

Novel Development of Chitosan-Based Nanocomposites

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Abstract—Chitosan polyacrylic acid composite membranes were prepared by a bulk polymerization method in presence of N, N'-methylene bisacrylamide (crosslinker) and ammonium persulphate as initiator. Membranes prepared from this copolymer in presence and absence of Ag nanoparticles were characterized by measuring mechanical and physical properties, water up-take and antibacterial properties. The results obtained indicated that the prepared membranes have antibacterial properties which increase with adding Ag nanoparticles.

Keywords—Ag nanoparticles, antimicrobial, composites, Membrane, physical properties.

I. INTRODUCTION

CHITOSAN (CS) is considered as a weak cationic polysaccharide produced by deacetylation of the natural polymer chitin; it has several useful biological properties, such as biocompatibility, biodegradability, and bioactivity. Due to the existence of amino groups, CS is a polycation and is able to form intermolecular complexes with many polyanions including hyaluronic acid, [1]-[3] poly(galacturonic acid), [4] alginate, [5] gelatin, [6] dextran sulfate, [7] and poly(acrylic acid) [8]-[10]. Commercially available CS has a quite large molecular weight (Mr) and it is dissolved in an acetic acid solution at a pH value of approximately 4.0. However, there are potential applications of CS in which a low Mr would be essential. Given a low Mr, the polycationic characteristics of water-soluble chitosan can be used together with a good solubility at pH value close to physiological ranges. Nanostructure noble metals have a wide-range of applications in the fields of photonics [11], catalysis [12], microelectronics [13], biosensing [14] and antimicrobial [15], etc. silver nanoparticles (AgNPs) are used as a substrate for enhancing the surface, plasmon resonance [16]. In the past reports, many effective methods have been used for the synthesis of Ag nanoparticles [17]-[19]. According to environmental and biological risks by using of noxious reducing and/or stabilizing agents in the synthesis procedures, like sodium borohydride, hydrazine [16] and N,N-dimethyl formamide [20], People are inclined to focus on the green chemistry. For this purpose, the natural compounds like D-glucose [21] and

chitosan [22], [23] was used to stabilize Ag nanoparticles with other reducing agents. In addition, the soluble starch was used as both the reducing and stabilizing agents to prepare the Ag nanoparticles via a one-pot green method [24]. Green synthesis of Ag-NPs involve three main steps, which must be evaluated based on green chemistry perspectives, including selection of solvent

medium, reducing agent, and nontoxic substance for AgNPs stability [25].

The aim of the present work is the preparation and characterization of chitosan polyacrylic acid composite membranes in presence of antibacterial agents such as Ag nanoparticles and chlorohexidindiacetate.

II. EXPERIMENTAL

A. Materials

Acrylic acid obtained from Merk-Schuchardt, was used without further purification. Chitosan, high molecular weight as determined by Brookfield viscometer (800.00 cps in 1% w/w chitosan aqueous 1% w/w acetic acid at 25°C), degree of deacetylation 87% [26] was kindly supplied by Aldrich Chemical Company (Germany). N, N'-methylene bisacrylamide and ammonium persulphate were purchased from Sigma-Aldrich Chemie GmbH, Germany. Chlorohexidindiacetate was supplied by Synopharm GmbH Co, Germany. All other chemicals were of laboratory grade reagents.

B. Preparation of Silver Nanoparticles

In recent studies, it was reported that silver nanoparticles was prepared using hydroxypropyl starch acting as both reducing and stabilizing agent. The aldehyde terminal of soluble starch is used to reduce silver nitrate while starch itself stabilizes the formed silver nanoparticles. Silver nanoparticles were prepared using soluble starch as reducing and stabilizing agent with the range of 6-8 nm [27].

C. Preparation of Composite Membranes

The copolymer was prepared by a bulk polymerization method. A typical copolymerization procedure was described as follows [28]-[30]. Chitosan (0.5-2%) (w/w) was dissolved with stirring in acrylic acid (10-25%) (w/w) aqueous solution at room temperature and then stirred overnight. The solution was filtered to remove insoluble impurities. To the prepared solution N, N'-methylene bisacrylamide (crosslinker), ammonium persulphate and tetramethyl ethylene diamide were added to initiate the polymerization.

A calculated amount from the antimicrobial agent was added with stirring. The solution was kept in stirring for 2 h at 70°C. The solution was transferred to the mold, where it was left for 1 hour to complete polymerization. After that the samples were allowed to swell in water to remove the water soluble impurities and then the final product was vacuum dried.

D. Evaluation of the Prepared Samples

1. Mechanical Properties of Composite Membranes

The sheets prepared for mechanical tests were cut into four dumb-bell-shaped specimens by a steel die of constant width (4

mm). The thickness of the test specimens were determined by gauge calibrated in hundredth of a millimeter. The working part of size 15 mm was chosen for each test specimen. The mechanical properties (tensile strength, elongation at break and young's modulus) of the samples were determined according to standard methods using an electric Zwick tensile testing machine, model 1425, in accordance with ASTM D 412-06.

2. Water-up-take

Water up-take was determined gravimetrically. The samples dried to constant weight (W^0) were immersed in water at room temperature, for 24 h. The excess water was removed with a filter paper and the samples were reweighed (W^1) [31], [32]. The water up-take was calculated, using the following equation:

$$\text{Water up-take} = (W^1 - W^0) / W^0 \times 100$$

3. Estimation of Antimicrobial Activity

The antimicrobial activity of the prepared membranes was measured according to the diffusion disk method [33], [34], against *Escherichia coli* (*E. coli.*) and *Staphylococcus aureus* (*Staph.*).

III. RESULTS AND DISCUSSION

A. Mechanical Properties

The mechanical properties of the poly (AA)/ chitosan samples having different poly (AA) content in dry state are given in Fig. 1 (a). It can be observed that with increasing poly (AA) content in the samples from 10-25% in presence of 1% chitosan the tensile strength (TS) increased by increasing poly (AA) to 15%, TS slightly decreases with further increase of poly (AA) content and at 25% a sharp decrease in TS occurs. The improvement of the tensile strength can be attributed to the strong interaction between the two polymers plus the intrinsic high mechanical properties of poly (AA) [35]. The same behavior is observed for young's modulus and modulus at 100% elongation. This observation may be due to an increase in the crosslinking density, which indicates reinforcement of the resulting copolymer. On the other hand, elongation at break (ϵ_R , %) decreases as poly (AA) content increases up to 15% of poly (AA). The value of ϵ_R , % is nearly the same up to 20%, and then at 25% of poly (AA) content a sharp increase occur (Fig. 1 (b)), which can be explained by the formation of complex between amino groups of chitosan and carboxylic groups of poly (AA). This may lead to the break of the hydrogen bonds between amino groups and hydroxyl group in chitosan and then results in amorphous structure of the polymer complex [36], [37] which may lead to decrease in the ϵ_R , %. Also, this may be attributed to the fact that ductility decreases when stiffness is increased by increasing p poly (AA) concentration.

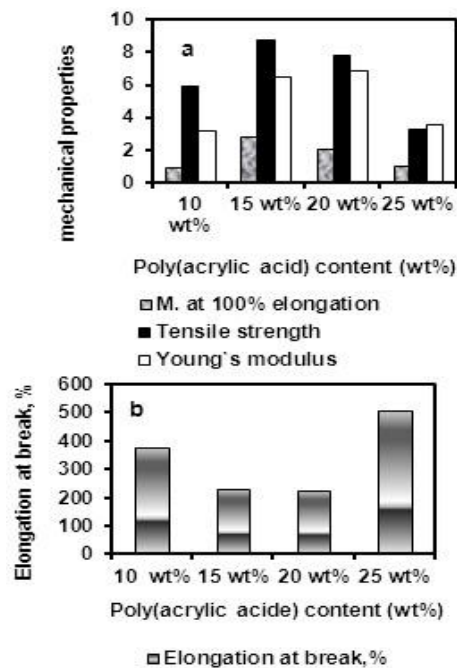


Fig. 1 (a) Mechanical properties of prepared membranes, (b) elongation at break of the prepared membranes; 1% chitosan, different concentration of acrylic acid

Tensile strength of the poly (AA) / chitosan copolymer having different chitosan (CS) content is given in Fig. 2 (a). It is observed that an increase of the chitosan content up to 1% leads to increase of tensile strength. At 1.5 and 2% of chitosan, slightly decline of tensile strength can be observed. The same behavior is observed for young's modulus and modulus at 100% elongation. This may be attributed to the ionic crosslinking between NH_3^+ of chitosan and COO^- of poly (AA) which may lead to an increase in rigidity. It was found that when the concentration of CS increases, the elongation at break of the copolymer decreases (Fig. 2 (b)). The mechanical properties thus prove the existence of some partial miscibility between the poly (AA) and chitosan in the copolymer [38].

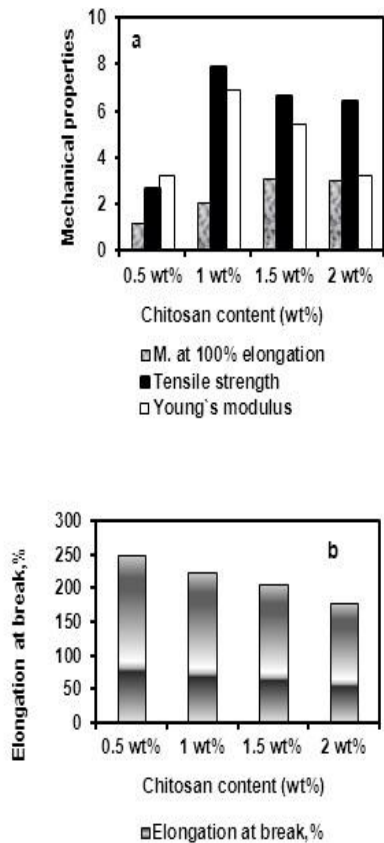


Fig. 2 (a) Mechanical properties of prepared membranes, (b) elongation at break of the prepared membranes; 20% acrylic acid, different concentration of chitosan

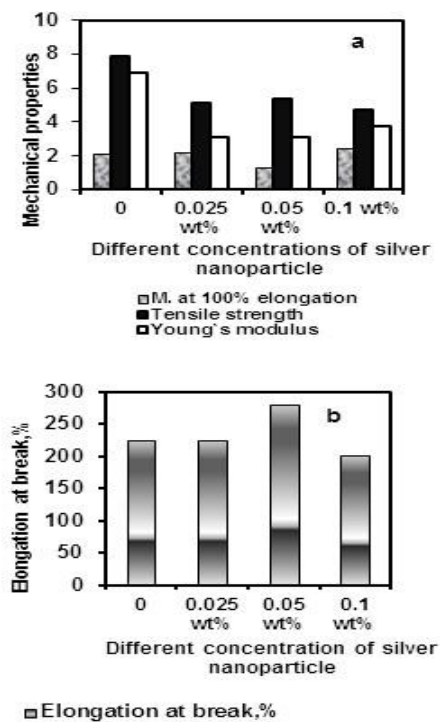


Fig. 3 (a) Mechanical properties of prepared membranes, (b) elongation at break of the prepared membranes; 1% chitosan, 20% acrylic acid

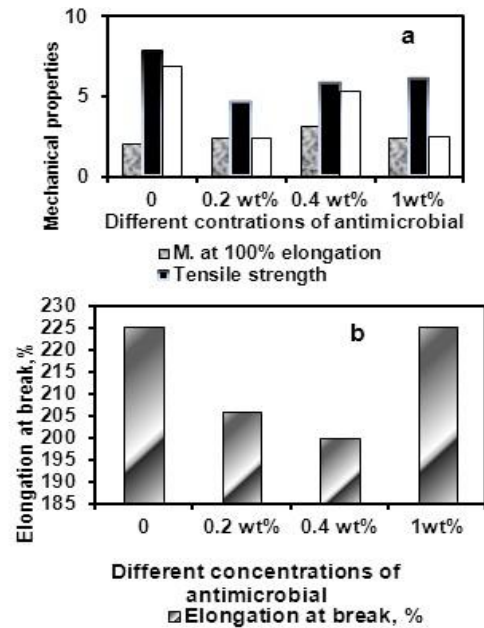


Fig. 4 (a) Mechanical properties of prepared membranes, (b) elongation at break of the prepared membranes; 1% chitosan, 20% acrylic acid

Fig. 3 (a) shows that when silver nanoparticles are used, TS decreases and stay nearly in the same value regardless to the silver concentration. The same behavior is observed for young's modulus and modulus at 100% elongation while elongation at break increases by using up to 0.05% of the silver nanoparticles and then decreases (Fig. 3 (b)). Figs. 4 (a) and (b) show that, the tensile strength decreased when a small amount of antimicrobial ingredient (chlorohexidindiacetate) was added to poly (AA) / chitosan copolymer. Other properties (Modulus at 100% elongation, Young's modulus and elongation at break) showed an irregular or clear character. These results can be interpreted in terms of the interaction between polymers in the presence of antimicrobial ingredient. Also, it is known that polymer chains in copolymer having electrostatic interactions with another polymer chain which may exhibit restriction in mobility due to ionic crosslinking, resulting shift in glass transition (T_g) and rigidity [39], [40].

B. Water Up-Take

To study the effect of different concentration of poly (AA), on the network structure of the poly (AA) / chitosan copolymers. The water up-take of the copolymer formed was determined as shown in Fig. 5 (a). It is observed that the water up-take % increases with the increase in the concentration of poly (AA) in the copolymer matrix. This can be explained by the hydrophilic nature of poly (AA) which causes more hydrogen bonding between the poly (AA) molecules and water, and leads to increase in the water up-take %. This can also be explained in terms of the decrease of the crystallinity of chitosan segments, due to the bulky units of poly (AA). Also, the presence of poly (AA) in polymer solution reduces the probability of radical combination during preparation of copolymer, which reduces the cross-linking density of the copolymer. This leads to more free volumes in the polymer

network, and consequently, more water can be absorbed [41]. Fig. 5 (b) shows that as concentration of chitosan increases the value of water up-take decreases, which may be due to the decreased mobility of polymer chains in chitosan filled copolymer. Moreover after adding Ag nanoparticles, the water up-take % of the copolymer increases with increasing Ag nanoparticles content up to 0.05% and then it is slightly decreases (Fig. 5 (c)). This character may be due to the fact that the copolymer prepared contains both chitosan as cationic and poly (AA) as anionic polyelectrolyte counterparts. Naturally, interchain salt bonds between amino groups of chitosan and carboxylic groups of poly (AA) forms. Therefore, complex behavior resulting from inter and/or intera molecular as well as possible electrostatic interactions should take place. On the other hand, the water up-take of poly (AA)/chitosan copolymer increases in the presence of antimicrobial ingredients. The ionic interaction between both polymers may cause changes in the porosity of the network, thus the water up-take varied [42], [43] as shown in Fig. 5 (d).

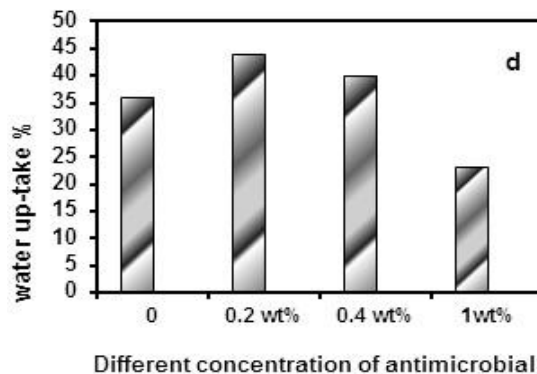
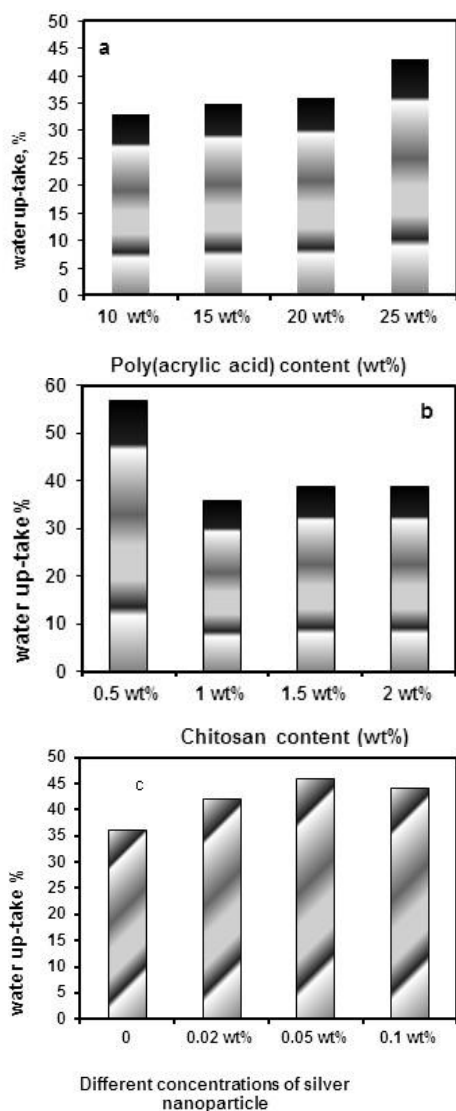


Fig. 5 Water up-take of prepared membranes (a) effect of acrylic acid content (b) effect of chitosan concentration (c) effect of Ag nanoparticles concentration (d) Effect of antimicrobial agent concentration

C. Antimicrobial Assessment

In order to overcome microbial infection, chitosan was incorporated in the formulation of copolymer solution used for the production of copolymer membranes. It is seen from Table I that the antimicrobial activity is greatly affected by chitosan and the antimicrobial ingredients added to the prepared copolymer solution.

TABLE I (A)
ANTIBACTERIAL PROPERTIES AS A FUNCTION OF ACRYLIC ACID AND CHITOSAN CONCENTRATION

Weight ratio Water: AA: chitosan	Inhibition zone diameter (mm/1 cm)	
	G ⁻	G ⁺
control		
blank		
90: 10: 1		
85: 15: 1		
80: 20: 1		
75: 25: 1		
80: 20: 0.5		
80: 20: 1		
80: 20: 1.5		
80: 20: 2		

TABLE I (B)
ANTIBACTERIAL PROPERTIES AS A FUNCTION OF AG NANOPARTICLES AND CHLOROHEXIDINDIACETATE

Weight antimicrobial added	Inhibition zone diameter (mm/1 cm)	
	G ⁻	G ⁺
Chlorohexidindiacetate (1g)		
Chlorohexidindiacetate (0.4g)		
Chlorohexidindiacetate (0.2g)		
nano Ag (0.1g)		
nano silver (0.05g)		

nano silver (0.025g)

Blank

20% acrylic acid and 1% chitosan

The effect of chitosan concentration on the antimicrobial properties of the prepared membranes is shown in Table I (A). The results indicate that the antimicrobial activity expressed as inhibition zone diameter, is strengthened as the concentration of chitosan increases [44]. In order to enrich the antimicrobial activity of the prepared copolymer, silver nanoparticles and chlorohexidindiacetate were added to the copolymer formulation used for preparing the membranes. The results (Table I (B)) show that adding silver nanoparticles induces antimicrobial properties to the prepared samples. This is probably due to high surface area to volume ratio of silver nanoparticles. The size and shape of silver play an important role in the antimicrobial properties [45].

Adding chlorohexidindiacetate to copolymer solution containing chitosan raised the antimicrobial activity. Results in Table I (B) show that the antimicrobial activity increases by increasing the concentration of chlorohexidindiacetate.

IV. CONCLUSION

Chitosan polyacrylic acid copolymer was prepared by a bulk polymerization method in presence of N, N'-methylene bisacrylamide (crosslinker) and ammonium persulphate as initiator. Membranes prepared in presence and absence of Ag nanoparticles was characterized by measuring mechanical and physical properties, water up-take and antibacterial properties. The results obtained indicated that the water up-take % increases with the increase in the concentration of poly (AA) in the copolymer matrix. The antibacterial properties enhanced by increasing chitosan concentration of the prepared membranes and Ag nanoparticles.

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