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ORIGINAL ARTICLE

Synthesis of New Glycine Cephalexin Condensed Polymer as Peptide Biopolymer for Controlled Release of Cephalexin

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KEYWORDS
Glycine;
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ABSTRACT: A new peptide-based polymer was synthesized via polymerization of cephalexin acid chloride with glycine acid chloride with molar ratio 1:1 in condensed polymer solution. This Glycine Cephalexin peptide biopolymer was characterized by different analyses of UV, FT-IR and ^{1}H NMR spectroscopy. Also, physical properties of new synthesized Glycine Cephalexin peptide biopolymer were studied with measurement of its intrinsic viscosity at 30°C, swelling percentage in water and studying drug release in pH 4-10 at 37°C.

INTRODUCTION

Peptides are considering as a new class of bio-substances that have unique chemical, physical and biological properties [1]. Synthetic and natural biopolymers are with many applications in biointerface engineering, such as tissue engineering scaffolds, drug delivery, as detectors and transducers in biosensors. Contrasted to naturally occurring biopolymers, there are peptide-based biopolymer which is named under word" engineered". This peptide based biopolymers have attracted much attention many researchers as a new class of materials such as elastin-like polypeptides, poly-amino acids, silk-like proteins, tropoelastin-based peptides, coiled-coil domains, peptide amphiphiles, leucine zipper-based peptides, beta-hairpin peptides, and beta-sheet forming ionic oligopeptides [2]. For example, Spider-silk is a remarkably strong various materials that act as peptide based biopolymer [2], Another example of Peptide-based polymers is Collagen that has been found inside the human body, it forms major components of extracellular matrix in cell tissues to give mechanical strength to cells [3]. In the same manner, Elastin, peptide based biopolymer, has unique property of allowing body tissues to resume their shape after stretching or contracting [4-6]. Also, there are various peptide based biopolymer; poly-amino acid-based methacrylamide [7-9], biocompatible materials such as artificial skins and fibers [10].

Conventional synthesis of solid-phase peptide is not efficient in synthesis of large peptide-based polymer (>100 amino acid residues) together with conventional solution-phase peptide synthesis. These methods result in overall poor yield (10–40%) in 36–48 h. So, there are various methods that used in synthesis of polypeptides such as polymerization of α-amino acid N-carboxyanhydrides as an economical and expedient process for the synthesis of relatively high molecular weight polypeptides [11] or rapid microwave-assisted solution-phase peptide

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synthesis [12-14], for example, Chitosan hydrogels can be used as carriers for drug release and as bioactive molecules as shown in Figure 1 [15-17].

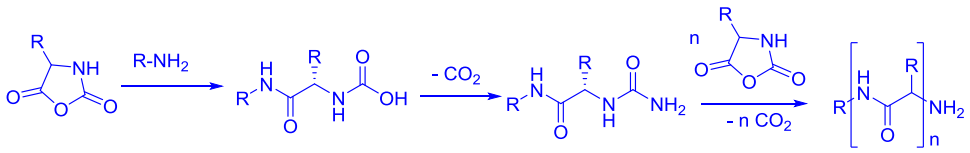

Figure 1. Chitosan hydrogels as carriers for drug release and as bioactive molecules

Our research aims to synthesize new peptide based biopolymer that derived from glycine or cephalexin and determine some of its physical properties such as intrinsic viscosity, effect of pH and percentage of swelling.

MATERIALS AND METHODS

Instrumentation

Melting point were measured using Gallen Kamp M.F.B-600 melting point apparatus. Infrared spectrophotometer measurements were performed using Pyeunicam SP3-100. UV. Visible double beam scanning spectrophotometer-260 at room temperature. Differential Scanning calorimetry (DSC) and Thermo-ravimetric analysis (TGA) were recorded using (Pt- STA1500, Rheometric Sentific UK). All chemicals were purchased from Fluka and BDH. All analytical solvents were used without further purification.

Preparation of Glycinoyl chloride C_1

In a round bottom flask equipped with a magnetic stirrer, a thermometer and a condenser, 5g glycine (1mmole) in 15 ml of Dioxane were added then 1 mmole thionyl chloride was added dropwise with rate 10 min at 0°C . Orange oily product was formed, isolated, washed with diethyl ether for several times, and dried at 50°C to give C_1 with yield 75%.

Preparation of Cephalexinoyl chloride C_2

Compound C_2 was prepared by applying the same method of preparation of compound C_1, the yield was 80%.

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

A 100 mL round bottomed two necked flask equipped with a thermometer and a reflux condenser was charged with (0.01mole) of dissolved cephalexinoyl chloride C_2 the solvent was used (1m1) of 1:10 volume of DMF: Dioxane mixture were added to the flask dissolved Glycinoyl chloride C. Then stirred and refluxed continuously for 1h., the mixture was cooled to room temperature. The condensed polymer C_3 was obtained then washed with ether and dried at 50°C the yield was 85% with tli_n=0.56.

Swelling percentage

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% was calculated according to the following relationship:-

$$S\% = M_1 - Mo/M0 \times 100$$

Where Mo is the mass of dry polymer at time 0

M_1 is the mass of swollen polymer at t time.

Release studies

Condensed Cephalexin-glycine polymer [C_3J] (50mg) was kept in a cylinder containing 50:50 ml of buffer-dioxane in a water bath at 37 °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 glycine unites .A calibration curve was constructed with software built in the computerized UV. Spectrophotometer the pH4 and 10 were used at 37°C.

RESULTS AND DISCUSSION

Thionyl chloride is reacted with glycine or cephalexin to afford their acid chloride derivatives (C_1 and C_2) to increase

reactivity of carboxylic acids because of presence of chloride as good leaving group (Figure 2).

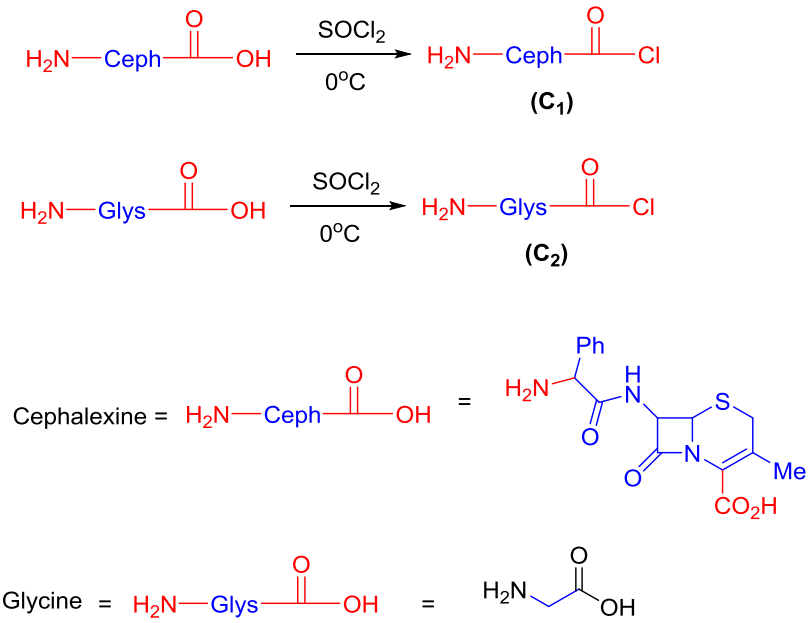

Figure 2. Mechanism reaction of Acid chloride derivatives (C_1 and C_2) with glycine or cephalexin.

A direct condensation between glycine chloride (C_1) and cephalexin chloride (C_2) has been produced to afford condensed polymer (C_3) as illustrated in Figure 3.

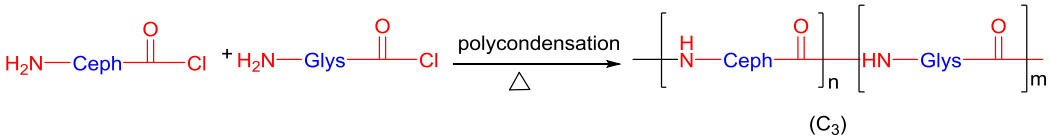

Figure 3. Mechanism condensation between (C_1) and (C_2) to produced condensed polymer (C_3).

The mechanism of the reaction is depicted in Figure 4. A nucleophilic attack of amino group to the carbonyl carbon with expulsion of HCl to afford the new Glycine-cephalexin based biopolymer (C_3).

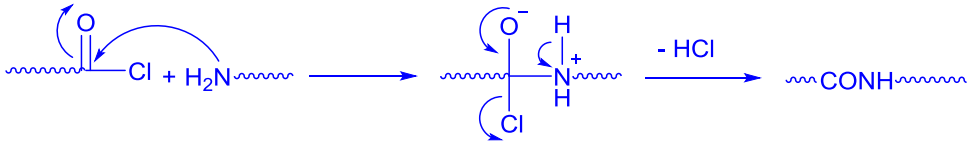

Figure 4. The mechanism of the reaction of A nucleophilic attack of amino group to the carbonyl carbon with expulsion of HCl.

Structure of new biopolymer is elucidated by using FT-IR, ^{1}H NMR as illustrated in Figures 5 and 6. IR spectrum of C_3 revealed presence of strong absorption band at 3200 (amide NH), 1740, 1732 (amide C=O) of cephalexin and glycine, respectively. Also, CH aliphatic and aromatic has absorption bands at 2960-2620 and 3060. 1324 (C-N), 1114 (C-O) cm^{-1} (Figure 5).

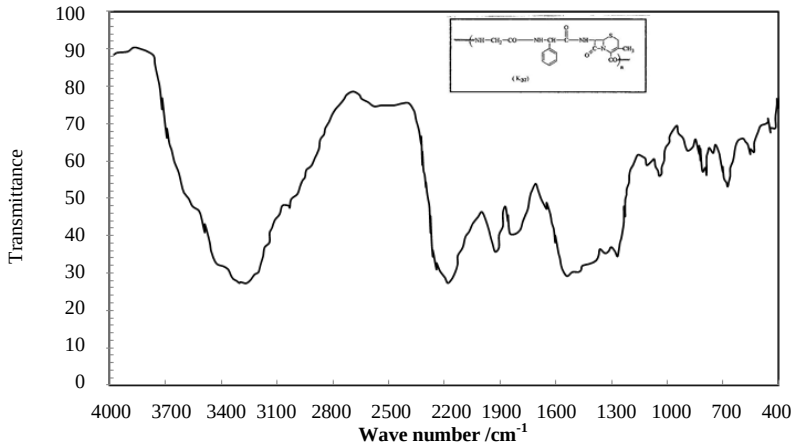

Figure 5. FTTR spectra of glycine-cephalexin polymer.

^{1}H NMR spectrum of C_3 revealed presence of signals at 8.1-8.3ppm of aromatic protons, 3.2, 3.3 ppm (NH protons), and aliphatic protons of CH-Ph, CH_2, CH, cyclic CH_2 and CH_3 groups revealed strong signals at 2.8, 2.7, 1.9, 1.3, and 1.2 ppm, respectively (Figure 6).

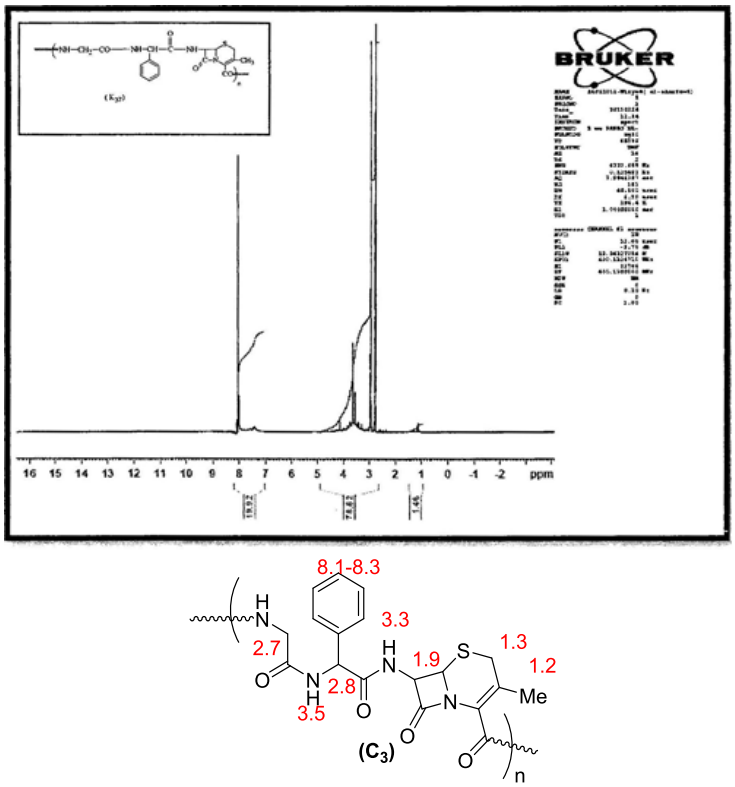

Figure 6. ^{1}H NMR spectrum of C_3

Physical Properties of C_3

The intrinsic viscosity

By using Ostwold viscometer, intrinsic viscosity of new prepared polymer (Figure 7) was calculated in dioxane [η_{in}] = 0.4d1/g. this result indicates that low molecular weight polymer is proportional with Tim (Table 1).

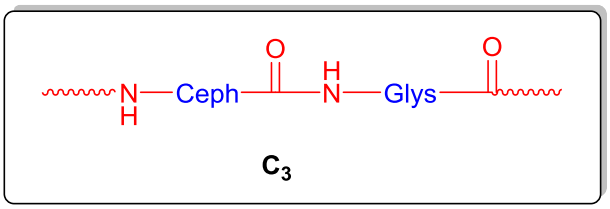

Figure 7. condensed polymer (C_3)

Table 1. The Physical properties of condensed polymer C_3

Poly. No.	Color	η_{in} dl/g	Softening point ^oC	Yield %
C_3	Yellow	0.56	120-130	85

Effect of pH

Figure 8 shows the effect of pH values on the rate of release and profiles of mole fraction ratio to total moles present in the sample versus time at pH4 and 10 at 37°C (Table 1).

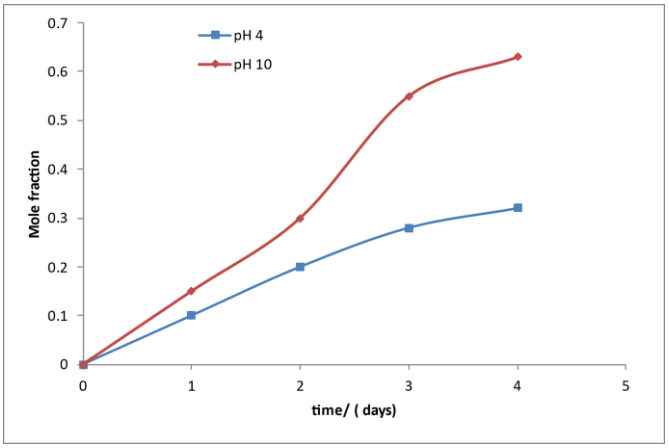

Figure 8. Effect of pH values and controlled drug release of condensed polymer (C_3) in different medium

New formed amide group was hydrolyzed in basic medium to afford cephalexin and glycine units, this occurred with sustained controlled release and to prolonged time, as shown in the following mechanism (Figure 9).

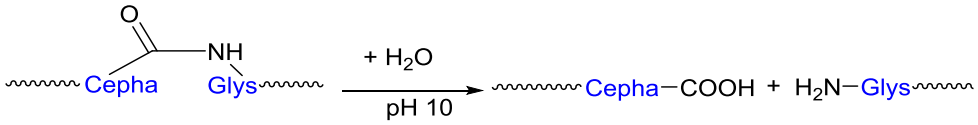

Figure 9. Mechanism reaction to hydrolyzed amid group in basic medium to afford cephalexin and glycine units.

Swelling percentage

Swelling % was measured for C_3 polymer in water was high S% = 25% (Table 1). swelling. The gradually hydrolyzed in different pH values, could enhanced the controlled release of bioactive units.

CONCLUSIONS

New synthesized peptide-based biopolymer was characterized with measuring of some physical properties such as intrinsic viscosity, effect of pH and percentage of

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Conflict of interests

The author declares no conflict of interest.

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