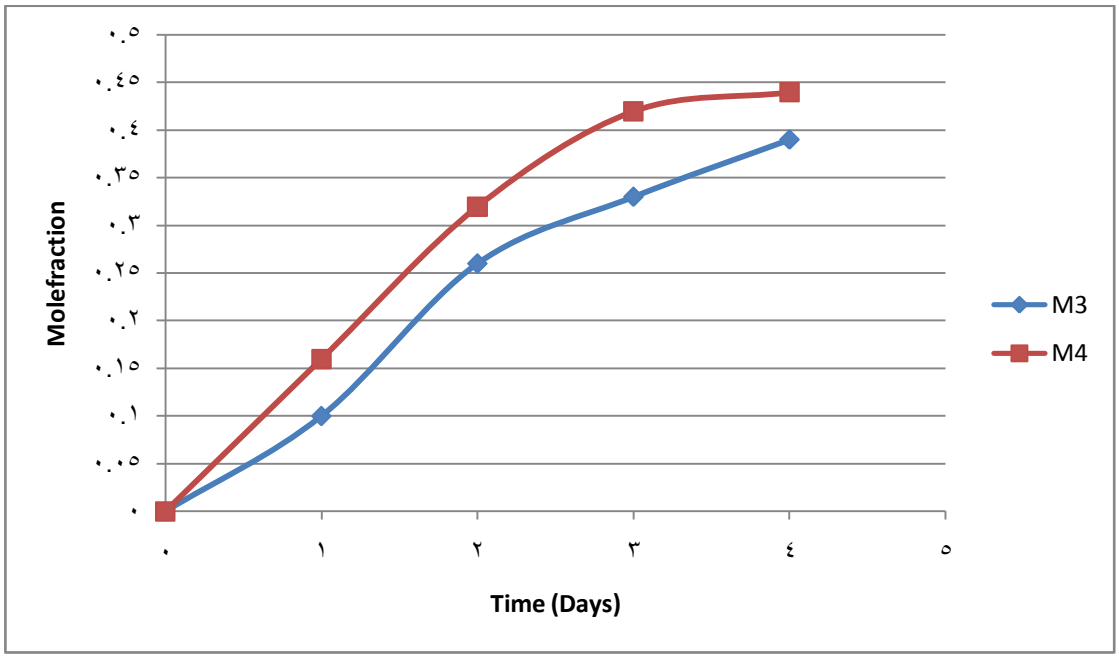


Fig. (3): Release Profile of Asparagine from the Prepared Polymers M_3 and M_4 in pH 1.1 Dissolution Media at 37°C

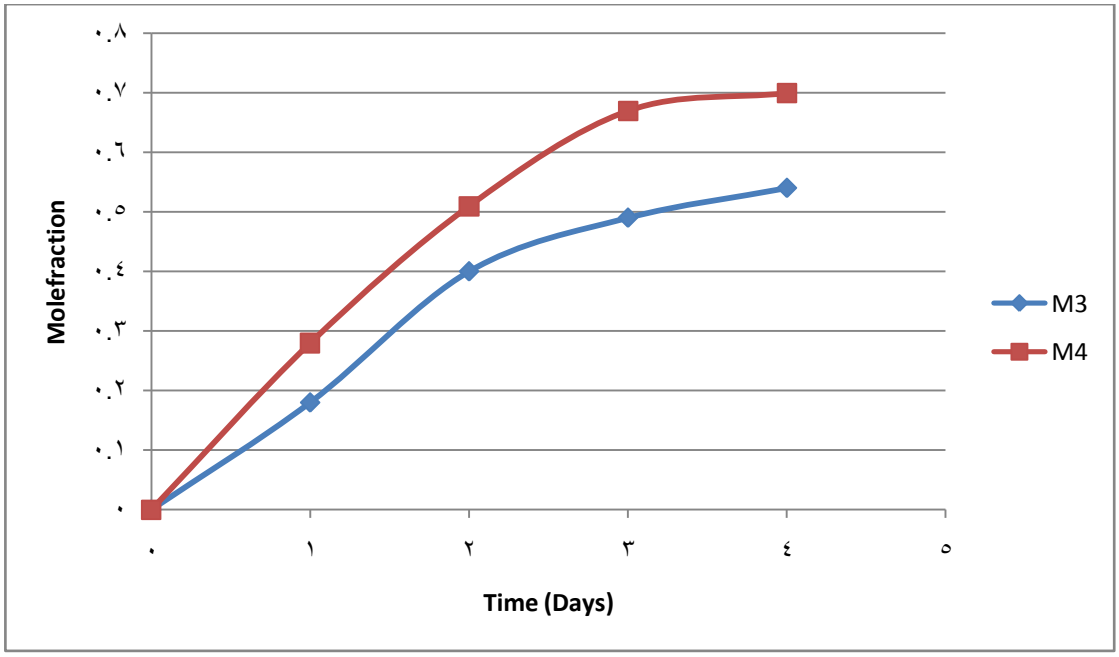


Fig. (4): Release Profile of Asparagine from the Prepared Polymers M_3 and M_4 in pH 10 Dissolution Media at 37°C

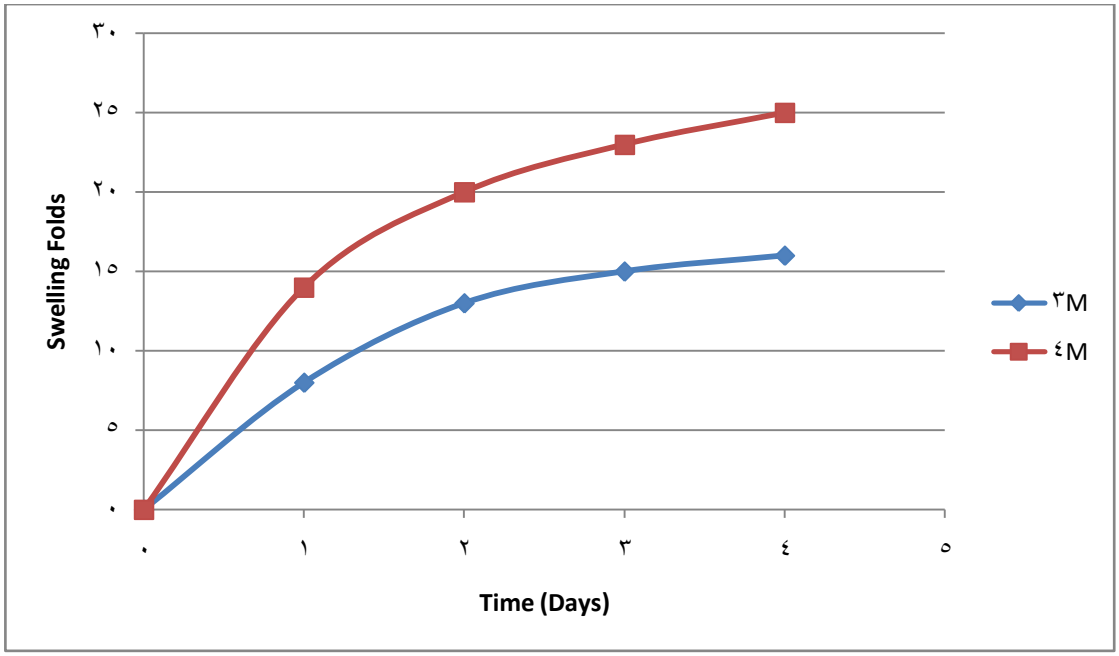


Fig. (5):- Swelling percentage of Polymers M_5 and M_6 in pH 1.1 at Room Temperature

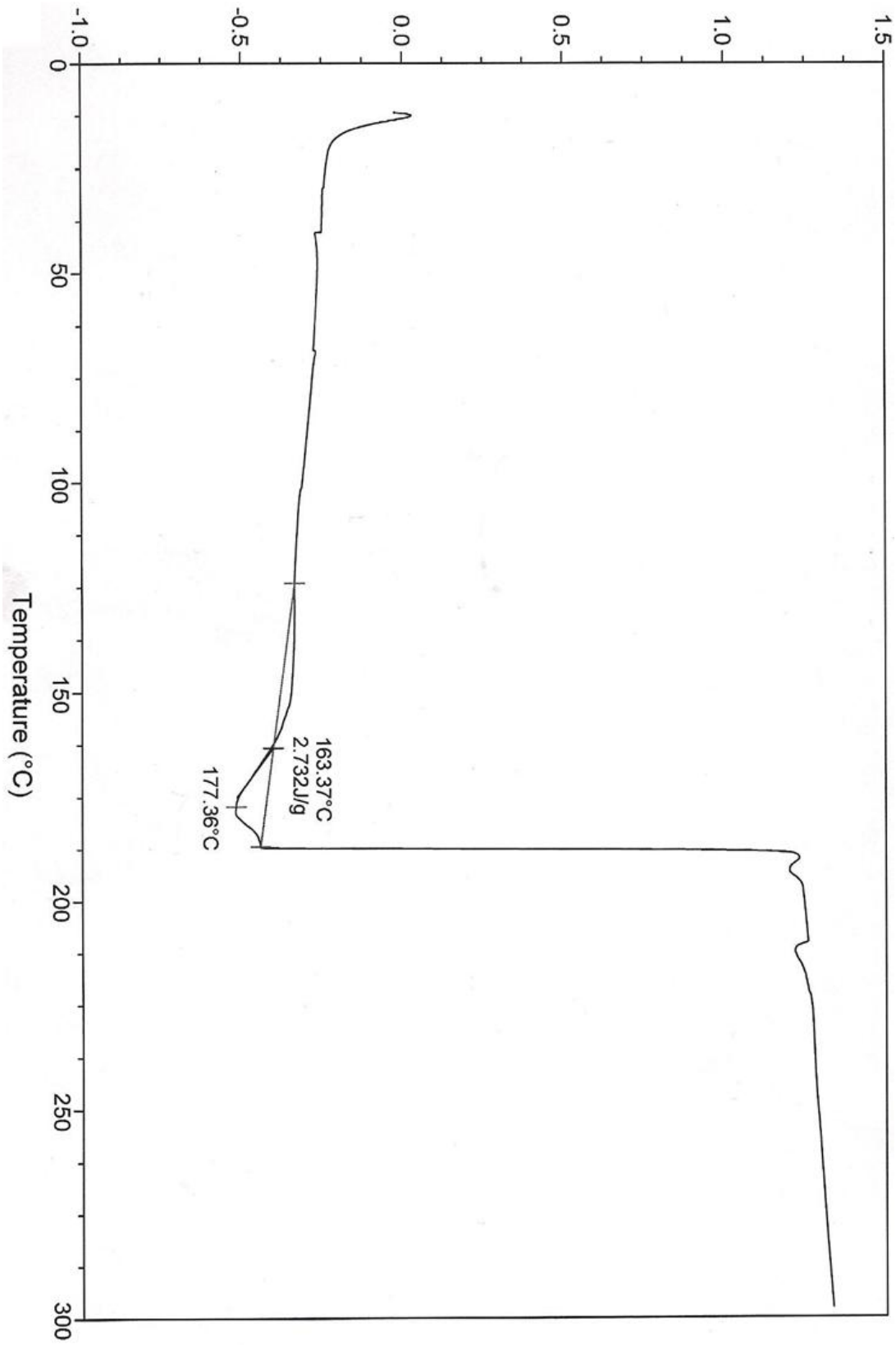


Fig. -6: Thermal Analysis of Polymer [M_3]

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