Mechanical Properties and Release Studies of Chitosan Films Impregnated with Silver Sulfadiazine

Eduardo P. Azevedo,[1] Tásia D. P. Saldanha,[1] Marco V. M. Navarro,[1] Aldo C. Medeiros,[2] Marconi F. Ginani,[3] Fernanda N. Raffin[1]

[1]*Department of Pharmacy, Federal University of Rio Grande do Norte, Natal, Rio Grande do Norte, Brazil*
[2]*Department of Surgery, Federal University of Rio Grande do Norte, Natal, Rio Grande do Norte, Brazil*
[3]*Department of Chemistry, Federal University of Rio Grande do Norte, Natal, Rio Grande do Norte, Brazil*

Received 3 February 2005; accepted 18 March 2006
DOI 10.1002/app.24537
Published online in Wiley InterScience (www.interscience.wiley.com).

ABSTRACT: The purpose of these studies was to evaluate chitosan films impregnated with silver sulfadiazine as potential wound dressings, based on their mechanical properties and the controlled-release phenomenon. The mechanical properties of such films were investigated under varying plasticizers (glycerol or sorbitol) concentrations, as well as in the presence of a crosslinking agent (formaldehyde). The drug release was also determined under such varying conditions, as well as using different thicknesses of film and drug concentrations. The results showed that the additives decreased the tensile strength of the chitosan films (except for sorbitol at 20% w/w), while at the same time remarkably enhancing the percentage elongation of the films. This elongation was especially pronounced in the case of glycerol. The type of plasticizer also influenced the release of silver sulfadiazine. Glycerol had a greater effect than sorbitol on the release rate, regardless of the amount used, probably due to leakage of this additive from the film, which leaves pores that enhance the water uptake of the film. As might be expected, increased concentrations of entrapped silver sulfadiazine yielded increasingly higher release rates. Decrease in thickness of the film also enhanced the release rate. © 2006 Wiley Periodicals, Inc. J Appl Polym Sci 102: 3462–3470, 2006

Key words: biodegradable polymer; chitosan films; silver sulfadiazine; solubility; mechanical properties; controlled release

INTRODUCTION

Biodegradable polymers are a well-established field with widespread applications in the pharmaceutical and cosmetic industries. A vast amount of research has been undertaken with the purpose of optimizing the rational use of biopolymers in different areas.[1–3]

Chitosan is especially recommended as a multifunctional material because this natural polymer has an excellent array of properties such as biocompatibility, biodegradability, nontoxicity, and high adsorption capacity.[2] Chitosan is a linear copolymer of β(1→4) linked 2-acetamido-2-deoxy-β-D-glucopyranose and 2-amino-2-deoxy-β-D-glycopyranose. This biopolymer is easily obtained by deacetylation of chitin, a polysaccharide that is widely distributed in nature, being a key component of the shells of crustaceans.[1–4]

Pharmacologically, chitosan is reported to have useful hypocholesterolemic, hemostatic, antiulcerative, antimicrobial, and wound-healing properties.[4] Chitosan can be used as an artificial skin to hasten the healing of wounds and its positive surface charge and biocompatibility enable it to support cell growth very effectively.[1,5] These properties together with its film-forming ability make chitosan a promising biomaterial for application in burn wounds.

A severe burn causes a serious disruption of normal skin barrier functions and of host defense mechanisms against infections. The patient remains highly vulnerable to invasive microbial infection until complete re-epithelialization or recovery of the wound area has occurred. Consequently, wound sepsis is a major cause of mortality among such patients.[6] In addition, infected wounds also scar more severely and are associated with the need for more prolonged rehabilitation.[7] In order to prevent or retard this process, the excision of burnt tissues, closure of the wound, and prophylactic treatment with topical antimicrobial should be undertaken rapidly.[7–9]

Topical antimicrobial agents have an important therapeutic role in the treatment of burns because they maintain the wound flora at low levels. Silver sulfadiazine (4-amino-*N*-2-pyrimidinylbenzenesulfonamide monosilver (1+) salt) disintegrates in the burn wound, thereby providing a slow and sustained release of silver ions. These inhibit the growth and multiplication of bacterial cells without affecting the cells of the skin and subcutaneous tissue. The drug is effective against a wide range of gram-negative and gram-positive bacteria including *Escherichia coli*, *Pseudomonas aeruginosa*,

Correspondence to: E. P. Azevedo (azevedoep@hotmail.com).

Staphyloccocus aureus, strains of *Proteus* and *Klebsiella*, as well as *Candida albicans* and other fungi. The emergence of resistant strains is rare and allergies to this drug are unusual. For these reasons, silver sulfadiazine is currently the most extensively used topical antiinfective agent for burn care.[9,10]

The traditional treatment of burn wounds consists of the application of 1% silver sulfadiazine cream over the injured area followed by protection with dressings. These dressings should be changed frequently, because the antibacterial action of the cream lasts only about 12 h. However, the cream eventually dries and the dressing then sticks to the wound surface, leading to pain and considerable damage of the newly formed epithelium when the dressing is removed.[7,11]

The need for alternative, rapidly effective and permanent wound dressings has attracted the interest of the pharmaceutical industry. Research and development has resulted in the production of a wide variety of synthetic and biological skin substitutes for wound closure and wound healing.[12]

Chitosan, which has structural characteristics similar to glycosaminoglycan, can be considered as a good skin substitute.[2] Apart from its reported activity as an accelerator of wound healing,[5,12,13] chitosan has been documented as having considerable antibacterial activity against a broad spectrum of bacteria.[12,14,15]

Thus, the proper combination of topical agents with wound coverings that are both biocompatible and biodegradable (like chitosan) can decrease the probability of infection and enhance wound healing.[7]

Recently, topical antibiotics have been incorporated in wound dressings or artificial skins, taking advantage of the property of controlled release over a period of days to protect the wound effectively against infection.[6,16]

Chitosan is widely used for the effective delivery of many pharmaceuticals.[1,16,17] Furthermore, the fact that chitosan can be turned into films which are flexible, elastic, and adherent[2,18] suggests it may be a suitable matrix for the incorporation of silver sulfadiazine for the preparation of long-acting antibacterial wound dressings.

For this study, chitosan films impregnated with silver sulfadiazine were prepared using the method described below. The solubility of silver sulfadiazine in chitosan solution was evaluated. As the ultimate aim of this work was to use these films in burn patients, it was necessary to check if the material had adequate mechanical properties to survive the whole period of treatment, maintaining its properties, such as protection of the wound site, provision of a barrier against bacterial penetration and controlled release of silver sulfadiazine. The mechanical properties of the films were studied using crosslinking agent and increasing quantities of plasticizers (glycerol or sorbitol). The influence of drug concentration, type of plasticizer, and film thickness on the release rate were investigated.

MATERIALS AND METHODS

Materials

Chitosan (molecular weight = 50,550 Da; deacetylation degree = 87.5%) was obtained from Polymar Indústria e Comércio Ltda (Fortaleza, CE, Brazil). Formaldehyde and sorbitol 70% were purchased from Labsynth Produtos para Laboratórios Ltda (Diadema, SP, Brazil), glycerol was obtained from Vetec Química Fina Ltda (Rio de Janeiro, RJ, Brazil).and silver sulfadiazine was generously provided by Galena Química e Farmacêutica Ltda (Campinas, SP, Brazil).

Solubility study of silver sulfadiazine

The experiments were performed following the method described by Higushi and Connors.[19] This involves adding successive portions of the sample to constant volumes of a solvent in which it is slightly soluble. Therefore, fixed amounts of silver sulfadiazine (0.0015, 0.0030, 0.0045, 0.0060, 0.0075, 0.0090, 0.0105, and 0.0120 g) were added to 100 mL of 2% lactic acid and to 1% chitosan solution (chitosan was dissolved in 2% lactic acid). The systems were brought to equilibrium by prolonged agitation for 60 min at 25±2°C. The solution was then analyzed for total solute content by a Varian Cary 1E UV spectrophotometer (Cary, NC) at 256 nm. A curve was obtained by plotting the total amount of drug dissolved (vertical axis) against the amount of drug added (horizontal axis). The analyses were performed in triplicate.

Preparation of silver sulfadiazine impregnated films

First, 1% (w/w) chitosan solution was obtained by dissolving chitosan in 2% (v/v) lactic acid at room temperature. The solution was then filtered and a fixed amount of formaldehyde was added in the proportion of 1 mole of formaldehyde to 5 moles of chitosan monomers. Then, 20% or 40% (w/w) of plasticizer (glycerol or sorbitol) was added to samples of this solution, alongside untreated control samples, with the purpose of evaluating its influence on the mechanical properties of the films and also on the drug release. After that, silver sulfadiazine was added obeying the proportion of 0.6% or 1% in relation to the weight of the chitosan. The solution was agitated for 60 min and was then cast on a Teflon® plate and dried in an oven at 50°C for 48 h. Finally, the films were detached from the plate manually and conditioned in desiccators at 25 ± 1°C containing silica gel for about 24 h before each experiment. The thickness of the dry films was deter-

mined in five locations with a micrometer, model DCF-900, Check-Line (Cedarhurst, NY).

Swelling studies

Film samples (2 × 3 cm) were cut from the bulk chitosan film crosslinked with formaldehyde. Samples with thicknesses in the range 250–300 μm were tested by immersing in PBS (pH 7.4) at room temperature. The sample weights were determined at 0.5, 1, 2, 24, and 48 h after carefully blotting the film with filter paper to remove adsorbed solution on the surface, then weighed immediately on an electronic balance. Swelling was expressed as the percentage swelled:

$$\% \text{ swelled} = [(W_t - W_o)/W_o] \times 100$$

where W_o is the weight of the dry sample (g) and W_t is the weight of the wet sample at time t.

Amount of water adsorbed

The amount of water adsorbed at 75% RH was reported for the various chitosan films crosslinked with formaldehyde and plasticized with glycerol or sorbitol. The weights of the completely dried samples (2 × 3 cm) were measured directly and then introduced into containers containing saturated sodium chloride solution to create an environment with 75% of relative humidity. After 24 h, the samples were weighted immediately and the amount of water adsorbed was calculated according to the following equation for each weighted sample (WS):

$$[(W_{\text{wet}} - W_{\text{dry}})/W_{\text{dry}}] \times 100$$

Mechanical properties

Tensile strength and percentage elongation were measured with an EMIC Universal Testing Machine, model DL-500 MF (São José dos Pinhais, PR, Brazil), at 25 ± 0.5°C and relative humidity of 75 ± 2%. The effects of the presence of formaldehyde and silver sulfadiazine, as well as the presence, type, and quantity of plasticizer, were investigated. Five specimens consisting of 8 × 3 cm strips were cut from each type of film and mounted between the grips of the machine. The initial grip separation was set to 50 mm and the grips were moved at a crosshead speed of 200 mm/min until the film broke. A microcomputer was used to record and determine the tensile strength, elongation and the percentage elongation at rupture.

In Vitro drug release studies

The release of sulfadiazine from chitosan films was evaluated using the USP dissolution apparatus (paddle method) made by Nova Ética Indústria e Comércio Ltda, CDC 44 (Vargem Grande Paulista, SP, Brazil). Each film (2 cm^2) was placed in individual cells containing 200 mL of phosphate-buffered saline (pH 7.4) and the paddles were rotated at 50 rpm at 37 ± 0.5°C. Samples of 2 mL were withdrawn from these solutions at fixed time intervals of 0.6, 2, 4, 6, 8, 24, 48, 72, 96, 120, and 168 h (for seven days). The amount of sulfadiazine released from the films was determined spectrophotometrically at 256 nm using a Varian Cary 1E spectrophotometer (Cary, NC). Equivalent volumes of fresh phosphate-buffered saline were placed into the cells after each sampling to maintain constant medium volume. Release studies were carried out in triplicate.

Statistical analysis

One-way analysis of variance (ANOVA) was performed to determine the significant effects of the amount of drug, the film thickness, the type and amount of plasticizer on the swelling percentage, and the mechanical properties, as well as the drug release. The analyses were performed using the GraphPad Prism program, version 4, where the differences were considered to be significant at a level of $P < 0.05$.

RESULTS AND DISCUSSION

Solubility study of silver sulfadiazine

Figure 1 shows the dissolution profile of silver sulfadiazine in two different media: 2% lactic acid and 1% chitosan solution. It is well known that 2% acetic acid is used frequently as the chitosan solvent. However, studies performed by Bégin and Calsteren[14] showed that chitosan films prepared from acetic acid were hard

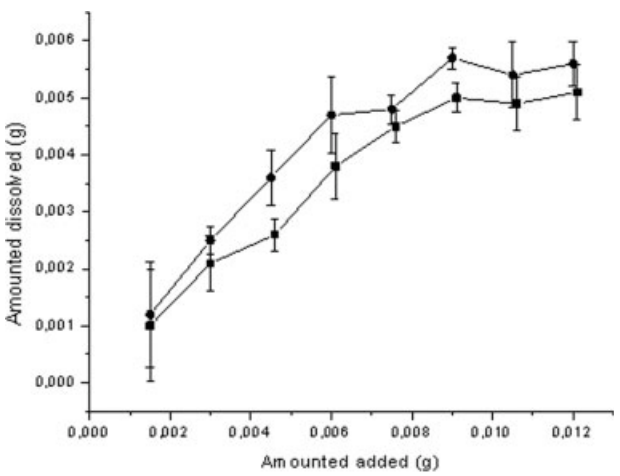

Figure 1 Dissolution profile of silver sulfadiazine in 2% lactic acid (■) and in 1% chitosan dissolved in 2% lactic acid (●). $n = 3$. Error bar: standard deviation.

and brittle, whereas those from lactic acid were soft and could be stretched. This way, lactic acid would be more appropriate to produce films that will be used as wound dressings.

The dissolution profile of silver sulfadiazine in both media was very similar, whereas the quantity of dissolved drug was directly proportional to the amount added. However, adding further quantities of the drug above 0.0090 g did not increase the amount dissolved, indicating that the solubility limit had been reached. At this point, the solution became saturated with the drug. Interpolation of the plateau of the graph to the vertical axis yielded the solubility of the silver sulfadiazine in 2% lactic acid (50 mg/L), and in 1% chitosan (57 mg/L).

Therefore, chitosan did not significantly affect ($P < 0.05$) the solubility of silver sulfadiazine. Indeed, the silver sulfadiazine was probably dissociated into silver as the cation and sulfadiazine as the anion.[20] Moreover, sulfadiazine does not react with chitosan,[16] which would explain why its solubility in the chitosan solution is similar to that in lactic acid solution.

SWELLING STUDIES

Swelling of chitosan films in PBS were investigated at pH 7.4 and the influence of each plasticizer and its concentration were evaluated. In our experiments, we did not neutralize the chitosan films with alkalis because the drug is soluble in basic medium. Actually, we obtained chitosan lactate which is water soluble. Therefore, all chitosan films were previously crosslinked with formaldehyde to prevent its dissolution.

Figure 2 shows the percentage of swelling of chitosan films as a function of time. Chitosan films swelled considerably in PBS, imbibing up to 100% of their own weight. All the profiles obtained show that the percentage of swelling is at maximum at 0.5 h and then very slowly decreases steadily with time after this.

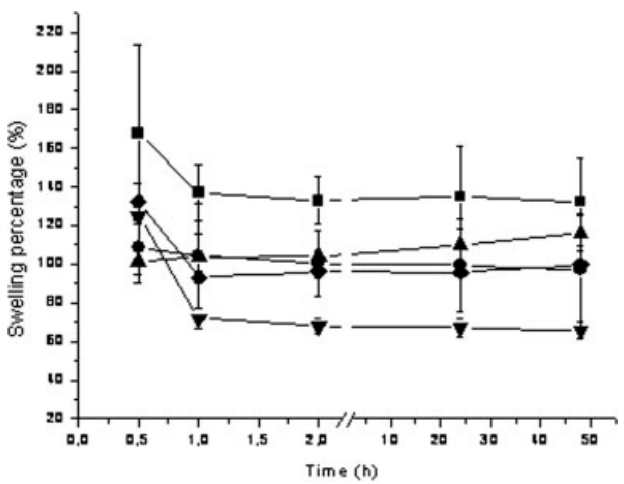

Figure 2 Swelling percentage of chitosan films without plasticizer (●), plasticized with 20% of glycerol (▲), 40% of glycerol (▼), 20% of sorbitol (◆), and 40% of sorbitol (■). $n = 3$. Error bar: standard deviation.

The percentage of swelling of chitosan films after immersion in PBS ranged form 132% at 30 min to 100% at 48 h. According to analysis of variance, there were no significant differences ($P > 0.05$) between the swelling percentages of films plasticized with glycerol or sorbitol (regardless of the amount added) and those without plasticizers.

Films plasticized with 20% of glycerol swelled 101% at 0.5 h and slightly increased this percentage during the experiment, reaching 116% at 48 h. However, the percentage of weight gained decreased severely after 1 h of experiment with films plasticized with 40% of glycerol. Films plasticized with 40% of glycerol swelled 125% at 0.5 h and decreased to 72% at 1 h. Similar results were obtained by Brown et al.,[21] who concluded that the steady fall in the swelling of their chitosan/glycerol films was due to the glycerol linkage. Comparable conclusions were reached by Lopez and Bodmeier[22] and Okor.[23] On the other hand, increasing the sorbitol concentration caused a noticeable effect ($P < 0.05$) on the film swelling. As can be seen from Figure 2, comparing the swelling percentage of films plasticized with 20% or 40% showed that the later swelled more than the former and that this difference was statistically significant ($P < 0.05$). Films plasticized with 20% of sorbitol increased 101% its own weight at 0.5 h and reached 97% at 48 h. Films plasticized with 40% of sorbitol, in comparison, had a relatively higher value of 168% at 0.5 h and 132% at 48 h.

AMOUNT OF WATER ADSORBED

The percentage of water adsorbed of various films is shown in Figure 3. All films increased its weight after 24 h in a high RH environment (75%). Chitosan is a hydrophilic polysaccharide, and thus chitosan film would be expected to progressively hydrate and swell when exposed to a high RH environment. Water molecules adsorbed in the film would be expected to behave as a plasticizer along with the plasticizer molecules. During hydration of the film, newly added water molecules may have developed water–polymer hydrogen bonds, reducing the interchain interactions and enhancing the chain mobility. According to Figure 3, the presence of plasticizer did not affect the water adsorbed of films without additives, regardless of its concentration ($P > 0.05$). On the other hand, films crosslinked with formaldehyde adsorbed less water than films without additives ($P < 0.01$). Lòpez and Bodmeier[22] suggested that the extent of swelling was smaller in crosslinked films due to the reduction in water adsorbed induced by such agents. This was probably due to a decrease in chitosan chain mobility.

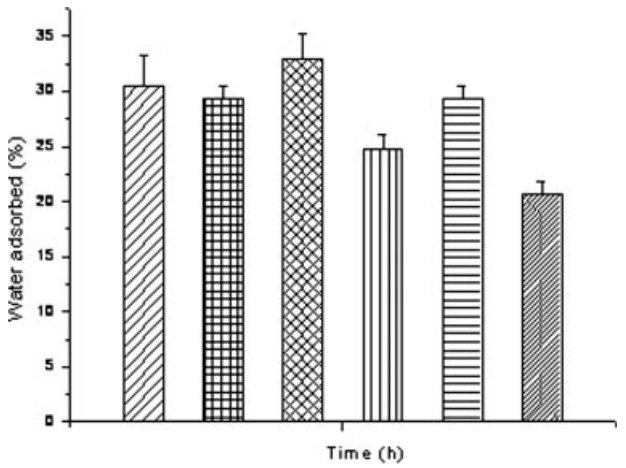

Figure 3 Percentage of water adsorbed: chistosan without additives; chitosan plasticized with 20% of glycerol; chitosan plasticized with 40% of glycerol; chitosan plasticized with 20% of sorbitol; chitosan plasticized with 40% of sorbitol; chitosan crosslinked with formaldehyde. $n = 3$. Error bar: standard deviation.

MECHANICAL PROPERTIES

According to Luterman,[24] some of the properties required for a wound dressing are: flexibility to permit conformation to irregular wound surfaces, as well as elasticity to linear and sheer stresses.

The selection of a plasticizer for biopolymer films is of importance since it strongly affects its physicochemical properties.

Table I shows the results obtained from the mechanical property studies (the film thickness was varied from 250 to 300 μm). The tensile strength values showed that all additives (plasticizers, drug, and crosslinking) added to the chitosan solution decreased the resistance of the films, except for sorbitol at 20%.

Formaldehyde decreased the tensile strength of the chitosan films by more than 60%, compared to those without additives. In addition, it decreased the percentage elongation of the films. Lòpez and Bodmeier[22] have reported that the tensile strength and percentage elongation of films decreased with the addition of crosslinking agents due to the reduction in water uptake induced by such agents. This finding can be confirmed by the water adsorbed results, whereas films crosslinked with formaldehyde adsorbed less amount of water than films without additives and plasticized. It is known that water has a significant plasticizer effect. Thus, these findings can explain the fact that these films were weaker and less elastic than those without additives.

Our results showed that plasticizers (glycerol and sorbitol) decreased the tensile strength of the films when compared to those without additives. Films plasticized with glycerol became more flexible and elastic. Evidences from other experiments suggest that the addition of plasticizer leads to a decrease in intermolecular forces along the polymer chains, which produces the observed improvement in flexibility. Polar groups (—OH) along the plasticizer chains are believed to develop polymer–plasticizer hydrogen bonds, replacing the polymer–polymer interactions in the biopolymer films.[25]

On the other hand, films plasticized with 20% (w/w) of sorbitol showed similar tensile strength values to those without additives, but 40% (w/w) of sorbitol was capable of decreasing the tensile strength by half. As can be seen from Table I, increasing the total plasticizer content resulted in a considerable decrease in the tensile strength and an increase in the percentage elongation of the films. This effect was more pronounced with the films plasticized with sorbitol, where an increase from 20% to 40% of this plasticizer resulted in a decrease in tensile strength of 55% and increase in percentage elongation of 193%. These findings are in accordance with the results of swelling percentage showed in Figure 1, whereas increasing the sorbitol content on the chitosan films increased the swelling percentage. Arvanitoyannis et al.[26] studied the influence of glycerol and sorbitol on the mechanical properties of chitosan/gelatin films. They concluded that both plasticizers decreased the tensile strength and increased the percentage elongation due to an enhancement in the water uptake by the films, leading to higher chitosan chain mobility.

TABLE I
Mechanical Properties of Chitosan Films[a]

Composition	Tensile strength (gf)	Elongation (mm)	Percentage elongation (%)
Films without additives	3189 (33.5)	65 (15.2)	144 (15.2)
0.6% (w/w) of Silver sulfadiazine	1859 (23.9)	74 (18.6)	164 (18.6)
Formaldehyde	1152 (6.2)	38 (18.1)	84 (18.1)
20% (w/w) of glycerol	1548 (9.2)	89 (12.4)	197 (12.4)
40% (w/w) of glycerol	461 (24.4)	109 (12.4)	242 (12.4)
20% (w/w) of sorbitol	3444 (16.2)	28 (8.4)	61 (8.4)
40% (w/w) of sorbitol	1552 (35.1)	75 (28.3)	180 (28.3)

[a] Variation coefficient (%) is given in parentheses; $n = 5$.

On the other hand, an increase in the glycerol content decreased the tensile strength of the films by only 30% and increased the percentage elongation by 23%. Although the increase in the glycerol content had less influence than sorbitol, the former was able to produce the most elastic films, permitting 242% deformation.

According to both elongation and percentage elongation results showed in Table I, films plasticized with glycerol showed better flexibility than those plasticized with sorbitol, regardless of the amount added. According to Sothornvit and Krochta,[27] the mechanical properties of plasticized films are influenced mainly by the number of functional hydroxyl groups and the molecular weight of the plasticizer. Glycerol and sorbitol have similar structures, however, glycerol is a smaller molecule with a molecular weight of just 92 in comparison with that of sorbitol (182).[28] Therefore, sorbitol has more hydroxyl groups than glycerol and hence could be expected to enhance the mechanical properties of the films to a higher extent than glycerol. However, at an equal percentage concentration, the total number of smaller glycerol molecules in the film-forming solution is greater than that of the higher molecular weight sorbitol molecules, and therefore glycerol has more functional hydroxyl groups than sorbitol, which should promote more pronounced plasticizer–polymer interactions.

In addition, Yang and Paulson[25] suggest that the effectiveness of glycerol is most likely due to its small size, which allows it to be more readily inserted between the polymer chains, and consequently exert more influence on the film's mechanical properties than larger molecules.

In the case of films plasticized with sorbitol, there was a significant difference in the mechanical properties between the two cases (20% w/w and 40% w/w). As can be seen from Table I, 20% of sorbitol showed the lowest percentage elongation: 61%. However, increasing the amount added to 40% enhanced the percentage elongation to 179%, lower only than those plasticized with glycerol.

Gaudin et al.[29] studied the effect of sorbitol content on the mechanical properties of starch films. According to the results obtained from the maximum stress and yield at break curves, sorbitol content below 27% produced rigid and brittle films and sorbitol did not have the classical effect of a plasticizer. On the other hand, films with sorbitol content above 27% showed mechanical properties similar to those observed with currently used plasticized materials, exhibiting high flexibility. The best results were those containing around 39% of this plasticizer.

The addition of silver sulfadiazine to chitosan solution produced films with lower tensile strength than those without additives. On the other hand, the percentage elongation was very similar. This effect on tensile strength was probably due to the fact that the drug molecules are inserted among the chitosan chains, leading to an increase in the distance between them. In addition, the large silver sulfadiazine molecules probably prevent perfect tight chains.

IN VITRO DRUG RELEASE STUDIES

The percentage of sulfadiazine released from the films crosslinked with formaldehyde was plotted against time. Figures 4–6 show the effect of the plasticizer, film thickness and loading concentration of the drug on the amount released.

Figure 4 shows the release profile of sulfadiazine in different films containing glycerol or sorbitol, as well as without plasticizer.

The use of glycerol led to a significant increase ($P < 0.001$) in the amount of sulfadiazine released. This effect can be attributed to two main factors. Firstly, glycerol is extremely miscible with water (which was the predominant component in the chitosan solutions). In addition, this plasticizer has low molecular weight, which allows it to fit easily among the chitosan chains. In this way, the hydroxyl groups of glycerol develop polymer–plasticizer interactions that can enhance the flexibility and mobility of the chitosan chains, leading to an increase in the amount of drug released.[23]

Secondly, the high affinity of the glycerol for water allows leakage of a proportion of this plasticizer to the medium (PBS). The mechanism probably involves the opening of channels in the films which facilitate the solvent uptake, leading to an enhancement in the swel-

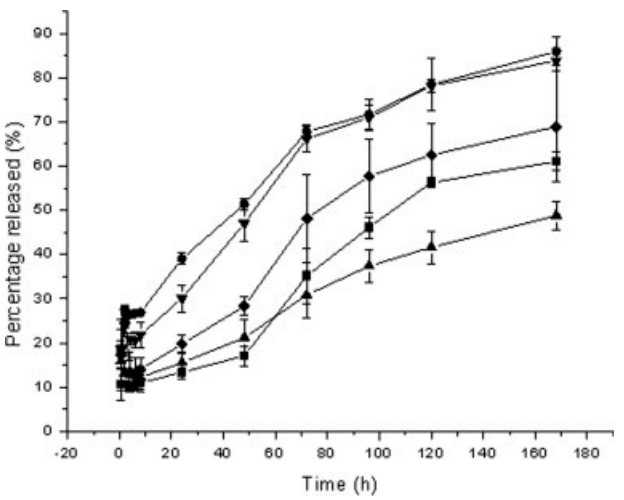

Figure 4 Role of plasticizers on the sulfadiazine release. Film plasticized with 20% (w/w) of glycerol (●), 40% (w/w) of glycerol (▼), 20% (w/w) of sorbitol (▲), 40% (w/w) of sorbitol (◆), and without plasticizer (■) (silver sulfadiazine concentration in the films was 0.6% based on the polymer weight). $n = 3$. Error bar: standard deviation.

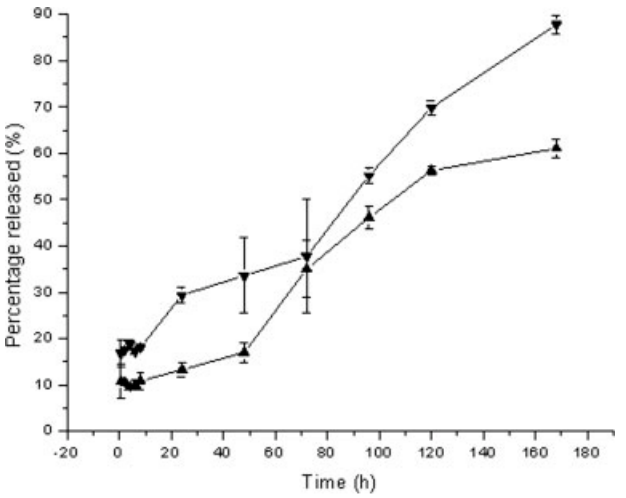

Figure 5 Release of sulfadiazine from membranes of different thickness. 230 μm (▲) and 115 μm (▼) (silver sulfadiazine concentration in the films was 0.6% based on the polymer weight). Error bar: standard deviation.

ling properties of the chitosan matrix.[22,29] This hypothesis can be strengthened by the swelling studies discussed previously. Chitosan films plasticized with glycerol showed an increase in the weight gain at 30 min and showed a marked decrease in the weight gain after 1 h of experiment. This was probably due to the passage of a considerable amount of glycerol from the film to the PBS solution. In addition, the initial glycerol leakage was probably the main cause of the high amount of sulfadiazine released from the films plasticized with glycerol in the beginning of experiment. After 2 h, the films plasticized with 20% (w/w) of glycerol released almost twice more than those plasticized with 20% (w/w) of sorbitol, maintaining this difference until 48 h of experiment. Films plasticized with 20% (w/w) of glycerol released 86% of the drug in total, against 69% in those plasticized with 20% (w/w) of sorbitol.

Statistically, there was no difference in the drug release from films plasticized with sorbitol and from those without plasticizer. As may be seen from the percentage elongation data given in Table I, the addition of 20% (w/w) of sorbitol decreased the film elasticity in 57%. In fact, a reduction in the elasticity leads to lower mobility of the polymer chains. Possibly as a result of this, sorbitol did not increase the drug release significantly.

On the other hand, the analysis of variance reveals a significant difference ($P < 0.01$) between the amount of drug released from films plasticized with 20% (w/w) of sorbitol and from those plasticized with 40%. The increase in the amount of sorbitol resulted in an increase in the quantity released. This finding is similar to that obtained in the mechanical property studies where an increase in the amount of sorbitol enhanced the elasticity of the films, as a possible result of higher chain mobility.

Figure 4 also shows the influence of the amount of glycerol on the release of sulfadiazine. The increase in the glycerol content from 20% to 40% did not result in a significant ($P > 0.05$) increase in drug release.

The effect of glycerol on the release has been attributed to the facilitation of the solvent's access to the $-NH_3^+$ groups of the chitosan chains. Probably, 20% of glycerol is sufficient to reach the majority of these groups, which would suggest that this concentration is sufficient to produce the plasticizing effect.

The influence of the film thickness on the drug release is shown in Figure 5. Statistically, there is a significant difference ($P < 0.001$) between the release rate in films of 230 μm and 115 μm (without plasticizer).

According to Ritger and Peppas,[30] for a constant drug diffusion coefficient D, with one-dimensional diffusion in the x direction and the solute concentration C, Fick's second law, along with the appropriate initial and boundary conditions, may be expressed as

$$\frac{\partial C}{\partial t} = D\frac{\partial^2 C}{\partial x^2} \tag{1}$$

The solution to Fick's law in the form of a trigonometric series is given by

$$\frac{M_t}{M_\infty} = 1 - \sum_{n=0}^{\infty} \frac{8}{(2n+1)^2 x^2} \exp\left(\frac{-D(2n+1)^2 x^2 t}{l^2}\right) \tag{2}$$

where M_t is defined as the mass of drug released at time t, and M_∞ is the mass of drug released as time approaches infinity.

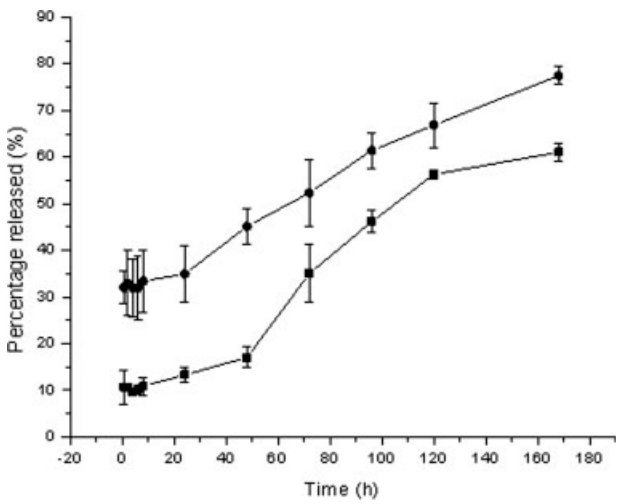

Figure 6 influence of the drug concentration on its release. Film loaded with 0.6% (■) and 1% (●) of silver sulfadiazine. Error bar: standard deviation.

In order to interpret short-time behavior, defined as the first 60% of the total released drug, Fick's law can be approximated by

$$\frac{M_t}{M_\infty} = \frac{4[Dt]^{1/2}}{xl^2} \quad (3)$$

Thus, considering D and x as constants in the system, the mass of drug released is inversely proportional to the film thickness. Therefore, doubling the film thickness resulted in a 30% decrease in the amount of drug released. This is probably due to the increase in the distance in which the drug has to diffuse through the polymeric matrix. In addition, a marked increase in the lag time on the thicker films was observed. This occurred as a result of the longer time that these films took to swell.

In fact, the swelling process occurs through the polymer–solvent contact, followed by water entrance and, consequently, an increase in the hydrostatic pressure within the polymeric matrix followed by the expansion of the polymeric network. However, this process takes a certain time to occur. Therefore, higher thickness films tend to swell slowly because of the higher polymer mass content.

Figure 6 shows the influence of the drug concentration on the release rate. There was a significant increase ($P < 0.001$) in the drug release using films loaded with 1% of silver sulfadiazine as compared to those loaded with 0.6% of the drug. This is probably due to the total amount of silver sulfadiazine above the solubility limit dispersed into the polymeric matrix. According to the results obtained from the solubility study of silver sulfadiazine in chitosan solution, the solubility of this drug is around 60 mg/L, which means 0.6% of silver sulfadiazine in relation to the total polymer mass. Thus, it was assumed that at this concentration, the total amount of drug was completely dissolved in the chitosan matrix. On the other hand, films loaded with 1% of silver sulfadiazine presented an excess amount of drug dispersed in the chitosan matrix, since the solubility limit was exceeded.

As a result, the ''nondissolved'' fraction that is dispersed into the chitosan matrix acts as a reservoir, maintaining the Cs value (concentration of saturated solution) always higher than C (concentration existing in the solution). Consequently, the $Cs - C$ value was higher in the films loaded with 1% than those loaded with 0.6% which led to an increase in the release rate.[31]

The plasticized chitosan films were efficient in controlling the drug release. All the films studied were found to release amounts of drug above the silver sulfadiazine Minimal Inhibitory Concentration (MIC) of 18 mg/L, determined by Modak and Fox[32] against *Pseudomonas aeruginosa* strains.

CONCLUSION

In conclusion, it was satisfactorily demonstrated that chitosan did not affect the solubility of silver sulfadiazine in 2% lactic acid. Furthermore, the additives had useful effects on the mechanical properties of the films. The crosslinking agent decreased both the tensile strength and percentage elongation, but these parameters were mainly affected by the type and concentration of plasticizers. The mechanical properties of films plasticized with sorbitol showed a strong relationship to its concentration in the chitosan solution. Films plasticized with glycerol showed the best mechanical property results. The type of plasticizer and the drug concentration influenced the drug release. Amounts of silver sulfadiazine that were added above its solubility coefficient in chitosan solution increased its release rate. In addition, the drug release was significantly influenced by the film thickness, whereas a decrease in the thickness enhanced the sulfadiazine release.

Therefore, the effective control of drug release and good mechanical properties make these films a promising resource for further development as a wound dressing, especially for severe burn patients.

References

1. Dodane, V.; Vilivalam, V. D. Pharm Sci & Technol Today 1998, 1, 246.
2. Majeti, N. V.; Kumar R. React Funct Polym 2000, 46, 1.
3. Chandra, R.; Rustgi, R. Prog Polym Sci 1998, 23, 1273.
4. Felt, O.; Buri, P.; Gurny, R. Drug Dev Ind Pharm 1998, 24, 979.
5. Ueno, H.; Yamada, H.; Tanaka, I.; Kaba, N.; Matsuura, M.; Okumura, M.; Kadosawa, T.; Fujinaga, T. Biomater 1999, 20, 1407.
6. Loke, W.; Lau, S.; Yong, L.; Khor, E.; Sum, C. K. J Biomed Master Res (App Biomater) 2000, 53, 8.
7. Ward, C. G. Am Coll Surg 1998, 186, 123.
8. Tunkel, A. R. In Principles and Practice of Infectious Diseases, 5th ed.; Mandell, G. L.; Bennett, J. E.; Dolin, E., Eds. Churchill Livingstone: Philadelphia, PA, 2000, p 428.
9. Howard, P. A.; Cancio, L. C.; McManus, A. T.; Goodwin, C. W.; Kim, S. H.; Pruit, B.A. Curr Surg 2000, 56, 397.
10. Monafo, W. W.; West, M. A. Drugs 1990, 40, 364.
11. Kim, H.; Choi, E.; Oh, J.; Lee, H.; Park, S.; Cho, C. Biomater 2000, 21, 131.
12. Balasubramani, M.; Kumar, T. R.; Babu, M. Burns 2001, 27, 534.
13. Cho, Y.; Cho, Y.; Chung, S.; Yoo, G.; Ko, S. Biomater 1999, 20, 2139.
14. Bégin, A.; Calsteren, M. V. Int J Biol Macromol 1999, 26, 63.
15. Sudarshan, N. R.; Hoover, D. G.; Knorr, D. Food Biotechnol 1992, 6, 257.
16. Mi, F. L.; Wu, Y. B.; Shyu, S. S.; Chao, A. C.; Lai, J. Y.; Su, C. C. J Membr Sci 2003, 212, 237.
17. Pillai, O.; Panchagnula, R. Curr Opin Chem Biol 5, 447–451.
18. Kas, H. S. 1997. J Microencapsulation 2001, 14, 689.
19. Higushi, T.; Connors, K. In Advances in analytical chemistry and instrumentation; Wiley: New York, 1965, p 117–212.
20. Tsipouras, N.; Rix, C. J.; Brady, P. H. Clin Chem 1995, 41, 87.

21. Brown, C. D.; Kreilgaard, L.; Nakakura, M.; Lelham, N. C.; Pettit, D. K.; Gombotz, W. R.; Hoffman, A. S. J Controlled Release 2001, 72, 35.
22. Lopez, C. R.; Bodmeier, R. J Controlled Release 1997, 44, 215–225.
23. Okor, R. S. Int J Pharm 1982, 11, 1.
24. Luterman, A. J Am Coll Surg 2000, 190, 104.
25. Yang, L.; Paulson, A. T. Food Res Int 2000, 33, 563.
26. Arvaniitoyannis, I. S.; Nakayama, A.; Aiba, S. Carbohydr Polym 1998, 37, 371.
27. Sothornvit, R.; Krochta, J. M. J Food Eng 2001, 50, 149.
28. Raymond, C. R.; Paul, J. S.; Paul, J. W. Handbook of Pharmaceutical Excipients; Washington, DC: American Pharmaceutical Association, 2001.
29. Gaudin, S.; Lourdin, D.; Botlan, D.; Ilari, J. L.; Colonna, P. J Cereal Sci 1999, 29, 273.
30. Ritger, P. L.; Peppas, N. A. J Controlled Release 1987, 5, 23.
31. Parrot, E. L. Pharmaceutical Technology: Fundamental Pharmaceutics; Minneapolis: Burgess Publishing Company, 1970; p 158–169.
32. Modak, S.; Fox, C. J Trauma 1985, 25, 27.