Synthesis and modification of some new prodrug polymer based on carboxymethyl cellulose and study some of their application

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ABSTRACT

This study includes the preparation of new polymers for carboxymethyl cellulose and chitosan with different drugs carrying carboxylic groups (amoxicillin, cephalexin, ampicillin) after conversion into acid chloride derivatives. Synthesis of a new various drug delivery systems (DDS) and smart polymer (SP) could develop to provide us the modification and remarkable improvement the new therapeutic efficiency and safety of drug polymer, it eliminates the toxic side effects medications and diagnosis of the resulting compounds using the FT-IR and HNMR spectrum.
INTRODUCTION

Polymers are large molecules that consist of a large number of repeating units bound together called monomers (1) Such as sugars, proteins, nucleic acids and other basic and major components of living systems (2) They can be used in medical, biological, pharmaceutical and genetic transmission applications (3) and industrial areas as an important renewable resource can be used to reduce pollution in the environment (4).

The polymer is bonded to the carboxylic drug by a covalent process to form a biologically active polymer that is important in drug delivery systems (5).

The behavior of pharmacological polymers in the body depends on the possibility of hydrolysis of the enzyme loaded with the polymer, which contains hydrophilic and hydrophobic groups (6) This reduces the side effects of the drug in the long term. Medication delivery systems are in constant development because it controls the delivery of medicine to the body (7) The medicine can be stored in a dry powder form inside the polymer, and it can be delivered to the disease area, depending on the pH, temperature, electric and photovoltaic field, or oxidizing and reducing chemicals (8). This system works to reduce the accumulation of medicine in the lung (9), The drug delivery systems are of great molecular weight due to their ease of use, high conductivity and less toxicity (10-11).

Chitosan is a natural polymer (a chain of carbohydrates) consisting of chitin that represents the outer structure of crustaceans, linear connection and the formation of glycoside bonds (4-1) It has important biological, economic, degradable and renewable properties that can be used in the pharmaceutical, cosmetic, livestock and agricultural industries (12). The amine group can be linked to it with other
groups and load drugs on them\(^{13-14}\). Carboxymethyl cellulose is a cellulose derivative consisting of frequent anhydrous glucose units bound together with the \(\text{g bonds glycoside bonds N}(4\text{-1})\), used as a treatment for dry eyes, and the ability to add it to foods and food products for a coherent consistency.

**Measurements**

The intrinsic viscosity (\(\eta\)) measurement were carried out in water using an Ostwald Viscometer.

The IR spectra measurements were recorded using a device Fourier trans Infrared.

\(^{1}\text{HNMR} was taken at 300 MHz in DMSO as reference

**Experimental**

The reaction of Carboxymethyl cellulose (CMC) with chitosan

(0.4 g) of Carboxymethyl cellulose (CMC) was dissolved in (4ml) ethanol, (2ml) DMSO and two drops of \(\text{SOCl}_2\) were added to it and reflux temperature 50\(^\circ\text{C}\) at half hour with constant stirring to obtain acetyl chloride. took (1 gm) of chitosan and dissolve it in glacial acetic acid (0.1 N) (2 ml) and add drops of water to it and add it to the previous mixture and make reflux of 50\(^\circ\text{C}\) a half-hour with continuous stirring, the precipitate was taken and dried, called polymer \((R_1)\).
Scheme (1): preparation of polymer R₁

**Preparation of drug polymers A₁- A₃**

(0.3 gm) of carboxylic drug (ampicillin) was taken and dissolved in (4 ml) of dioxan with (4 drops) of SOCl₂ and reflex for half hour at a degree of 50 °C with continuous stirring, (0.3 gm) of polymer (R₁) was taken and dissolved in dioxan, add the medicine and reflex for half hour at a degree of 50 °C with constant stirring and Leave the precipitate to dry

The experiment was reused with other carboxylic drug (amoxicillin and cephalexin)
Scheme (2): preparation of drug polymer

| Sample | drug | Color  | Yield % | Viscosity=dL/g | Solvent               |
|--------|------|--------|---------|----------------|-----------------------|
| A1     | Amp  | orange | 60      | 0.70           | Distilled Water + heat |
| A2     | Amox | yellow | 35      | 0.85           | Distilled Water       |
| A3     | Ceph | Light yellow | 58 | 0.65         | Distilled Water + heat |

The table (1) shows some of the physical properties of the compounds (A1 – A3)
Drugs:

\[ A_1 = \text{Ampicillin} \quad A_2 = \text{Amoxicillin} \quad A_3 = \text{Cephalexin} \]

\[ \text{Ampicillin} \]

\[ \text{Amoxicillin} \]

\[ \text{Cephalexin} \]

**RESULTS AND DISCUSSION**

Preparation and diagnosis of polymer \((A_1 - A_3)\)

![FT-IR spectrum of polymer A1](image)

Figure (1): FT-IR spectrum of polymer \(A_1\)
FT-IR spectrum of A1 shows: (3332) NH amide, (2939 -2986) CH aromatic, (2836) CH alkane, (1767) C=O Ester, (1692) C=O Amide, (1581-1480) C=C aromatic.

$^1$HNMR spectrum shows: signal at 0.9 ppm for proton of CH$_3$, signal at 1.2- 1.6 ppm for proton of CH$_2$, signal at 2 ppm for NH amine, signal at 4.57 -4.35 ppm for CH, signal at 7.33 ppm for proton of CH (aromatic), signal at 7.7 ppm for NH(amide).
Figure (3): FT-IR spectrum of polymer A₂

Figure (4): ¹HNMR spectrum of polymer A₂
FT-IR spectrum of A2 shows : (3521) OH alcohol, (3438) NH amide, (3154) amine, (3016-2919) CH aromatic, (2709-2619) CH aromatic, (1742) CH alkane, (1683) C=O ester, (1575-1480) C=C aromatic, (2116) C-S.

$^1$HNMR spectrum shows: signal at 1 ppm for proton of CH$_3$, signal at 1.17 ppm for proton of CH$_2$, signal at 2 ppm for NH amine, signal at 3.8 ppm for CH, signal at 6.4 ppm for proton of CH (aromatic), signal at 7.3 ppm for NH(amide), signal at 8.5 ppm of OH (aromatic).

Figure (5) : FT-IR spectrum of polymer A$_3$
FT-IR spectrum of A₃ show: (3434) NH amide, (3278) amine, (3029-2943) CH aromatic, (2878) CH alkane, (1756) C=O ester, (1690) C=O amide, (1594-1454) C=C aromatic.

¹HNMR spectrum shows: signal at 0.9 ppm for proton of CH₃, signal at 1.3 ppm for proton of CH₂, signal at 2 ppm for NH amine, signal at 3.5 – 4.5 ppm for CH, signal at 7.4 ppm for proton of CH (aromatic), signal at 8.1-8.71 ppm for NH(amide).

**Swelling ratio% and drug release**

Drug release the polymers A₁-A₃ was studied, acid and base functions were used where hydrolysis was gradual. As a pharmaceutical unit of the hydrolysis of the polymer loaded with the drug where PH= 1.2, PH= 7, PH=8.
Table (2): drug release of polymers $A_1$-$A_3$ at PH= 1.2 at 37 $^\circ$C

| time | $A_1$  | $A_2$  | $A_3$  |
|------|--------|--------|--------|
| 1    | 2.112  | 0.234  | 0.599  |
| 2    | 2.899  | 0.403  | 3.032  |
| 3    | 0.118  | 0.429  | 0.138  |
| 4    | 0.055  | 0.134  | 0.212  |

Figure (7): drug release of polymers $A_1$-$A_3$ at PH = 1.2

Table (3): drug release of polymers $A_1$-$A_3$ at PH= 7 at 37 $^\circ$C

| time | $A_1$  | $A_2$  | $A_3$  |
|------|--------|--------|--------|
| 1    | 2.111  | 0.255  | 0.601  |
| 2    | 3.123  | 2.889  | 3.112  |
| 3    | 4.208  | 3.599  | 4.312  |
| 4    | 1.987  | 1.123  | 1.231  |

Table (3): drug release of polymers $A_1$-$A_3$ at PH= 7 at 37 $^\circ$C
Figure (8): drug release of polymers A1-A3 at PH = 7

| time | A1    | A2    | A3    |
|------|-------|-------|-------|
| 1    | 2.198 | 1.484 | 0.685 |
| 2    | 3.731 | 4.349 | 3.121 |
| 3    | 4.165 | 3.194 | 3.923 |
| 4    | 0.976 | 1.234 | 2.012 |

Table (4): drug release of polymers A1-A3 at PH= 8  at 37 °C

Figure (9): drug release of polymers A1-A3 at PH = 8
The swelling of polymers was measured using water for 24 hour at 25°C.

| sample | Swelling ratio % |
|--------|------------------|
| A_1    | 10               |
| A_2    | 14               |
| A_3    | 16               |

Table (5): The swelling ratio % of polymers A_1-A_3

The swelling of the polymers occurs because of the spread of the solvent molecules in the polymers with a high molecules weight and they cause changes in the size, polymer causing to collapse during mechanical stress through the process of swelling or degree of entanglement of the polymers because the greater the degree of entanglement, where the process of overlap occurs between the solvent and the polymer molecules so that the polymer chain is not completely.

**Biological Activity**

MCF-7 cells were maintained in RPMI-1640 supplemented with 10% Fetal bovine serum, 100 units/mL penicillin, and 100 µg/mL streptomycin. Cells were passaged using Trypsin-EDTA reseeded at 80% confluence twice a week, and incubated at 37°C.

**Cytotoxicity Assays**

To determine the cytotoxic effect of (A_1, A_2, A_3), the MTT assay was done using 96-well plates. Cell lines were seeded at 1 × 10^4 cells/well. After 24 hrs. or a confluent monolayer was achieved, cells were treated with tested compounds at different concentration. Cell viability was measured after 72 hrs of
treatment by removing the medium, adding 28 µL of 2 mg/mL solution of MTT and incubating the cells for 2.5 h at 37 °C. After removing the MTT solution, the crystals remaining in the wells were solubilized by the addition of 130 µL of DMSO (Dimethyl Sulphoxide) followed by 37 °C incubation for 15 min with shaking (17). The absorbency was determined on a microplate reader at 492 nm; the assay was performed in triplicate. The inhibition rate of cell growth (the percentage of cytotoxicity) was calculated as the following equation (18):-

**Statistical analysis:**

The obtained data were statically analyzed using an unpaired t-test with GraphPad Prism 6. The values were presented as the mean ± SEM of triplicate measurements (19).

![Graph showing cytotoxic effect](image)

**Figure (10): Cytotoxic effect of A1 in MCF-7 cells. IC50=54.18 µg/ml**
Figure (11): Cytotoxic effect of A2 in MCF-7 cells. IC50= 53.11 µg/ml

Figure (12): Cytotoxic effect of A3 in MCF-7 cells. IC50= 18.24 µg/ml
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