Synthesis and Characterization co-Polymer of Aspargine and Cephalexin

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Abstruct

In this work a new peptide-base polymer were synthesized by polycondensation of cephalexinyl chloride and asparginyl chloride. This synthesized biopolymer is especially interesting because its primary structure allows the specific cleavage through amide group , can also be used for site specific release of drug as we expected.

The physical properties of [C_3] were measured. , FT-IR , ^{1}H-NMR and UV. Spectroscopy used to characterized the polymer [C_3]. S % was calculated in water, and controlled release was studied in different pH values at 37^{0}C.

الخلاصة

حضر في هذا البحث البوليمر التكاثفي بين السيفالكسينيل كلورايد والاسبارجينيل كلورايد، كبوليمر احيائي ذي اهميه خاصة نتيجة حصول الانفلاق من خلال مجموعة الامايد ، ونتيجة التحرر الدوائي بمواقع محددة كما توقعنا .

درست الصفات الفيزيائية، وشخص البوليمر المحضر بواسطة مطياف الاشعة تحت الحمراء والاشعة فوق البنفسجية وطيف الرنين النووي المغناطيسي.

حسبت نسبة الانتفاخ المئوية للبوليمر ودرست سرع التحرر بدوال حامضية مختلفة بدرجة حرارة 37م^0 .

Introduction

Two different approaches for the synthesis of such peptide-based polymers have been used . In the first approach. Peptides or amino acids are N-terminlly coupled onto a polymerizable group. The amino acid-based monomers are polymerized via radical, anionic, and cationic polymerization, which yield polymers with amino acid and peptide moieties in the side chains [1-2]. In the

second approach, peptide-based polymers consist of peptides in the backbone[3]. This approach implies that at several positions of the peptide polymer backbone, the native amide bond is replaced by a suitable amino bond isostere , e.g. a triazole, moiety. Various examples of α-amino acid-based methylmethacrylamide polymers

have been reported in literature [4] for example, L-leucine based methacrylamide (N-methacryloyl-L-leucine methyl ester) via radical polymerization to obtain high molecular weight polymers. The synthesized polymers formed highly ordered structures that might be used as biocompatible materials such as artificial skins and fibers.

Two acrylamides were synthesized containing proline and hydroxyl proline moieties [5]. The acrylamides were polymerized by reversible addition fragmentation chain transfer polymerization to yield well-defined amino acid based polymers with thermosresponsive properties. Thermosensitive polymers are characterized by a so-called cloud point (CP) or lower critical solution temperature. Thermoresponsive polymers with CP around human body temperature are under investigation for biomedical and pharmaceutical application[6-8].

However this class of polymers with amino acid moieties in the side chains, often have a synthetic backbone with rather low biodegradability, which might lead to cylotoxicty, especially when the molecular weight of the polymer is above the rehal threshold valve (typically 30 k Da)[9,10].Further, some peptide-based biopolymers derive their structural, mechanical and biological properties from their backbone sequential repetitions, these properties might be lost when the peptides are N-terminally grafted onto a synthetic polymer backbone [11,12].

Experimental

Instrumentation:

Fourier Transition Infrared Spectroscopy analysis were performed using pyeunicam sp3-100. UV. Visible double beam scanning spectrophotometer-250 at room temperature. Thermal analysis were recorded using PL-STA 1500, Rheometric scintific UK. The inherent viscosities were measured at 30^0C, using Ostwald viscometer , Swelling % of prepared polymer was determined in water for one day, swollen gels removed from the water at regular intervals were dried superficially with filter paper, weighed and placed in the same sample. S% were calculated according to the following relationship:-

$S\% = M_1\text{-}M_0/M_0 *100$

Where M_0 is the mass of dry polymer at time 0

M_1 is the mass of swollen polymer at t time[13].

Release studies

Condensed Cephalexin -Aspargine polymer [C_3](50 mg) was kept in a cylinder containing 50:50 ml of buffer-dioxane in a water bath at 37 ^{0}C without stirring. A sample from the release medium was periodically with draws and analyzed by UV. At 300 nm to determine

the amount of the released cephalexin and aspargine unites .A calibration curve was constructed with a software built in the computerized UV. Spectrophotometer the pH 4 and 10 were used at 37^0C.

Conversion of Aspargine to its acid chloride [C_1] or Cephalexin to its acidchloride [C_2]

A 100 ml round bottomed two necked flask equipped with a condenser and a funnel was charged with (4g. ,0.05mole) of aspargine and (1.1ml, 0.05mole) of thionyl chloride was added dropwise for about 10 mins.

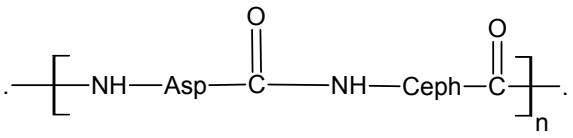
The mixture was stirred at 0 ^{0}C, and the yellow product was obtained. The precipitate was collected and rapidly washed with diethyl ether, dried at room temperature. The yield of C_1 was about 90% and the a cephalexinyl chloride C_2 was prepared as the same procedure.

Polycondensation of [C_1] with cephalexinylchloride [C_2] to [C_3]

The prepared [C_1] (0.001 mole) was added in a round bottomed flask equipped with condenser , 10 ml of dioxane with 0.5 ml of DMF were added to the flask with dissolved cephalexinyl chloride [C_2](0.01 mole), The mixture was refluxed with stirring for 1h. the solid polymer was obtained washed with diethyl ether and derided at 50^0C with 90% was obtained .

The physical properties of [C_3] condensed polymer were listed in Table - 1-

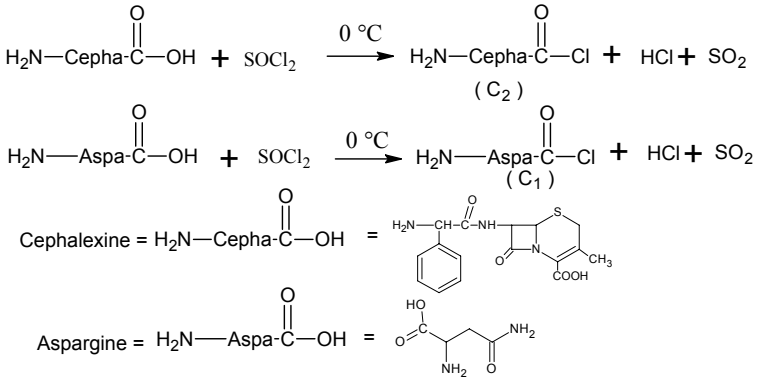
Table 1: Physical properties of condensed polymer [C_3]



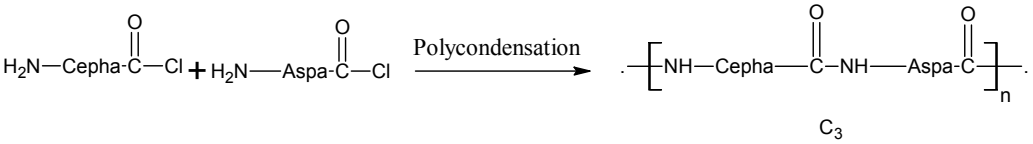
Poly . No.	Color	Conversion%	$[\eta]_{in}$=dl/g	Softening point ^{0}C
C_3	brown	85	0.45	>300

Result and Discussion

Aspargine as amino acid and cephalexine as β-lactam antibiotic were converted to their acid chloride by using thionyl chloride. This reaction converts a less reactive a carboxylic acid into a more reactive an acid chloride as a good leaving group. The reactions were illustrated as follow:-



Polycondensation between two prepared aspargenoyl chloride and cephalexinoyl chloride were polymerized and poly amide through backbone was obtained :-



C3

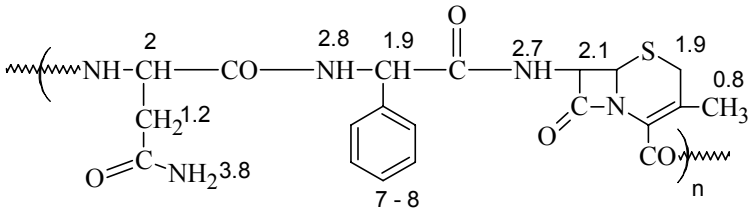
An amino group acts as nucleophilic group, which attacks to carbonyl group of the second monomers in an amino acidic chloride or antibiotic monomer.

The peptide-based biopolymer was obtained connected with amino acid and antibiotic moiety, could hydrolyzed in controlled release as delivery agent and as a drug polymer to enhanced the selectivity and to reach to specific site.

Fig.(1) shows the FT-IR spectra of prepared polymer, and the characteristic absorption was appeared at 3200 cm^{-1} due to υ(NH) amide. And 1700-1690cm^{-1} assigned to υ(C=O) stretching of amide of aspargine and cephalexine respectively. The absorption at 2960- 2650 cm^{-1} which attributed to CH aliphatic, and υ(-CN)at 1330 cm^{-1} and υ(C-O) or υ(C-N) at 1120cm^{-1}.

The H-NMR spectrum Fig.(2) of prepared polymer was gave the following proton signals:-

1- δ(0.8-2) ppm (CH_2,CH_3)
2- δ(7-8) ppm (for different environment aromatic protons)
3- δ(2.8) ppm (for HN-C=O amide)
4- δ(3.8) ppm (NH_2)



(C_3)

The swelling % of prepared polymer was studied in water at 37^0C and Fig(3) shows the ranged between 5-20%.

Fig(4) shows the controlled release drug polymer in different pH 4,10 at 37^0C. The intrinsic viscosity η_{in}= 0.45dl/g was

calculated by using Ostwald viscometer using dioxane as a solvent at 30^0C.

Conclusions

This a novel synthesized peptide-based polymer was characterized and the controlled release studying was illustrated the alkaline pH 10 is higher rate of hydrolysis of peptide bond through the backbone of the polymer contain cephalexine and aspargine compounds.

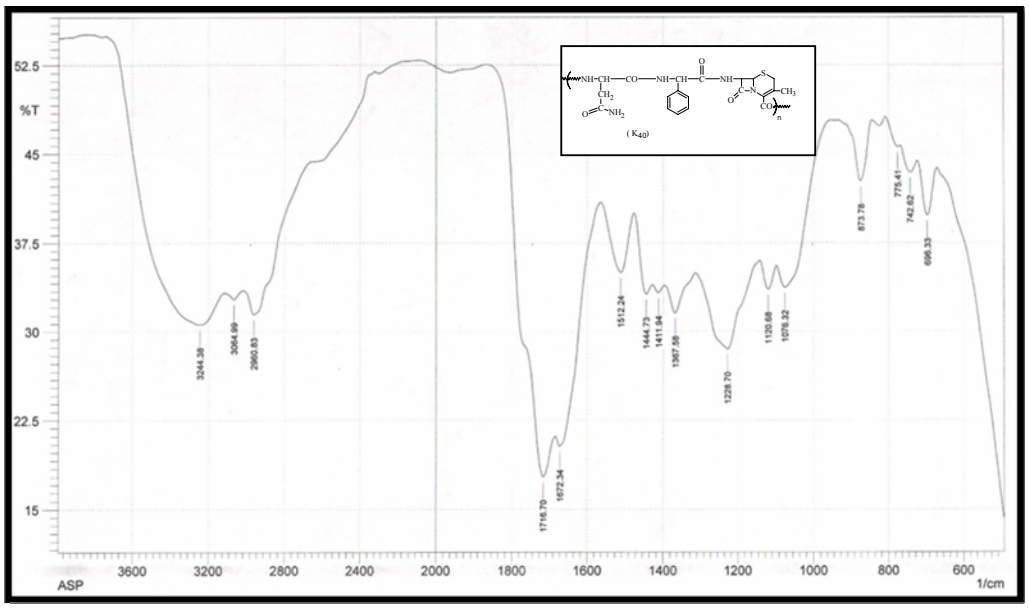
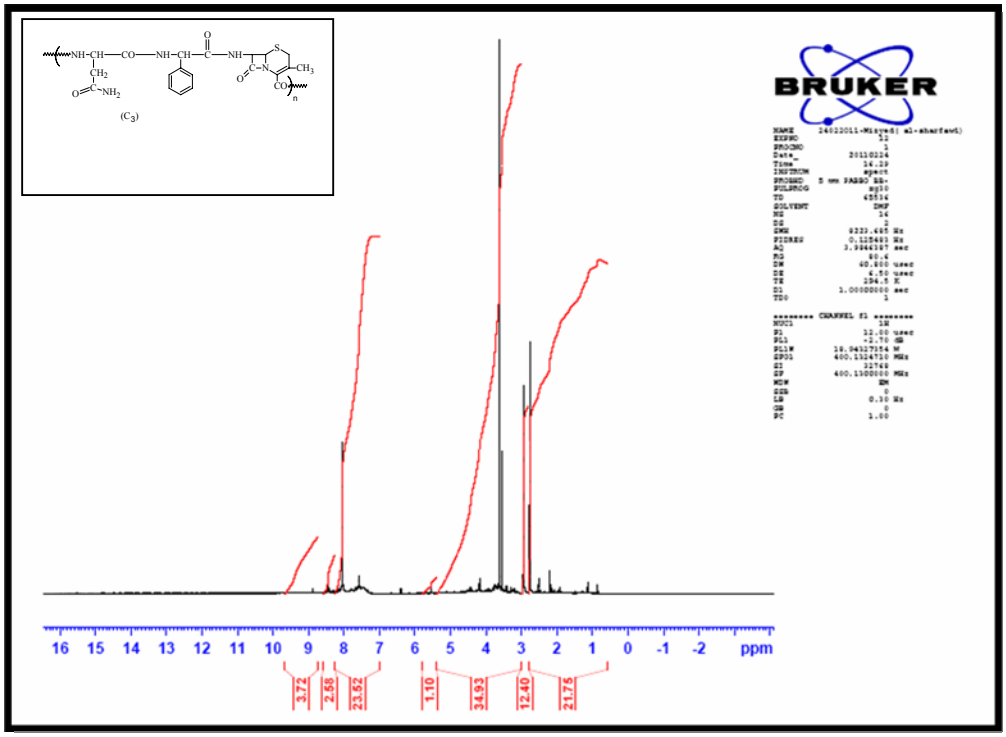


Fig.(1) FTIR spectra of aspargine-cephalexin condensed polymer.



Fig(2) ^{1}H-NMR spectra of prepared polymer at 37 ^{0}C.

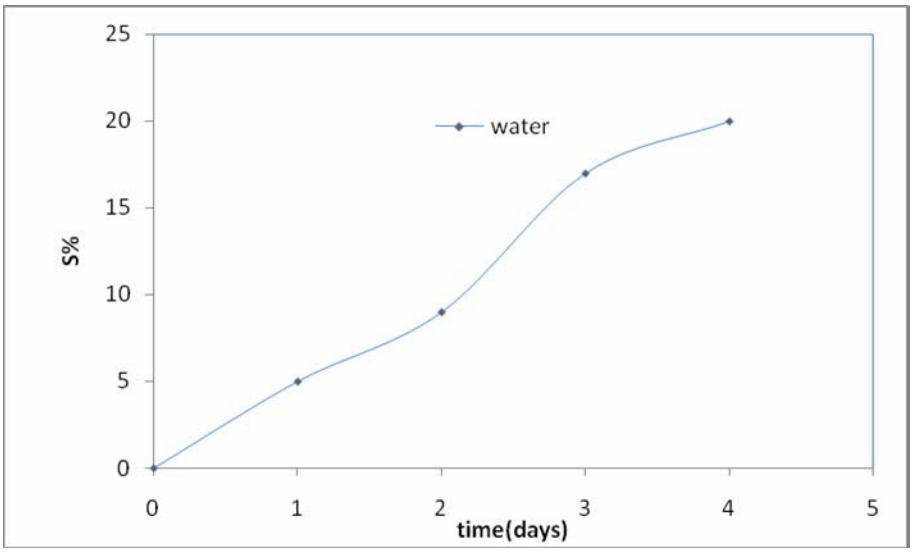


Fig.(3)swelling % respect to time of the prepared polymer (C_3) at 37 ^{0}C.

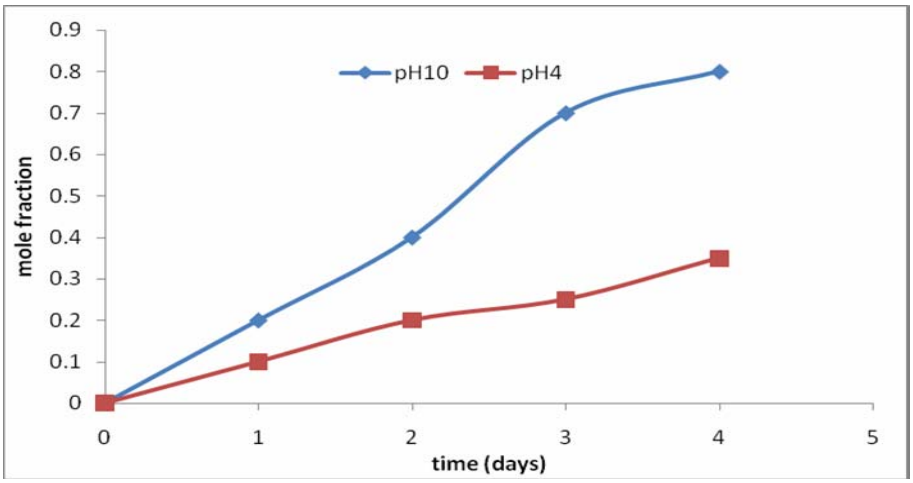


Fig.(4) Controlled drug release in different pH at 37^{0}C.

References

1- O'BrienSimpson,N.M.;Ede,N.J.;Brown,L.E.;Swan,J.;Jackson,D.C. ***J.Am.Chem.Soc***., 1997, **119**,1183-1188.

2- Ayres,L.;Adams,P.H.H.M.;Lowik, D.W.P.M.; van Hest,J.C.M. ***Biomaromolecules***, 2005, **6,** 825-831.

3- Sanda,F.; Endo,T., ***Chem.Physic***., 1999,**200**, 651-2661.

4- Sanda,F.;Nakamura,M.;Endo,T.;Takata,T.;Handa,H. ***Macromolecules,*** 994, **27**,7928-7929.

5- Mori,H.;Kato,I.;Matsuyama,M.;Endo,T.RAFT ,***polymers Macromolecules*** 2008,**41**, 5604-5615.

6- Jeong,B.;Bae,Y.H.;Lee,D.S.;Kim, S.W. ***systems Nature***, 1997,**388**,860-862.

7- Chilkoti,A.;Dreher,M.R.;Meyer,D .E. ***Drug. Deliv. Rev.***2002,**54**,1093-1111.

8- Luten,J.;van Nostrum,C.F.;De Smedt,S.C.;Hennink,W,E. ***Control.Release*** 2008,**126**,97-110.

9- Petersen,H.;Medan,T.;Kunath,K.; Fischer,D.;Kissel,T.Poly(ethylenimine-co-L-lactamide-co-succinamide): a biodegradable polyethylenimine derivative with an advantageous pH-dependent hydrolytic degradation for gene delivery ***Bioconjugate Chem.***, 2002, **13**, 812-821.

10- Kricheldorf,H.R.,Alpha-Aminoacid-N-Carboxyanhydrides and Related Heterocyclic;Springer,Berlin,1987 .

11- Gallot,B. ***Polym.Sci.***, 1996, **21**, 1035-1088.

12- Deming,T.J.Facile, ***Nature***, 1997,**390**,386-389.

13- Firyal M. , Abbas N. and Khudheyer J..(Preparation of (N-Procainyl amic acid) polymers. Fourth Scientific Conference University of Wasit. Iraq,(2010).