SYNTHESIS AND CHARACTERIZATION OF CELLULOSE GRAFTED MALEIC ANHYDRIDE AND SUBSTITUTED IT WITH AMOXICILLIN

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ABSTRACT
In this research the structural modification of cellulose was carried out with maleic anhydride (H) as a spacer by using ceric ammonium nitrate (CAN) as an initiator, and grafted copolymer was substituted with amino drug such as amoxicillin (HA), this design of carries for controlled delivery of therapeutic agent which could release the entrapped drug over an extended period of time, due to its biodegradable, non toxic and slow digesting nature, the new drug copolymer was characterized by FTIR and UV Spectroscopes. Differential scanning calorimetry (DSC) and thermo gravimetric analysis (TGA). The physical properties were measured. The prepared drug copolymer was analyzed in different pH values at (37°C) as in vitro study and controlled drug release was compared at zero time and after four days.

KEYWORDS: Cellulose, maleic anhydride, amoxicillin, Copolymer, Drug Copolymer.

INTRODUCTION
Cellulose is the most widely found natural polymer in nature, it is found in wood, cotton, hemp and other plant-based materials. It consists of repeating an hydro glucose units joined by β(1-4) linkages, forming the basic repeating unit. It is the structural component of the primary cell wall of green plants and many forms of algae. Cellulose is biocompatible and non-toxic, what makes it a good material candidate for medical applications e.g.: wound dressings, scaffolds for tissue engineering, soft tissue replacement and artificial blood vessels.[1] Cellulose is highly crystalline and generally insoluble, due to the intramolecular hydrogen bond networks extending from the O(3')-H hydroxyl to the O(5) ring oxygen of the next unit across the glycosidic linkage and from the O(2)-H hydroxyl to the O(6') hydroxyl of the next residue.[2]

Natural polymers are preferable due to low toxicity, renewability, flexibility to modification, biodegradability and low cost.[3] Natural polymers such as polysaccharides are hydrophilic, enzymatically degradable and are able to retain the stability of protein drugs incorporated in them as well as increase their (proteins) therapeutic effects.[4] Polysaccharides exhibit good haemo compatibility and interaction with living cells,[5] making them compatible and suitable biomaterials for long systemic circulation and targeting. Modification of natural polymers are a means to overcome their setbacks such as drop in viscosity, microbial degradation, and partial or low solubility. In addition, modification of natural polymers enhances their drug delivery properties and versatility. Modification should be undertaken such that the natural polymers do not lose their biological properties. Methods of modification include grafting, crosslinking, derivative formation and polymer-polymer blending.[6] Amoxicillin is a white powder, Soluble in water with molecular formula (C₁₆H₁₈N₅O₄S) maintained the broad-spectrum activity of Ampicillin, but with increased bioavailability . It is dissolve fast, has a high solubility and a good stability if the pH can be buffered at 8. Amoxicillin is one of the most important commercial antibiotics due to its high bacterial resistance and large spectrum against a wide variety of microorganisms.[7] It is effective against a wide range of infections caused by wide range of Gram- positive and Gram- negative bacteria in both human and Animals.[8] It act by inhibiting enzymes involved in bacterial cell wall synthesis. Drugs may also act by inhibiting extracellular reactions.[9] Graft polymerization of cellulose can be initiated by various initiator system such as potassium per sulfate, redox pair of Fe2+/H2O2, KMnO4/organic acid, transition metal ions/organic redactors, and also irradiation initiation, of the redox systems investigated so far the Ce (IV) ion has received considerable interest, because of its high graft yield and very low homo polymer formation[10]. In this research the structural modification of cellulose was carried out with maleic anhydride as a spacer by using ceric ammonium nitrate (CAN) as an initiator,[11] and grafted copolymer was substituted with amino drug such as amoxicillin (HA).
**EXPERIMENTAL**

**Instrumentation**

Melting points were measured using digital melting point, Stuart SMP 10. Infrared spectrophotometer measurements were performed using Perkin elmer spectrum 65. U.V-Visible double beam scanning spectrophotometer V-650, at room temperature. Differential scanning calorimetry (DSC) and Thermo gravimetric analysis (TGA) were recorded using Shimadzu, Japan.

**Preparation of cellulose graft maleic anhydride (H)**

(2 gm) of cellulose dissolved in (10 ml) of acetone, (0.1 gm) (1ml) of ceric ammonium nitrate (CAN), (2gm) of maleic anhydride (H) was added, the mixture was introduced in polymerization bottle, and heated about (30) minutes at (60 °C), using water bath, the white color product was produced (92%), S.P (122-132 °C).

**B-Substituted of (H) with amino drugs (HA)**

(0.30 gm) of cellulos - g-maleic anhydride (H) was dispersed in (5ml) of Acetone, (0.50 gm) of amoxicillin dissolved in (5ml) of dioxane, (0.5 ml) of DMF was added to the mixture, the mixture was refluxed with stirring about 1 hour at (90 °C), the solution was filtered, the filtrate was isolated and the solvent was evaporated, the pink product (HA) cellulose-g-[N-Amoxicillinyl male ame acid] was dried at (50 °C) in a vacuum, conversion (80%), S.p. (135-138 °C). all physical properties were listed in table (1).

**Table (1) Physical properties of prepared Polymer**

<table>
<thead>
<tr>
<th>Pol. No</th>
<th>-Drug</th>
<th>Conversion %</th>
<th>Softening point °C</th>
<th>Color</th>
</tr>
</thead>
<tbody>
<tr>
<td>HA</td>
<td>Amoxicillin</td>
<td>80</td>
<td>135-138</td>
<td>Pink</td>
</tr>
</tbody>
</table>

**RESULT AND DISCUSSION**

Chemical modification of cellulose by grafting with maleic anhydride, cellulose can be grafted as main chain of backbone of polymer, it was polymerized and initiated by various initiators.\[12\] Among the various types of redox initiators, ceric ion offers many advantages because of its high grafting efficiency. \[13\] when (Ce⁺⁴) salts such as cerium ammonium nitrate (CAN) is used as initiator in the grafting of vinyl monomers onto glucose, at first a ceric ion–glucose complex occurs, and then it decomposes to cerous (Ce⁺³) ion and glucose radicals created by hydrogen abstraction from glucose. Thus, The radical formation on the glucose backbone occur on the oxygen atom.\[14,15\] The –OH group present on the backbone of cellulose polymer acts as the active sites for the graft copolymerization.

The mechanism of grafting monomer onto cellulose as shown below in equations (1)

*Initiation:*

\[
\text{Cellulose-OH + Ce(IV) $\rightarrow$ Cellulose-OH-Ce(IV) \rightarrow Cellulose-O$^-$ + Ce(III) + H}^+ \quad (1)
\]

*Propagation:*

\[
\text{Cellulose-O$^-$ + M $\rightarrow$ Cellulose-O-M$^*$} \quad (2)
\]

*Termination:*

\[
\text{Cellulose-O-M$_n$ + M $\rightarrow$ Cellulose-O-M$_{n+1}$} \quad (3)
\]

Graft co polymer was prepared by the reaction of cellulose with maleic anhydride by using ceric ammonium nitrate as a radical initiator. New drug polymer was prepared by the reaction of cellulose with maleic anhydride and substituted with amoxicillin in scheme below.
The presence of –NH2 group in the drug, which acts as a strong nucleophile attack on the C=O group of maleic anhydride produced N-drug substituted, the mechanism of reaction was described as shown below.\cite{16}

Scheme (2) cellulose-g- maleic anhydride and Substituted it with amoxicillin.

Figure (1) FTIR spectrum of natural polymer (cellulose) showed absorption bands at (3345 cm\(^{-1}\)) of (O-H) group and (C-O-C) ether absorption band at (1025-1117 cm\(^{-1}\)), band at (2904) cm\(^{-1}\) due to (C-H aliphatic) stretching.

Figure (2) FTIR spectrum of (H) cellulose grafted maleic anhydride gave the characteristic absorption of carbonyl group of anhydride band was appeared at (1740-1775 cm\(^{-1}\)) in addition to the cellulose backbone absorptions.

Figure (3) FTIR spectrum of (HA) Cellulose-g-[N-Amoxicillin] male amic acid] copolymer containing (-NH amide) as characteristic absorption was appeared at (3283 cm\(^{-1}\)) in addition absorption for carbonyl of amide (CONH) appeared at (1658 cm\(^{-1}\)), band at (1720) cm\(^{-1}\) due to (C=O) stretching vibration of acid. Other bands of the compounds are listed in table.\cite{2}

Table (2): FT-IR absorptions of grafted natural polymers (cellulose) with maleic anhydrides and substituted with drug compound (amoxicillin) [HA]

<table>
<thead>
<tr>
<th>Comp No.</th>
<th>v (O-H) cm(^{-1})</th>
<th>v (C=O) cm(^{-1})</th>
<th>v (O=O) cm(^{-1})</th>
<th>v (C=O) cm(^{-1})</th>
<th>v (C=O) cm(^{-1})</th>
<th>v (C=O) cm(^{-1})</th>
<th>v (C=O) cm(^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cellulose</td>
<td>3345 broad</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>1025-1117 Strong</td>
</tr>
<tr>
<td>H</td>
<td>3345</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>1709</td>
<td>2400-3500 Very broad</td>
</tr>
<tr>
<td>HA</td>
<td>3283 Strong</td>
<td>1658</td>
<td>1519</td>
<td>3056</td>
<td>1720</td>
<td>2400-3500 Very broad</td>
<td></td>
</tr>
</tbody>
</table>

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Controlled drug release
Release of (HA) was studied, (100 mg) was added continuously in (100ml) buffer solution at (37°C), the wave length of $\lambda_{max}$ was measured at different periods and different pH values (1.1 – 7.4) by using UV spectrometer. These samples were analyzer by UV-spectroscopes periodically withdrawn for every days, it was appeared the sustained release by measuring the mole fraction were constructed from UV, indicated the rate of hydrolysis in basic medium is higher than acidic medium.[16] Mechanism of these drug polymer were illustrated as shown in the scheme.[4,5]

\[
\text{Cellulose} \text{--NH-Drug} \xrightarrow{\text{H}} \text{Cellulose} \text{--NH-Drug} \\
\text{OH} \xrightarrow{\text{OH}} \text{Cellulose} \text{--NH-Drug} + \text{Drug-NH}_2 \\
\text{OH} \xrightarrow{\text{OH}} \text{Cellulose} \text{--NH-Drug} \\
\text{OH} \xrightarrow{\text{OH}} \text{Cellulose} \text{--OH} + \text{Proton transfer} \\
\text{Drug-NH}_2 = \text{Amoxicilline}
\]

Scheme (4): Mechanism of hydrolysis drug polymer in acidic medium.

\[
\text{Cellulose} \text{--NH-Drug} \xrightarrow{\text{Nucleophilic addition}} \text{Cellulose} \text{--NH-Drug} \\
\text{OH} \xrightarrow{\text{OH}} \text{Cellulose} \text{--NH-Drug} \\
\text{OH} \xrightarrow{\text{OH}} \text{Cellulose} \text{--OH} \xrightarrow{\text{Drug-NH}} \\
\text{Drug NH}_2 = \text{Amoxicilline}
\]

Scheme (5): Mechanism of hydrolysis drug polymer in basic medium.

Thermal Properties of polymer drug[13]
Thermal stability of prepared polymers were investigated by (TGA and DSC) table (3) TGA showed the results of some prepared drug polymers which indicated the high thermal resistance and showed their steps of weight loss-temperature. This high thermal resistance indicated the high interaction between amide hydrogen bonding through the polymer chains and led to best sustain drug release. Several thermal stability parameters were determined from TGA and DSC curves as shown in table (3) and table (4).
Table (3) TGA Analysis of some polymer drugs

<table>
<thead>
<tr>
<th>No. drug polymer</th>
<th>Temperature</th>
<th>Losses weight%</th>
</tr>
</thead>
<tbody>
<tr>
<td>H</td>
<td>219, 372, 594</td>
<td>37, 53, 8</td>
</tr>
<tr>
<td>HA</td>
<td>457, 593</td>
<td>71, 28</td>
</tr>
</tbody>
</table>

Table (4) DSC Analysis of some polymer drugs.

<table>
<thead>
<tr>
<th>No. drug</th>
<th>Polymer</th>
<th>Onset Temp. °C</th>
<th>End set Temp. 0°C</th>
<th>Peak Temp. 0°C</th>
<th>ΔH J/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>H</td>
<td></td>
<td>132.2</td>
<td>173</td>
<td>140.2</td>
<td>292.2</td>
</tr>
<tr>
<td>HA</td>
<td></td>
<td>122</td>
<td>175.3</td>
<td>130</td>
<td>64.1</td>
</tr>
</tbody>
</table>

It was concluded that the thermal stability of drug polymer was more than the drug alone this cause more expire date and more protection of the drug satiability.

![Figure (1): FTIR spectrum of cellulose.](image1)

![Figure (2): FTIR spectrum of cellulose-g-maleic anhydride (H).](image2)

![Figure (3) FTIR spectrum of cellulose-g-[N-Amoxicillinyl male amic acid] (HA).](image3)
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