Preparation of Chitosan Films Mixed with Superabsorbent Polymer and Evaluation of Its Haemostatic and Antibacterial Activities

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ABSTRACT: Blended and layered films were developed from chitosan and starch–poly (sodium acrylate-*co*-acrylamide) superabsorbant polymer (SAP) and were tested for haemostasis. The tensile properties of the films are reported. A simple *in vitro* test was used to peer rank the effect of the films on the ability of chitosan to aggregate blood cells. It was clear that the addition of SAP material to chitosan enhanced the ability of the resulted films to coagulate blood. When chitosan and SAP were blended with a ratio of 1 : 1 (v/v) using concentrations of 1–2% and 0.25–0.5% (v/v) of chitosan and SAP respectively, the resulted films reduced the erythrocyte sedimentation rate (ESR) by 22% as compared to the control, whereas the chitosan and SAP control films reduced ESR by 11% and 22% respectively. Also, the two layered films with 2% (v/v) chitosan exhibited the same percent of reduction. These haemostatic films were further investigated by FTIR, TGA, tensile, antimicrobial ability, and cytotoxicity. Some new peaks were observed by FTIR due to possible interaction between the —OH groups of the starch and $-NH_3^+$ groups of the chitosan. Also, the films showed good mechanical and thermal properties. Moreover, the films also expressed antibacterial activity against *Pseudomonas aeruginosa* with a bacterial reduction of 99%. All the film samples exhibited a viability percentage around 100% with no cytotoxic effect on the cells. Chitosan–SAP films can be described as biofilms with a homogeneous matrix, stable structure, and interesting mechanical properties, with possibilities of utilization in haemostasis. © 2010 Wiley Periodicals, Inc. J Appl Polym Sci 116: 3489–3496, 2010

Key words: chitosan; starch; superabsorbant; haemostasis; films; biopolymers; antimicrobial

INTRODUCTION

Chitosan consists of β-(1–4)-2-amino-2-deoxy-D-glucopyranose residues and can be obtained by the partial deacetylation of chitin, which is found naturally in the cuticle of a marine crustacean, fungal cell wall, and some insects. The deacetylated polysaccharide has gained a great interest in industrial and biomedical applications because of its abundance, biocompatibility, biodegradability, nontoxicity, and chemical inertness. Many endeavors have been made to use chitosan to produce new biofunctional materials such as films, fibers, sponges, and gels, which have many biological properties including homeostasis, antimicrobial activity, healing stimulation, tissue-engineering scaffolds and drug delivery. Malette and Quigley first noted the haemostatic effect of chitosan in their patent of 1983.[1] The haemostatic effect of chitosan films were reviewed although it is difficult to elucidate the mechanism of its biological activity because of its complex chemical structure.[2] Also, a chitosan dressing was used to reduce hemorrhage and to improve survival after severe liver injury in swine.[3] Furthermore, it was found that chitosan enhanced haemostatis of lingual incision in rabbits by a 32% decrease in bleeding time.[4] It was observed that chitin and chitosan reduce the blood coagulation time and enhance the release of platelet derived growth factor AB and the transforming growth factor-β1 from the platelets.[5] The haemostatic effect of chitosan was explained by its interaction with the erythrocytes, linking them together to establish a cellular clot or haemostatic plug.[6] This effect, not only due to erythrocytes aggregation but also, extended to platelet aggregation.[7]

Superabsorbent polymers (SAP) are hydrophilic materials that can absorb water by more than 100% of their weight. They are mainly used in disposable

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TABLE I
Coding System for Various Volumetric Blends of Chitosan and SAP

Blend volumetric ratio	Chitosan/SAP concentrations (% w/v)								
	0.5/0.12	0.5/0.25	0.5/0.5	1/0.12	1/0.25	1/0.5	2/0.12	2/0.25	2/0.5
1 : 1	11B1	11B2	11B3	11B4	11B5	11B6	11B7	11B8	11B9
1 : 2	12B1	12B2	12B3	12B4	12B5	12B6	12B7	12B8	12B9
2 : 1	21B1	21B2	21B3	21B4	21B5	21B6	21B7	21B8	21B9
Two layer film	2L1	2L2	2L3	2L4	2L5	2L6	2L7	2L8	2L9

diapers and other applications including agricultural uses. SAPs have two main types, natural (polysaccharide or polypeptide based) and synthetic (petrochemical-based). Starch-graft-polyacrylonitrile was the first commercial SAP that was produced in 1970s. Nowadays, most SAPs are produced by solution or inverse-suspension polymerization of acrylic acid, its salts, and acrylamide.[8] The addition of a SAP to the chitosan film may help accelerate the haemostatic properties of chitosan by adsorption of the blood plasma there by concentrating the suspended platelet and red blood cells. Hence, the objective of this study was to evaluate the *in vitro* haemostatic, antibacterial, mechanical, and thermal properties of chitosan films when blended or layered with the starch based superabsorbent with different concentrations and blend ratios.

EXPERIMENTAL

Materials

Chitosan was purchased from Vanson® (Redmond, WA) and Starch–Poly (Sodium Acrylate-*co*-Acrylamide) Superabsorbant Polymer (SAP) was obtained from Water Lock® (Muscatine, lowa). Deionized water was used for film preparation. All other chemicals were of high purity and were used as received. The test organism *Pseudomonas aeruginosa* ATCC 97 and the L929 cell fibroblast were obtained from the American type culture collection, Rockville, MD. The MTT test was performed with the Cell Titer 96® non-radioactive cell proliferation assay kit (Promega, Madison, WI).

Chitosan characterization

The IR spectra of chitosan and film samples were carried out using a Thermo Nicolet 510P FTIR Spectrometer with OMNIC software. Sixty-four scans were accumulated with a resolution of 4 cm^{-1} for each spectrum in the range of 4000–400 $cm^{-1.}$ The degree of deacetylation (DDA) was determined according to Roberts,[9] using the infrared absorbance ratio A_{1655}/A_{3450} and calculated according to the following equation:

$$DDA(\%) = [1 - (A_{1655}/A_{3450})] \times 100/1.33$$

Chitosan weight–averaged MW and dispersity (weight-averaged/number-averaged MW) were determined on a Waters 150-C ALC/GPC gel permeation chromatograph (Waters Chromatography Div., Millipore Corp., Milford, MA). All MW data are reported as weight-averaged MW. The calibration curve consisted of a series of the linear polysaccharide pullulan standards ranging in MW between 0.59×10^4 and 78.8×10^4 daltons (Shodex Standard P-82, Showa Denko). Each of the standards and the samples (0.01 gm) were dissolved in 5 mL of the solvent (0.5 mol/L acetic acid and 0.5 mol/L sodium acetate (1 : 1 v/v)) and injected into the column at a flow rate of 0.6 mL/min using the previous solvent as eluent.[10]

Chitosan–SAP film preparation

Chitosan (0.5, 1 and 2% w/v) and SAP (0.12, 0.25, and 0.5% w/v) solutions were prepared separately by dissolving each polymer into 30% (v/v) acetic acid aqueous solution at room temperature for 24 h. The solutions of chitosan and SAP were mixed together in volumetric ratio of 1 : 1, 1 : 2, and 2 : 1, respectively, with stirring for another 24 h to form a homogeneous casting solution. Films were casted by pouring 30 mL of each blend in polystyrene petri dishes (100 × 15 mm) and dried at room temperature. For the two layer films, SAP solutions were first casted by pouring 15 mL of each concentration in nine polystyrene petri dishes (100 × 15 mm) that were left to solidify for 24 h. Fifteen mL of each chitosan solution were poured onto the solidified SAP in a factorial design experiment. Control films were prepared as mentioned before using each polymer individually. Two replicates were made for each film. Table I illustrates the coding system for the various volumetric blends of chitosan and SAP.

Erythrocyte sedimentation rate

The erythrocyte sedimentation test was evaluated with heparinized samples of freshly drawn porcine whole blood obtained from the College of Veterinary

Medicine, NC State University. It was beyond the scope of this work to perform *in vivo* haemostatic testing on animals and instead a simple *in vitro* test was used to peer rank the behavior of the films with blood. It is known that chitosan acts as a haemostatic outside of the normal cascade that leads to blood coagulation.[2] The erythrocyte sedimentation test was conducted by measuring the time of red blood cell sedimentation or "erythrocyte sedimentation rate (ESR)," in anticoagulated blood. A standardized method called ESR has been widely used for one of the diagnosis of various diseases.[11] In this study, conventional glass tubes were used whether the blood sedimentation is observed or not, by addition of fabricated chitosan and layered films into the tube.

A total of 1 mL of blood was transferred to each glass tube (10× 75mm) and it was preincubated for 5 min at 37°C. Disks of 1-cm diameter were made of all of the blends, two layer and control films. Each film disk was put into the blood, and the tubes were placed in upright position and incubated at 37°C until the blood was separated into two phases, supernatant and red blood cell aggregation with a ratio of 1 : 1. The time of blood sedimentation was recorded for each sample. Three replicates were conducted. After 1.5 h, the test was stopped and films were taken out from the blood pool. The morphology of the red blood cells was examined by Nikon Labophot2-POL equipped with COOLPIX digital camera. A drop from the phase containing red blood cells was placed on a glass plate and covered with a glass cover and examined with the optical microscope.[12] The films that have been observed to coagulate blood were selected for further investigation and analysis.

Statistical analysis

All experiments were performed at least in duplicates. SPSS software (SPSS Software, Chicago, IL) was used for analysis of variance. Differences in the properties of the films were determined by Fisher's least significant difference (LSD) test, using $P < 0.05$ as level of significance.

Tensile strength and elongation at break

Chitosan–SAP blended and layered films thicknesses were measured using a micrometer. Five thickness measurements were taken along the gauge length of each specimen and the mean value was used in calculating the film cross sectional area to obtain the tensile strength. The films were dried at room temperature then cut into strips 0.5-cm wide and 5-cm length (ASTM Standard Method D882-91) and mounted between cardboard grips so that the final area exposed was 3 cm. A minimum of six strips were prepared from each film. The tensile strength and elongation at break were measured on an Instron tensionmeter (Model 5544P9181) with a 100N load cell and a crosshead speed of 10 mm/min. The load to break curve of the film was plotted as a function of crosshead and the values of break stress, %strain at peak load, modulus, and break energy were determined.[13]

Thermogravimetric analysis

Thermogravimetric analysis (TGA) was conducted with a Perkin-Elmer TGA pyris 1® from 25 to 900°C at a heating rate of 30°C/min under nitrogen atmosphere. The sample weight was about 9 mg.

Bactericidal assay

The test microorganism, *P. aeruginosa*, was grown in nutrient broth for 24 h at 30°C. The bacterial suspension (10^7 CFU/mL) was used to inoculate a set of 12 sterile 250-mL Erlenmeyer flasks, each flask containing 50 mL of nutrient broth and 0.1 gm of the selected films (11B4, 11B5, 11B6, 11B7, 11B8, 11B9, 2L7, 2L8, 2L9, CS, and SAP) and one flask without any samples served as the control. All flasks were agitated on an orbital shaker at 30°C for 24 h. Serial dilutions were made for each specimen and then plated onto nutrient agar plates. Three replicates were made for each specimen. The plates were incubated at 30°C for 24 h after which the numbers of colonies on each plate were counted. The percent of reduction was calculated compared to the control.[14]

Evaluation of the *in vitro* cytotoxicity

The cytotoxicity effect was evaluated by the modified indirect method of the ISO10993-5 protocol[15] using a reference cell line of rat lung fibroblast L929 (ATCC CCL-1) grown in Dulbecco's Modification of Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and maintained at 37°C under a humidified atmosphere with 5% CO_2. Media was replaced three times per week and cells were typsinized and subcultured every 5 days. The chitosan–SAP films were cut into pieces of 1 × 1 cm, sterilized in 70% ethanol for 30 min, and dried. Extracts were prepared by adding each sterilized film to 2 mL of culture medium and incubating at 37°C for 24 h without shaking.

Cells were seeded into a 96-well plate at the density of 2×10^4 cells/well in 100 μL of DMEM supplemented with 10% FBS. After incubation in a humidified atmosphere of 5% CO_2 at 37°C for 24 h, the culture medium was replaced by 100 μL of the film extracts (three replicates were conducted) and

TABLE II
Time (min) of Blood Separation Using Different Blends of Chitosan/SAP Films

Film code			CS control				SAP control		
Time (min)			64 ± 2.52				56 ± 2.08		
Time reduction %[a]			11				22		
Film code	11B1	11B2	11B3	11B4	11B5	11B6	11B7	11B8	11B9
Time (min)	64 ± 1	64 ± 0.58	64 ± 1.53	56 ± 1.53	56 ± 0.71	56 ± 1	56 ± 1.73	56 ± 2	56 ± 0.58
Time reduction %[a]	11	11	11	22	22	22	22	22	22
Film code	12B1	12B2	12B3	12B4	12B5	12B6	12B7	12B8	12B9
Time (min)	72 ± 0.58	64 ± 1.53	64 ± 2	64 ± 1.53	64 ± 2.65	64 ± 3.61	64 ± 2.52	64 ± 0.58	64 ± 1.15
Time reduction %[a]	0	11	11	11	11	11	11	11	11
Film code	21B1	21B2	21B3	21B4	21B5	21B6	21B7	21B8	21B9
Time (min)	72 ± 1.73	72 ± 1.53	72 ± 0.58	72 ± 2.52	72 ± 1.15	72 ± 2.52	72 ± 0.58	72 ± 2.08	64 ± 2
Time reduction %[a]	0	0	0	0	0	0	0	0	11
Film code	2L1	2L2	2L3	2L4	2L5	2L6	2L7	2L8	2L9
Time (min)	72 ± 2.08	72 ± 2.52	72 ± 0.58	64 ± 1.73	64 ± 1	64 ± 2.89	56 ± 1.15	56 ± 0.58	56 ± 2.31
Time reduction %[a]	0	0	0	11	11	11	22	22	22

Three replicates were conducted.
The blood separation time of the control (without any film disks) was 72 ± 0.58 minutes.
[a] Time reduction % = (control time − sample time)/control time × 100.

culture medium was used as control. The plate was incubated for 24 h at 37°C in a humidified atmosphere of 5% CO_2 after which the MTT test was performed to quantify cell viability. The colorimetric reaction starts by adding 15 μL of the dye solution to each well followed by incubation at 37°C in humidified 5% CO_2 for 4 h, then 100 μL of the solubilization solution was added to each well. One hour after the addition of the solubilization solution, the contents of the wells were mixed to get a uniformly colored solution. The absorbance was recorded at 570 nm using 96-well plate reader.

RESULTS AND DISCUSSION

DDA and molecular weight of chitosan

DDA and molecular weight of the chitosan are the most important characteristics that affect any application in which chitosan is involved in. The DDA of the chitosan was found to be 95.7%. The high DDA insures a high concentration of cationic ammonium salt sites on the surface of the film, which is thought to be the key to haemostatic effects of chitosan. It also allows for some crystallization that enhances the mechanical properties.

The average was 2×10^5 Da with a polydispersity of 2.35. This molecular weight is sufficient for film formation and contributes to the high extensibility of the films, which was observed. We can also speculate that the high polydispersity indicates the presence of the more soluble oligomeric chitosans which probably contributes to the haemostatic properties associated with chitosan.

Blood coagulation test

To evaluate the effect of chitosan–SAP films on blood, blood erythrocyte sedimentation test was conducted *in vitro*. As we investigated only those films that show substantial haemostatic activity, this allowed us to reduce the number of film samples for subsequent mechanical, biological, and thermal analysis. Table II shows the blood ESR for chitosan–SAP films. The time of sedimentation varied with both chitosan and SAP concentrations. Chitosan–SAP films separated the blood into two equal phases in a time ranging from 56–72 min while the control (with out any films) exhibited the same phenomenon in 72 min. The results showed that ESR was significantly reduced ($P < 0.05$) by 22% when whole blood was mixed with 11B4,11B5,11B6,11B7, 11B8, 11B9, 2L7, 2L8, and 2L9 chitosan–SAP films compared to the control. In contrast, other films had less or no effect on ESR in the view of the fact that they reduced the ESR by 0–11% compared to the control.

Surprisingly, the addition of chitosan control film into whole blood did not lower the ESR value much, whereas the SAP control film reduced the ESR by 22% compared to the control. After sedimentation, a drop of the red blood phase was examined by the optical microscope with 40× magnification. Significant red blood cell aggregation was observed for all films that reduced the blood coagulation time by 22% in contrast to the control that has a lower density of aggregated red blood cells (Fig. 1). Also, large clots were formed on the surface of these films. These samples were selected for further analyses.

The coagulation process can be divided into two main stages; the primary stage where the platelets form a spigot at the site of hemorrhage and at the same time the secondary stage starts by initiating the coagulation factors to form fibrin strands which support the platelet spigot.[16,17] The role of erythrocytes in the coagulation process is not confirmed. It

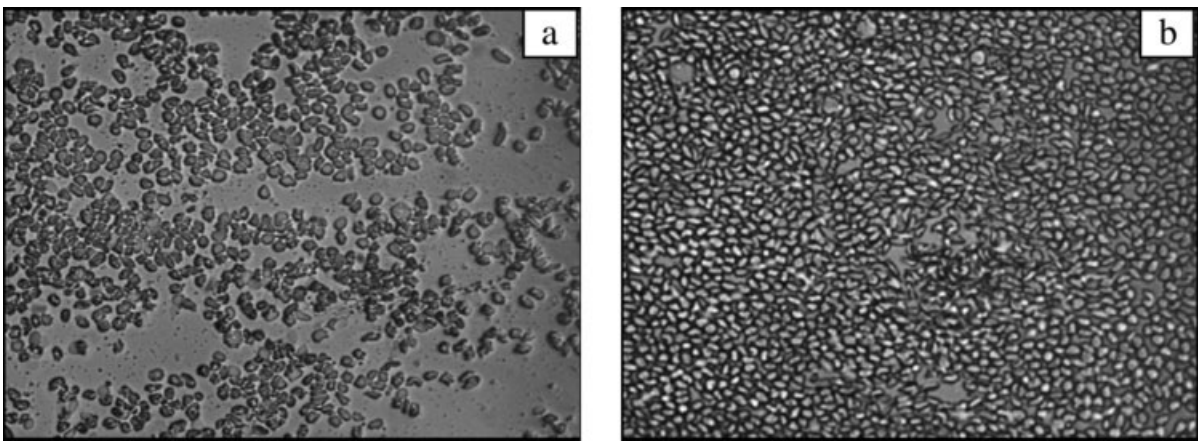

Figure 1 Optical microscope images (×40) of red blood cells after 1.5 h sedimentation; (a) blood control without film and (b) film sample.

was suggested that the erythrocytes can be the source of phospholipids needed for the coagulation mechanism.[18] Chitosan and SAP may induce not only the aggregation of platelets but also erythrocytes agglutination.

The chitosan films were prepared and left as the ammonium acetate salt. The positively charged chitosan is speculated to interact with the negative surfaces molecules on the platelets surface leading to its aggregation and therefore accelerating the coagulation process.[7]

The electrostatic interaction is also possible between the SAP, which has an anionic nature due to its hydroxyl groups, and the erythrocyte in the presence of ionic bridges. The role of chitosan in aggregating the erythrocytes has been suggested by many authors.[2,4,6,19–21] There could be a change in the surface charge of the chitosan and SAP as it interacts with the blood (pH 7.4) and the response would change with time. Interestingly though, the films have an observed effect upon the aggregation of blood cells. Only *in vivo* testing will determine the performance of these films compared to other currently available chitosan haemostatic dressings

FTIR analysis

The FTIR spectra of chitosan, SAP, and the representative chitosan–SAP blend and two layer films are shown in Figure 2. Four characteristic peaks for chitosan were observed, the O—H and N—H stretching at 3450 cm^{-1}, C—H stretching at 2920 cm^{-1}, amide I band at 1665 cm^{-1}, and the ammonium band at 1550 cm^{-1}. The SAP spectra can be divided into two parts, the first one is related to the acrylic/acrylamide residue that have bands located at 3360 cm^{-1} (O—H and N—H stretch), 2910 cm^{-1} (C—H stretch), 1710 cm^{-1} (C=O Stretch), 1550 cm^{-1} ($—NH_3^+$), 1467 cm^{-1} (CH_2), 1420 cm^{-1} (C—N stretch), and 1350 cm^{-1} (COO^- stretch), whereas the second part has the characteristic peaks of starch polysaccharide located at 3360 cm^{-1} (O—H stretch), 2910 cm^{-1} (C—H stretch), 1467 cm^{-1} (—OH bending), 1130 cm^{-1} (C—O stretch), and 1072 cm^{-1} (C—O—C stretch).

Both physical and chemical interactions can happen when chitosan and SAP materials are blended or layered with each other. The presence of a new peak in the spectrum of SAP film at 1710 cm^{-1}, which is absent in the spectrum of SAP powder, reveals the formation of C=O stretch as a result of the interaction between acrylic and acrylamide at low pH via hydrogen bonding.[22] Also, the peaks at 1665 cm^{-1} (amide I band) and 1550 cm^{-1} ($—NH_3^+$) appear at different positions and intensities.

Also, the starch peak at 1467 cm^{-1} and the chitosan amide peaks at 1665 cm^{-1} and 1550 cm^{-1} are shifted to higher frequencies indicating a possible interaction between the NH_3^+ of chitosan and the

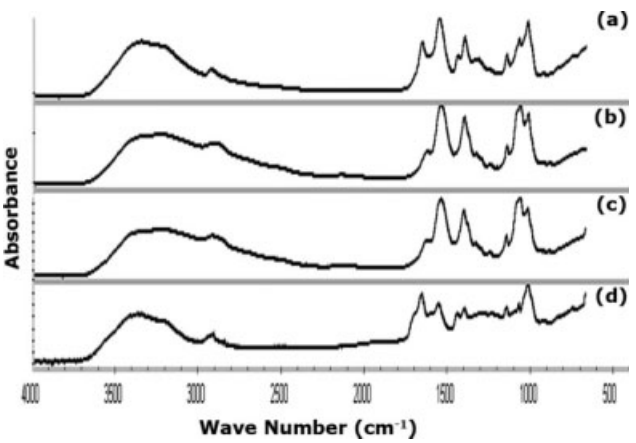

Figure 2 FTIR spectra of chitosan and SAP films: (a) 2L9, (b) 11B4, (c) CS, (d) SAP.

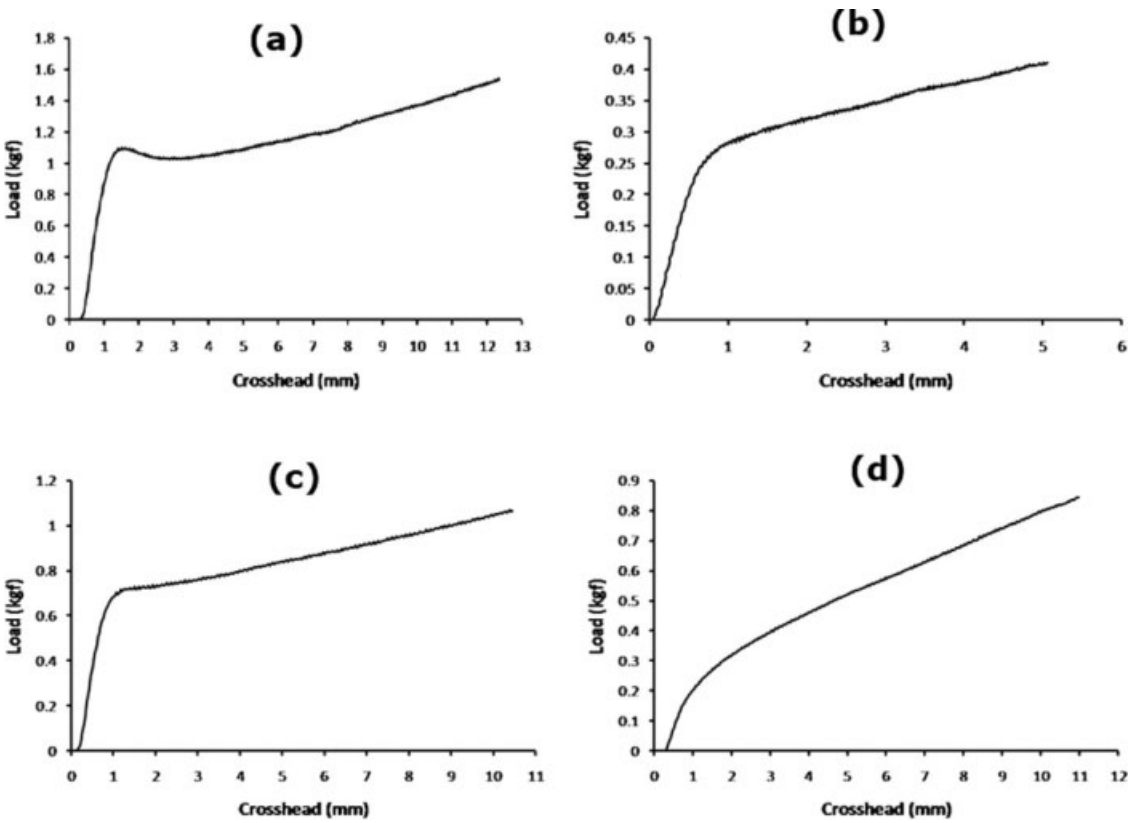

Figure 3 The load-extension curves of various CS–SAP films: (a) CS, (b) 11B5, (c) 11B8, and (d) 2L8.

—OH group of starch.[23] Furthermore, a difference was observed between the spectrum of the two layered films and that of the blended films. The two layered films have additional peak at 1467 cm^{-1} (CH_2), which is absent in the spectrum of the blended films.

Film tensile properties

Only those films that had the best effect for aggregating the blood cells were mechanically tested. Those results are reported here. It is known that the mechanical properties of the blend films are influenced by the interaction between its polymers. Representative load-extension graphs of chitosan–SAP films are shown in Figure 3. As summarized in Table III, the tensile strain of the mixed films ranged from 13.78 to 36.50% as the concentration of chitosan was doubled, in the films prepared by blending. The chitosan control film, as expected, had the highest strain at peak load (44.45%). The SAP itself is a poor film former and only brittle films, too weak to test, were obtained. SAP is not expected to be miscible with chitosan, so as observed, it decreased the tensile properties of the films, compared to the chitosan control. Surprisingly, the films obtained from the more viscous 2% chitosan solutions were stronger. This indicates that the films have a different morphological structure, perhaps characterized by a more continuous chitosan matrix, with domains of SAP included within its structure. The tensile strength results mirror those of the tensile strain for the blended films. The tensile results for the layered films indicate that the chitosan is the load bearing component and the SAP contributes little to the tensile strength of these films.

TGA studies

The TGA of some chitosan–SAP films are shown in Figure 4. The TGA of chitosan film is illustrated in

TABLE III
The Mechanical Properties of Blended and Layered Chitosan– SAP Films

Film code	Strain (%)	Stress (MPa)
CS	44.45 ± 8.72	34.10 ± 9.50
11B4	16.93 ± 3.27	12.87 ± 0.02
11B5	17.05 ± 1.98	12.05 ± 0.31
11B6	13.78 ± 1.17	13.44 ± 0.53
11B7	31.59 ± 14.49	20.44 ± 1.82
11B8	34.61 ± 9.28	21.96 ± 1.04
11B9	32.43 ± 6.01	15.22 ± 2.90
2L7	34.68 ± 5.02	19.99 ± 1.40
2L8	36.50 ± 1.90	20.47 ± 1.72
2L9	34.62 ± 7.70	27.31 ± 3.66

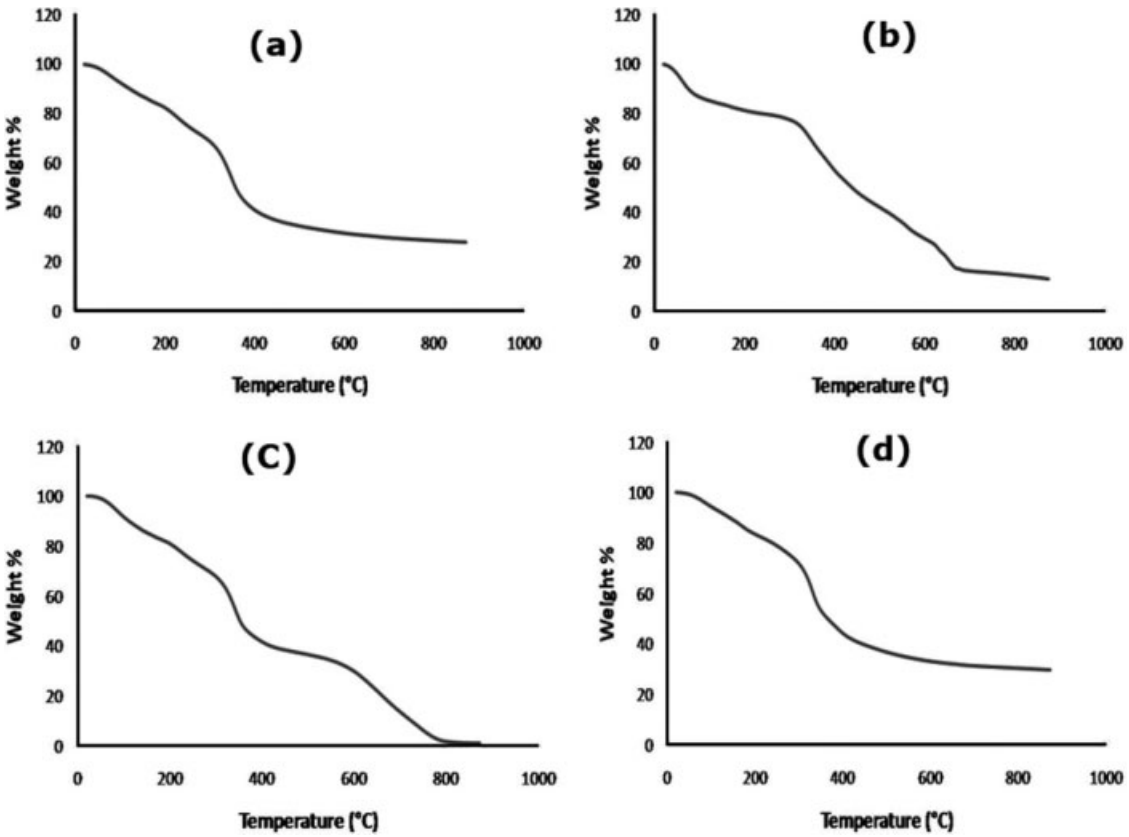

Figure 4 TGA curves of chitosan–SAP films: (a) CS, (b) SAP, (c) 11B4, and (d) 2L8.

Figure 4(a). It was observed that the curve is divided into three stages of decomposition in which the mass of the film decreased gradually because of water evaporation while the temperature increased from 30 to 300°C. In the second stage, which starts at 300°C and ends at 350°C, the saccharide ring is dehydrated and the polymer units are depolymerized, all of these effects are accompanied with weight loss of about 20%. A complete weight loss did not occur even after heating the material up to 800°C, as can be seen from the third stage that started above 350°C indicating the ash content. Also, the SAP film has three stages of degradation [Fig. 4(b)], but it was observed that the initial 30% loss of weight occurred at a higher temperature than the chitosan, which means that the SAP is more stable at a temperature of 350°C, and this relative stability extends to a temperature of 440°C at which 50% of the film weight was lost. However, the SAP material loses 70% of its weight at about 590°C in contrast to the chitosan film that loses the same percent at higher temperature (660°C). The TGA curves of chitosan–SAP films [Fig. 4(c,d)] are similar to the chitosan curve with slight differences between them because of the difference in solid material concentration. Increasing the total chitosan and SAP concentrations resulted in increasing the stability of the film material.

Antibacterial activity

The antibacterial activity of chitosan–SAP films was investigated against the Gram-negative bacterium *P. aeruginosa*, selected for its low antibiotic susceptibility and its ability to cause blood infections (sepsis) due to its opportunistic nature. As shown in Table IV, all the films showed high antibacterial activity with similar percent of reduction (99%), and this activity slightly decreased when SAP film was used to reach 88% reduction. The antibacterial activity of

TABLE IV
Bactericidal Effect of CS/SAP Films Against *Pseudomonas aeruginosa*

Sample	Surviving cells ($\times 10^8$ CFU/mL)	% Reduction
Control	548 ± 13.87	–
CS	4.1 ± 0.32	99
SAP	61.5 ± 7.68	88
11B4	49 ± 7.37	91
11B5	47 ± 3.79	91
11B6	45.5 ± 5.75	91
11B7	0.06 ± 0.04	99
11B8	0.03 ± 0.02	99
11B9	0.03 ± 0.02	99
2L7	2 ± 1.53	99
2L8	0.4 ± 0.21	99
2L9	0.1 ± 0.13	99

TABLE V
MTT Test of Chitosan Films Blended with SAP Material

Sample	MTT Value (%)[a]
Control[b]	–
11B4	100 ± 0.09
11B5	93 ± 0.03
11B6	103 ± 0.09
11B7	94 ± 0.06
11B8	89 ± 0.07
11B9	98 ± 0.17
2L7	102 ± 0.18
2L8	93 ± 0.07
2L9	101 ± 0.09
CS	96 ± 0.02

[a] Validity related to the control, which scored 100%.
[b] Control: cells with culture medium.

chitosan–SAP films is due to the $R\text{-}NH_3^+$ which form in the acidic solutions of both of chitosan.[24]

Cytotoxicity

The *in vitro* cytotoxicity test is based on the concept that toxic chemicals affect the basic functions of cells. Such functions are common to all cells, and hence the toxicity can be measured by assessing cellular damage. The MTT assay is used to measure the activity of mitochondrial reductase enzymes that reduce the MTT to purple formazan, which can be measured colorimetrically as an indication of cell viability. The results of the MTT test after 24 h of incubation with the films extract are shown in Table V. Normal morphology was observed when the cells incubated with the films extract medium were examined with the inverted light microscope. The cell viability percentage is related to the control which is considered being 100% viable. All the film samples exhibited a viability percentage around 100% with no cytotoxic effect on the cells. No significant difference was observed on the cell viability by increasing the chitosan or the SAP concentration. The results obtained can be considered as a good indicator of the biocompatibility of the tested materials.

CONCLUSIONS

Blended and layered films of chitosan and a starch–poly (sodium acrylate-*co*-acrylamide) (SAP) were prepared and characterized. As expected, the layered films had better mechanical properties than the blend films because the chitosan and SAP are not expected to be miscible polymers. A simple *in vitro* test demonstrated that the blend and layered films decreased the time it took for erythrocyte sedimentation to occur, suggesting a haemostatic potential. The presence of the SAP did not inhibit the antibacterial properties of the chitosan. All the film samples exhibited a viability percentage around 100% with no cytotoxic effect on the cells.

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