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Research Article

Preparation of Hybrid Films Containing Chitosan, Starch, Ascorbic Acid, and Different Metal Ions for Release of Doxorubicin

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ABSTRACT

As known, composite films containing different metal ions provide improvement in the properties of drug release systems. In this study, it was aimed to prepare composite films containing different metal ions for DOX release, and the effect of metal ions on drug release, swelling, and thermal properties were investigated. The structural characterization of the composite films was carried out using FT-IR, SEM, and TG analysis techniques. SEM images showed that the metal-free film was composed of a homogeneous structure while the calcium composite films consisted of a non-homogenous surface. Also, thermal analysis results showed that the thermal stability increased with the addition of metal ions to the composite film matrix. The swelling and drug-release behavior of the composite films were also studied, and metal ions-containing films exhibited a higher swelling performance and drug-release behavior than the metal-free composite.

1. Introduction

Biopolymers, which are also known as natural polymers, such as chitosan, starch, alginate, etc. are highly demanded in biomedical applications due to their biodegradability, biocompatibility, and non-toxic characteristics [1, 2]. Among these polymers, starch has attracted considerable attention due to its low cost, easy availability, and suitability for use as food [3]. Starch is also preferred in the production of large-scale biopolymer films and is used in food packaging, agriculture, and pharmaceutical distribution systems [4, 5].

On the other hand, starch-based films have shown poor thermal stability, low mechanical strength, and decomposition temperature [6]. Cao et al. stated that mixing starch with chitosan overcomes these disadvantages and improves some properties of starch such as easy film forming and antibacterial properties [7].

Chitosan is a biopolymer composed of linear polycationic polysaccharide units with inelastic behavior, and it has an excellent film-forming property [8, 9]. Chitosan, which also contains abundant amine ($-NH_2$) and hydroxyl (-OH) groups as its characteristic structural feature, is used as a biosorbent in dyes and organic pollutants [10]. It is also used in drug release applications due to its physical, chemical, and biological properties [11]. In addition, chitosan can easily chelate with metal ions due to its polycationic structure [12]. Although chitosan films have been shown good mechanical properties, they have poor flexibility and stress resistance [13]. Therefore, these features need to be developed.

Adding metal ions into a biocompatible material not only facilitates cross-linking and provides durability to the material, but also provides many benefits for the body's biological functions. As known, metal ions play an active role in many biological metabolisms such as protein, bone, and nucleic acid structure, electron transfer,

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charge balance, DNA signaling, redox, and acid-base catalysis. The use of metal ions in biomaterials positively affects cells in different aspects [14].

In the present paper, a series of ascorbic acid-based composite films were prepared by using chitosan, starch, L-ascorbic acid, and various cations such as Ag^+, Ca^{2+}, or Mg^{2+}. Metal ions were used as chelating agents. The usability of the fabricated films as drug delivery applications was also investigated. The structural characterization was carried out by using FT-IR, SEM, and TG analysis techniques. The swelling and doxorubicin release behavior of the composite films were also investigated.

2. Materials and Method

2.1 Reagents

Medium molecular weight chitosan (CS), starch (from potato, S), L-ascorbic acid (AA), calcium chloride dihydrate ($CaCl_2.2H_2O$), magnesium chloride hexahydrate ($MgCl_2.6H_2O$), silver chloride (AgCl), acetic acid (CH_3COOH), and doxorubicin (DOX) were obtained as commercially from Sigma Aldrich. All other chemicals were commercially supplied and they were used without any purification processes.

2.2. Preparation of the composite films

Composite films were fabricated by using chitosan, starch, ascorbic acid, and different metal cations (Ag, Ca, and Mg) as follows, and they were called the metal-free film, Ag, Ca, and Mg composite films according to containing cations (Table 1):

2.5 g of chitosan solution was prepared in distilled water containing 2.0% (v:v) acetic acid solution to prepare 2.5% (w:v) stock solution. Then, 2.0% starch (w:v), 1% L-ascorbic acid (w:v), and 5 mM of the cation (Ca, Mg, and Ag) solutions were prepared in distilled water and added into the chitosan solution as summarized in Table 1. Then, the solution was homogenized using a homogenizer for 3 minutes, and it was mixed for 4 hours at room temperature. The composite films were poured into the petri dish, and the solvent was completely evaporated under ambient conditions. Finally, the fabricated films were stored at +4 °C for further experiments [15, 16].

Table 1. Composition of the composite films

Composite film	**CS (v:v)**	**S (v:v)**	**AA (v:v)**	**Ca (v:v)**	**Mg (v:v)**	**Ag (v:v)**
Metal-free	80	5	10	-	-	-
Ca composite	80	5	10	5	-	-
Mg composite	80	5	10	-	5	-
Ag composite	80	5	10	-	-	5

2.3.Characterization

Fourier Transform-Infrared spectroscopy (FT-IR, Perkin-Elmer Spectrum Two FT-IR spectrometer) with an ATR unit was used to confirm functional groups in the starting materials and composite films. These measurements were carried out in the range from 500 to 4000 cm^{-1} under ambient conditions. The thermal degradation behavior of the composite films was also investigated using a Perkin-Elmer Diamond Thermogravimetric Analysis (TGA) with a heating rate of 10 ^oC min^{-1} in the range from 20 to 700 ^oC under a nitrogen atmosphere. The surface morphology of the composite films was determined using a Zeiss EVO LS 10 Scanning Electron Microscopy (SEM) under the high electron voltage (10 kV).

2.4. Swelling behavior of the composite films

A certain amount (20-25 mg) of the fabricated composite films was weighed and immersed into the freshly prepared phosphate buffer solution (PBS, pH 7.4, 25 mL) to investigate the swelling performance at room temperature. The composite films were removed from the solution at predetermined times, and their weights were recorded. The swelling percentage was calculated using the following equation (Eq.1) [17, 18]:

$$\%\text{Swelling} = \frac{m_2 - m_1}{m_1} \text{ x } 100 \qquad (1)$$

where m_1 and m_2 are the weights of the dry and wet films, respectively.

2.5. Drug delivery behavior

Doxorubicin (DOX), which is one of the most effective chemotherapy drugs used in various cancer treatments such as breast, and prostate, etc., was used as a model drug for drug release experiments in the present paper [19, 20]. The drug release performance of the composite films was determined in 20 mL PBS (pH 7.4) as the following equation (Eq. 2) [21]:

$$\%\text{Released drug} = \frac{Cumulative\ DOX\ weight}{Total\ weight\ of\ DOX} \text{ x } 100 \qquad (2)$$

About 10 mg of the composite film was added to the 20 mL 0.01 M PBS containing DOX and placed in a shaking incubator at 37 ^{o}C. At certain time intervals, 3 mL of DOX-containing PBS was removed from the solution medium, and the same amounts of the freshly prepared buffer solution were also added. The drug release amount was measured with the help of an UV-Vis spectrophotometer at 480 nm, and the released drug % was found.

3. Results and Discussion

3.1. Characterization of the composite films

FT-IR spectra of chitosan (CS), starch, L-ascorbic acid (AA), magnesium chloride hexahydrate (Mg), and Mg composite film were represented in Figure 1, and all FT-IR data were given in Table 2. In the FT-IR spectrum of CS, the characteristic peaks at 3279, 2864, 1646, 1414, 1372, and 1025 cm^{-1} were associated with the stretching vibrations of the hydroxyl (-OH), aliphatic –CH (Al-CH), -NH, -C-O, aromatic –CH (Ar-CH), and –C-O-C, respectively [22]. In the FT-IR spectrum of the starch, -OH, Al-CH, -C-O, and Ar-CH stretching, and –C-O-H bending vibrations were seen at 3280, 2920, 1418, 1329, and 991 cm^{-1}, respectively [23].

In the spectra of the Ca, and Mg, the stretching and bending vibration of H_2O were seen at 3322-3356 and 1608-1610 cm^{-1}, respectively [24]. According to the FT-IR spectrum of L-ascorbic acid, -OH, Al-CH, -C-O, enol hydroxide (-C=C-OH), and –C-O-C stretching vibrations were recorded at 3205, 2916, 1429, 1317, and 1023 cm^{-1}, respectively [25]. Also, the mentioned stretching vibrations were observed in the range from 3245 to 3267 cm^{-1}, 2885 to 2936 cm^{-1}, 1588 to 1639 cm^{-1}, 1386 to 1410 cm^{-1}, 1328 to 1305 cm^{-1}, and 1012 to 1018 cm^{-1} in the structure of composite films. These findings showed that the most of stretching vibrations of the composite films were shifted to the lower values compared to the chitosan and starch. This tendency was evidenced by the successful preparation of composite films [18]

Table 2. FT-IR data of the using materials and composite films

Materials	**1**	**2**	**3**	**4**	**5**	**6**
Chitosan	3279	2864	1646	1414	1372	1025
Starch	3280	2920	-	1418	1329	991
L-Ascorbic acid	3205	2916	-	1429	1317	1023
Metal-free	3228	2874	1638	1407	1306	1017
Ag composite	3245	2917	1589	1386	1328	1012
Ca composite	3250	2936	1588	1410	1305	1014
Mg composite	3267	2885	1639	1408	1305	1018

1: Hydroxy (-OH)
2: Aliphatic -CH (Al-CH)
3: -NH
4: -C-O
5: Enol hydroxy (-C=C-OH) or aromatic -CH (Ar-CH)
6: -C-O-C or -C-O-H

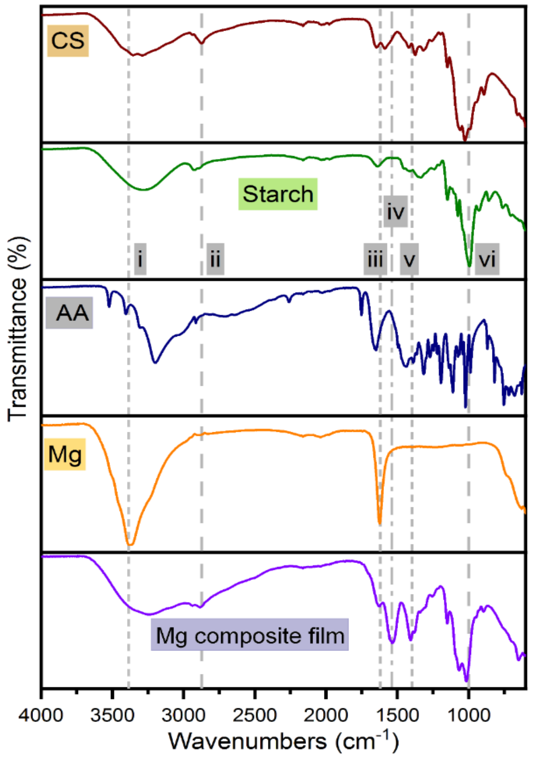

Figure 1. FT-IR spectra of chitosan (CS), starch, L-ascorbic acid (AA), Mg and Mg composite film

The surface of the fabricated metal-free composite and Ca composite film was investigated by using Scanning Electron Microscopy (SEM), and SEM images were shown in Figure 2. As can be seen in Figure 2, the metal-free film has a homogeneous and smooth surface whereas Ca composite film has been composed of a non-homogenous surface. Also, the surface roughness of the Ca composite film was increased after the addition of Ca^{2+} ions into the starch, chitosan, and L-ascorbic acid-containing composite matrix.

The thermal degradation behavior of the metal-free and composite films was clarified by using Thermogravimetric Analysis (TGA). Thermograms of the films were represented in Figure 3 and the results were summarized in Table 3. According to the degradation behavior of the composite films, they exhibited the two-step decomposition behavior.

The first degradation step, which takes place between room temperature and about 200 °C, is due to the evaporation of water, and acetic acid used in the fabrication of the composite films. The second degradation step, in which the main mass loss step was performed in the range from 200 to 350 °C, was due to the degradation of the saccharide rings in the structure of the composite films, the degradation of acetylated units or the decomposition of the biopolymer chains [26].

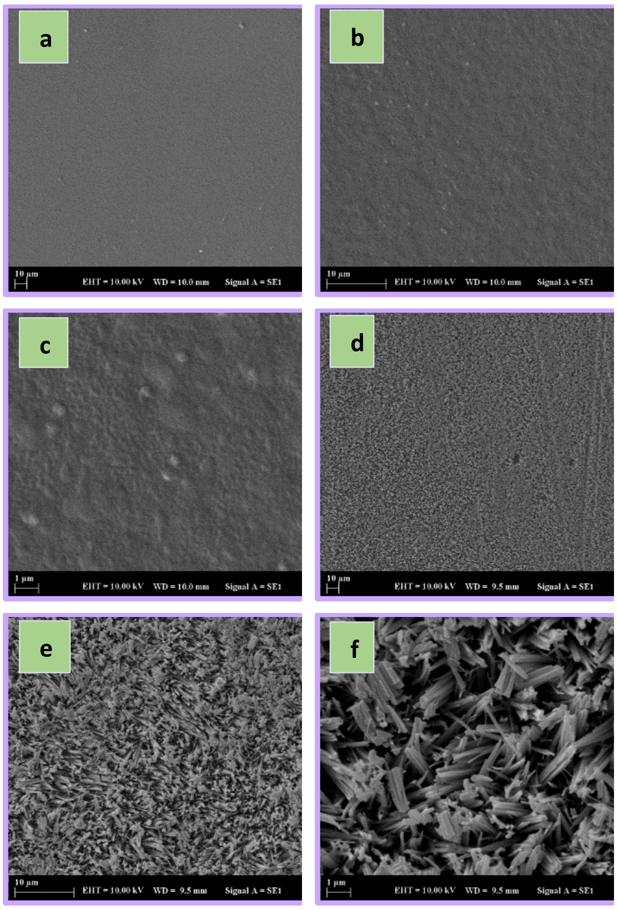

Figure 2. SEM image of metal-free film (a, b, c) and Ca composite film (d, e, f) at different magnitudes at 1, 5, and 20 kX

Also, Tmax, T20, T50, and char residual at 700 °C (char) of metal-free film, Ag, Ca, and Mg composite films were found in the range from 233 to 262 °C, 208 to 219 °C, 287 to 325 °C, and 30.77 to 35.99%, respectively. As can be seen in the char value of the films, the addition of cations into the composite film matrix, it was slightly increased.

Table 3. TG data of the composite films

Composite film	Tmax (°C)	T20 (°C)	T50 (°C)	%Char (at 700 °C)
Metal-free	262	208	307	30.77
Ag composite	235	215	325	35.99
Ca composite	233	219	307	35.96
Mg composite	237	208	287	33.63

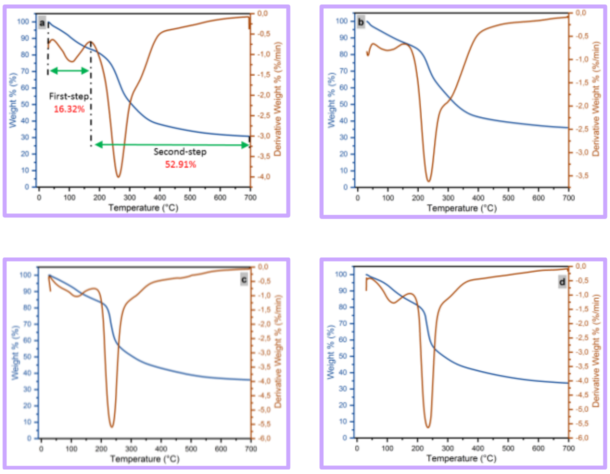

Figure 3. TG curves of metal-free film (a), Ag (b), Ca (c), and Mg (d) composite films

3.2. Swelling performance

The water uptake performance of the fabricated composite films was studied for 240 minutes, and the results were given in Figure 4. %Swelling capacity of metal-free film, Ag, Ca, and Mg composite films were determined as 58, 65, 79, and 94% after the 240 minutes. These results indicated that the % swelling performance of the composite films was increased after the addition of cations into the film matrix. The prepared chitosan, starch, and L-ascorbic acid matrix interact strongly with metal ions such as Ag^+, Ca^{2+}, and Mg^{2+}, and as a result, the swelling capacity of the composite films was increased due to the formation of a tight structure compared to the metal-free film [27].

3.3.Drug release behavior

Drug release behavior of the metal-free film and composite films was also studied, and doxorubicin was used as a model drug in these studies. DOX release performance of the films gradually increased within the first 90 minutes and it reached a constant value after the mentioned times (Figure 5).

%DOX release amounts of the metal-free film and composite films were calculated as 80.5, 55.0, 37.2, and 20.2% for Mg, Ca, Ag composite films, and metal–free films. Niazvand and coworkers stated that the desired properties of a drug release system can be achieved by the development of metal-containing composites. In addition, with the development of composite-containing cations for drug release systems, long-term controlled drug release can be achieved, the stability of the drug delivery system can be increased, and the preservation of drug bioactivity in the polymer-based composite system can be improved [28].

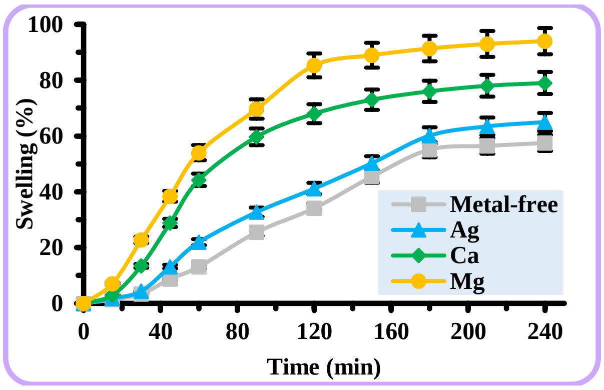

Figure 4. Swelling performance of the composite films

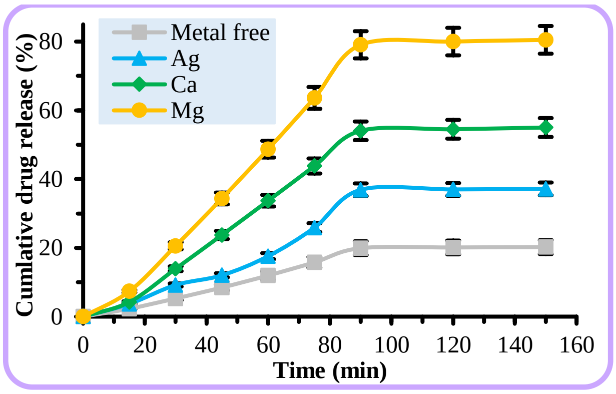

Figure 5. Drug release behavior of the composite films

4. Conclusion

In this paper, composite films based on chitosan, starch, L-ascorbic acid, and different metal ions such as Ag^+, Ca^{2+}, or Mg^{2+} were successfully fabricated to study the release of doxorubicin. The structural confirmation of the composite films was performed by FT-IR, SEM, and TG analysis techniques to clarify the structure, and chemical interactions, morphological and thermal properties. The swelling and drug release behavior of the composite films were also studied in 0.1 M PBS (pH 7.4). The results demonstrated that the addition of the metal ions into the composite film matrix increased the swelling, drug release, and thermal properties, and the developed composite films have the potential to be used for doxorubicin release.

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