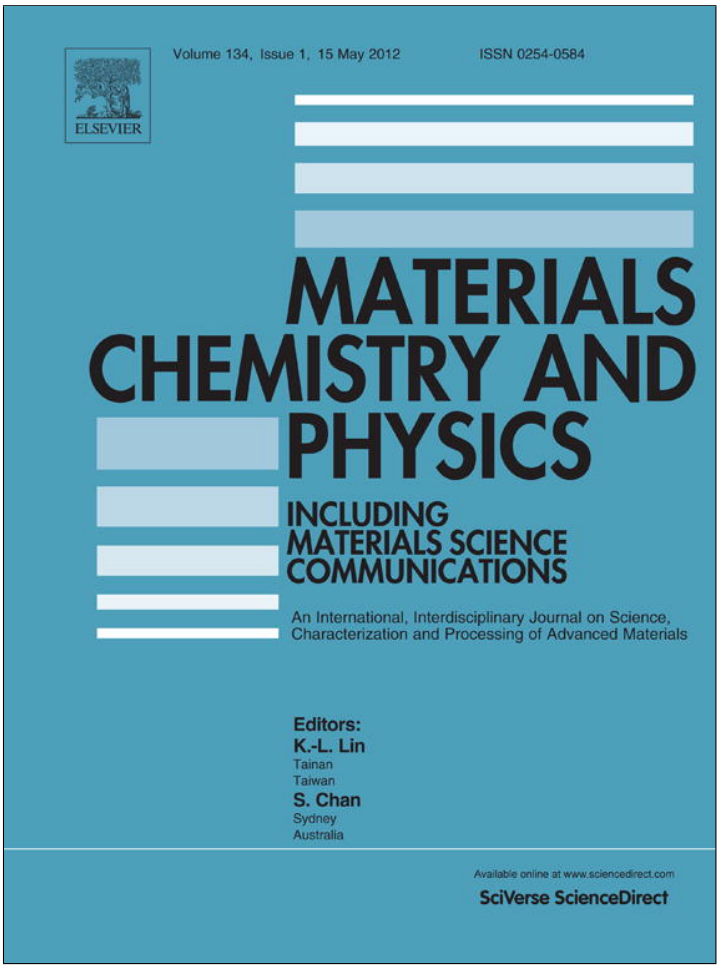

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Preparation and characterization of chitosan/genipin/poly(N-vinyl-2-pyrrolidone) films for controlled release drugs

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ABSTRACT

The study of the physicochemical and functional properties of chitosan films cross-linked with genipin and poly(N-vinyl-2-pyrrolidone) (PVP) was performed in this work. Cross-linked films were prepared by casting method from acetic acid solutions. The structure and physical properties of the films were analyzed by infrared spectroscopy (FT-IR), nuclear magnetic resonance spectroscopy (^{13}C NMR), differential scanning calorimetry (DSC) and mechanical testings. Propranolol hydrochloride was used like a model drug to determine the behavior of drug release from films. The drug release capacity was measured and compared with the degree of cross-linking, mechanical properties and swelling index.

There was an appropriate balance of hydrophilicity, mechanical properties and diffusion by the incorporation of PVP into the networks cross-linked with genipin. The combination of both cross-linkers allows obtaining a soft and tough material potentially applicable as a controlled release. This research represents the first report where both cross-linkers, chemical and ionic agents, are used for obtaining films.

These studies suggest that the chitosan films prepared here are promising drug delivery systems for buccal application, with thermal stability and acceptable mechanical properties. Buccal films may be preferred in terms of flexibility and comfort.

1. Introduction

Natural polymers represent a promising alternative as biodegradable and renewable materials. Chitosan has recently attracted much attention from researchers worldwide [1–5]. It is soluble in aqueous acidic media because of amino groups; its viscous solutions can be used to produce gels in various forms, e.g. beads, membranes, coatings and fibers [6–9].

Chitosan has been used in a broad range of applications in the fields of food, agriculture, water and waste treatment, cosmetics, material science, biotechnology, drugs and biopharmaceuticals, and more recently in gene therapy [10,11]. In addition, it is one of the materials mostly studied for drug delivery. It has been used for its good biocompatibility, film-forming availability, biodegradability to harmless products, non-toxicity, physiological inertness and hydrophilicity [12–14]. The biodegradability [15] and bioadhesivity [16] of chitosan are useful properties in formulations of oral drug delivery devices. The positive charge of chitosan generated under physiological conditions was found to be responsible for its enhanced bioadhesivity and site-specific applications in controlled delivery systems [17–20].

However, it is necessary to obtain cross-linked chitosan to improve the mechanical properties and get a material with potential applications in controlled release of drugs. Biodegradable polymers such as chitosan need to be cross-linked in order to modulate their general properties and last long enough to be used in drug delivery [21]. Modification of chitosan through its blending and cross-linking with other polymers is convenient and effective for improving its physical properties in practical applications. Various reagents, including glutaraldehyde and epoxy compounds, have been used as cross-linkers for chitosan [22–26]. The most common synthetic chemicals used as cross-linking reagents are glutaraldehyde, formaldehyde and dialdehyde starch [27–31]. However, most of these cross-linking agents are chemically synthesized and subject to the problems caused by physiological toxicity. Other cross-linkers such as genipin [9,32,33], glyoxal [34–36], and polymers such as poly(N-vinyl-2-pyrrolidone) (PVP) [35] have been used for their possible applications in the biomedical field.

The use of genipin as a cross-linking agent gave rise to improvements in the mechanical properties and stability of films in water [9,37]. Genipin [38,39] is a particularly non-toxic cross-linker and an effective naturally occurring cross-linking agent which can react spontaneously with amino acids or proteins to form dark blue pigments [40–42].

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Usually, covalent cross-linking exhibits good mechanical properties, but covalently cross-linked hydrogels would not necessarily be the best choice. Ionically cross-linked chitosan hydrogels offer more possibilities as drug delivery systems compared to covalently cross-linked hydrogels.

Blend films of chitosan and other polymers which improve its mechanical properties have been shown to be suitable for medical purposes. Chitosan and poly(ethylene oxide) (PEO) blends have been reported to be used in the preparation of membranes for haemodialysis [43] and semi-IPNs for pH-sensitive drug delivery [44]. In some cases, chitosan/PEO blend films were unstable since they partly dissolved in distilled water after having been immersed for some time. Blend films of PVP and chitosan may be used as a biomaterial for biomedical applications [45]. PVP is an ionic cross-linker which forms hydrogen bonds between amino and hydroxyl groups. In addition, it has been demonstrated that the addition of PVP was beneficial for the thermal stability of chitosan; however, it proved to have lessened strength performance [46]. Therefore, these blend films are limited in use and application. Much research has then been conducted to overcome such undesirable properties by the cross-linking method [47]. In order to obtain cross-linked chitosan hydrogels, some instances of applied genipin and PVP have been reported [48].Generally, in medical and pharmaceutical applications, chitosan is used as a component in hydrogels. The study of hydrogels composed of chitosan alone has proved to be somewhat limited by their poor tensile strength, poor elasticity due to their intrinsic chain rigidity, and lack of an efficient drug delivery control. Yet, cross-linked chitosan films could be applied in the biomedical area, for example as buccal drug delivery. Buccal films may be preferred in terms of flexibility and comfort; they can also circumvent the relatively short residence time of oral gels on the mucosa, since gels are easily washed away and removed by saliva. Moreover, the films are able to protect the wound surface, reducing pain and helping to treat oral diseases more effectively [49].

However, chitosan film has not yet been largely investigated as buccal drug delivery. Ideally, buccal films should be flexible, elastic, soft but strong enough to withstand breakage caused by stress from mouth activities.

The aims of this study were to synthesize cross-linked chitosan as potential buccal drug delivery agents; they also involved evaluating the effects of physical and chemical cross-linking of chitosan films in the mechanical properties, index and rate of swelling in water, and controlled release of the products obtained. Hence, chemical structure, swelling studies, thermal analysis and mechanical properties of the films yielded were researched.

The highlights in this research relate to the fact that it represents the first report where both cross-linkers, chemical and ionic agents, are used for obtaining films. In this study, the synthesis and properties of cross-linked chitosan films with different amounts of PVP and genipin cross-linker agents were studied. In addition, propranolol hydrochloride was used as the model drug to determine the behavior of drug release from these films.

2. Experimental

2.1. Reagents

The following chemicals were purchased and used: low molecular weight chitosan (Ch) (Aldrich); genipin (Gen) 98% (Wako); propranolol hydrochloride (PH) 99% (Aldrich); amine-acetic acid 98% (Anedra); ninhydrin (Anedra); sodium phosphate dibasic anhydrous (p.a. Cicarelli); sodium phosphate monobasic monohydrate (p.a. Cicarelli); poly vinyl pyrrolidone (PVP) (Todo Droga); sodium hydroxide beads (p.a. Cicarelli); glacial acetic acid analytical grade (Anedra). Solvents: absolute ethyl alcohol (Anedra); acetone (Taurus); milli-Q water.

2.2. Preparation and characterization of cross-linked chitosan films

Cross-linked chitosan films with different ratios of genipin were prepared. The ratios of genipin/chitosan studied were 0.10, 1.00, and 3.25%, w/w, and a constant proportion of PVP/chitosan (18%, w/w) was used.

All chitosan films were prepared by the casting method: around 1 g of chitosan were added to 45 mL of acetic acid (1.5%, v/v) and homogenized for 24 h at room temperature. Afterwards, different volumes of a genipin solution prepared in purified water (0.5%, w/v) were added to the chitosan solutions. After 2 h of stirring at room temperature, the solutions started to turn light blue and became increasingly viscous. They were immediately cast into Petri dishes (9 cm diameter) and dried to constant weight at room temperature. After 1 day, the cross-linked chitosan films became dark blue.

Cross-linked films with genipin and PVP were also prepared according to the above description. After adding the genipin solution, the corresponding mass of PVP (PVP/chitosan 18%, w/w) was added to the corresponding mixture under constant stirring. The films were carefully removed from the Petri dishes and analyzed by IR and ^{13}C NMR spectroscopy. They were also characterized by differential scanning calorimetry, mechanical properties and swelling studies. IR spectra were recorded on a Nicolet 5-SXC spectrometer, and Nuclear Magnetic Resonance Spectra (NMR) were obtained in $CDCl_3$ or D_2O using a Bruker 400 MHz NMR.

Chitosan–propranolol hydrochloride-loaded films were subsequently produced following the previously described steps. The model drug was added to the corresponding mixtures under constant stirring. Finally, the dried films were washed with water and ethanol to remove excess drugs. The films prepared were conditioned during 48 h at 25 °C and 45% relative humidity (RH) before measuring their properties.

2.3. Ninhydrin assay

Ninhydrin-based monitoring systems are among the most widely used for the quantitative determination of the amino acid content of proteins. Ninhydrin reacts with primary amines to form a colored complex known as Ruhemann's purple [50]. This reaction can also be used to measure the amount of free primary amino groups attached to an insoluble support.

The ninhydrin solution in ethanol (0.02 M) was freshly prepared on the day of the assays. For each assay, 1 mL of reagent was added to 0.002 g of the cross-linked chitosan films in a glass tube with 3 mL of water, and the mixtures were heated in boiling water for 10 min to allow the reaction to proceed. The absorbance of each solution was measured in a UV spectrophotometer (Shimadzu AEU-210) at 570 nm. The calibration curve was made using known amounts of glycine. The molar extinction coefficient calculated was 4233.62 M^{-1} cm^{-1}.

2.4. Swelling studies

The water sorption capacity of the cross-linked chitosan films was determined by swelling the films in buffer of pH 7 at room temperature. A known weight (200 mg) of each film was placed in the medium for 7 h, which is the appropriate dosage time. The swollen films were collected at different times, after having been superficially dried with tissue paper and weighed immediately on

Table 1
Quantities used for the film preparation and cross-linking percentages obtained.

Film	Ch (g)	PVP (g)	Genipin 0.5% p/v (mL)	Cross-linking (%)
Ch–PVP 18%	0.8185	0.1405	–	93.17
Ch–Gen 0.10%	1.0034	–	0.2	34.12
Ch–Gen 1.00%	1.0014	–	2.0	58.36
Ch–Gen 3.25%	1.0028	–	6.6	66.47
Ch–Gen 0.10%–PVP 18%	0.8034	0.1465	0.2	44.33
Ch–Gen 1.00%–PVP 18%	0.7969	0.1445	1.6	66.37
Ch–Gen 3.25%–PVP 18%	0.8080	0.1430	5.2	78.95

an analytical balance. The percentage swelling (E_{sw}) of the chitosan cross-linked films in the medium was calculated using Eq. (1):

$$E_{sw} = \frac{W_e}{W_o} \quad (1)$$

where W_e denotes the weight of the cross-linked chitosan films at equilibrium swelling, and W_o is the initial weight of the cross-linked chitosan films. Each swelling experiment was repeated twice, and the average value was taken as the E_{sw} value.

2.5. Thermal analysis

Differential scanning calorimetry or DSC measurements were taken using a Mettler Toledo DSC 823E (Facultad de Ciencias Agropecuarias, UNC) on samples with a mass of about 2 mg, at a heating rate of 10 °C min^{-1}, and at a testing temperature ranging from 40 to 360 °C.

2.6. Mechanical properties

Tensile strength and elongation at break were determined from the stress–strain curves for each sample (16 × 67 mm) according to ASTM D882-02. An Instron Universal Testing Machine (model 3342, Norwood, MA, USA) equipped with a 500 N capacity cell was used with an initial grip separation of 35 mm and crosshead speed of 0.1 mm s^{-1}. Each mechanical testing was performed in triplicate.

2.7. In vitro release

Drug-loaded films (50 mg, 10%, w/w) with a similar exposed area (1 cm^2) were soaked within a suitable volume (50 mL) of a dissolution medium (phosphate buffer solution, PBS) in glass vessels. These vessels were incubated at 37 °C with magnetic stirring at 100 rpm by 7 h. At appropriate time intervals, 2 mL of the solution were withdrawn from the glass vessels and the amount of propranolol hydrochloride released from the drug-loaded films was evaluated by UV spectrophotometry at 289 nm. An equal volume (2 mL) of the same dissolution medium was added back to keep a constant volume. The medium for the controlled release studies was a typical pH 7 phosphate buffer solution (PBS).

Experimental points were next approximated with Korsmeyer–Peppas and Gallagher–Corrigan models that have the following equations, respectively,

$$f_t = a \times t^n \quad (2)$$

$$f_t = f_{max} \times (1 - e^{-k_1 \times t}) + (f_{max} - f_b) \times (e^{k_2 \times t - k_2 \times t_{max}} / 1 + e^{k_2 \times t - k_2 \times t_{max}}) \quad (3)$$

where f_t is the fraction of drug released in t time; a is a constant incorporating structural and geometric dosage form; n is the release exponent indicating the drug release mechanism; f_{max} is the maximum fraction of drug released during process; f_b is the fraction of drug released during first stage – the burst effect; k_1 is the first order kinetic constant [$h - 1$] (1st stage of release); k_2 is the kinetic constant for second stage of release process – matrix degradation; and t_{max} is the time to maximum drug release rate.

In each case, maximum drug concentration in release medium was achieved within 7 h. Thus the scope of approximation was limited to the values of time in the range of (0–7 h). Correlation coefficient R^2 was chosen to define the approximation accuracy of an individual model. Acceptable correlation was achieved when R^2 values were equal to 0.970 or higher [51].

2.8. Statistical analysis

Data for each test were statistically analyzed. The analysis of variance (ANOVA) was used to evaluate the significance in the difference between means. Turkey test was used for comparing mean values. Differences between means were considered significant when $p \leq 0.05$.

3. Results

3.1. Preparation and characterization of cross-linked chitosan film

Cross-linked films were prepared using chitosan as the base matrix and genipin and PVP as cross-linkers (Fig. 1). The films were prepared using different ratios of genipin/chitosan (Ch–Gen) and PVP (Ch–Gen–PVP). Genipin is known to cross-link only chemically the amino groups of the chitosan chains [41] and PVP is physically entangled in the chitosan network.

Table 1 shows the variation in the degree of cross-linking at different ratios of genipin to chitosan (film Ch–Gen 0.10%, Ch–Gen 1.00%, Ch–Gen 3.25%) and the degree of cross-linking at a constant concentration of PVP (film Ch–Gen 0.10%–PVP, Ch–Gen 1.00%–PVP; Ch–Gen 3.25%–PVP).

Native chitosan (85% degree of deacetylation) was employed as blank and the degree of cross-linking (DC) of samples was calculated following Eq. (4):

$$DC = \frac{[(\text{NHN reactive})_{fresh} - (\text{NHN reactive})_{fixed}]}{(\text{NHN reactive})_{fresh}} \times 100 \quad (4)$$

where "fresh" is the mole fraction of free NH_2 in the non-cross-linked sample and "fixed" is the mole fraction of free NH_2 remaining in cross-linked samples. Three samples of each type of films were evaluated.

It can be observed that DC increases with an increasing concentration of genipin. The same behavior was also described in the literature for isolated soy protein film [37]. The addition of PVP to the chitosan–genipin films again increased the degree of cross-linking, because of the ease of formation of the hydrogen bridge between the two polymers. The cross-linked chitosan–genipin films are colored in different shades of blue, depending on their degree of cross-linking (Fig. 2). This change in color results from the formation of cross-linked chitosan derivatives by the reaction of genipin with primary amines of chitosan. The dark blue colored films are those (Ch–Gen–3.25% and Ch–Gen 3.25%–PVP) whose

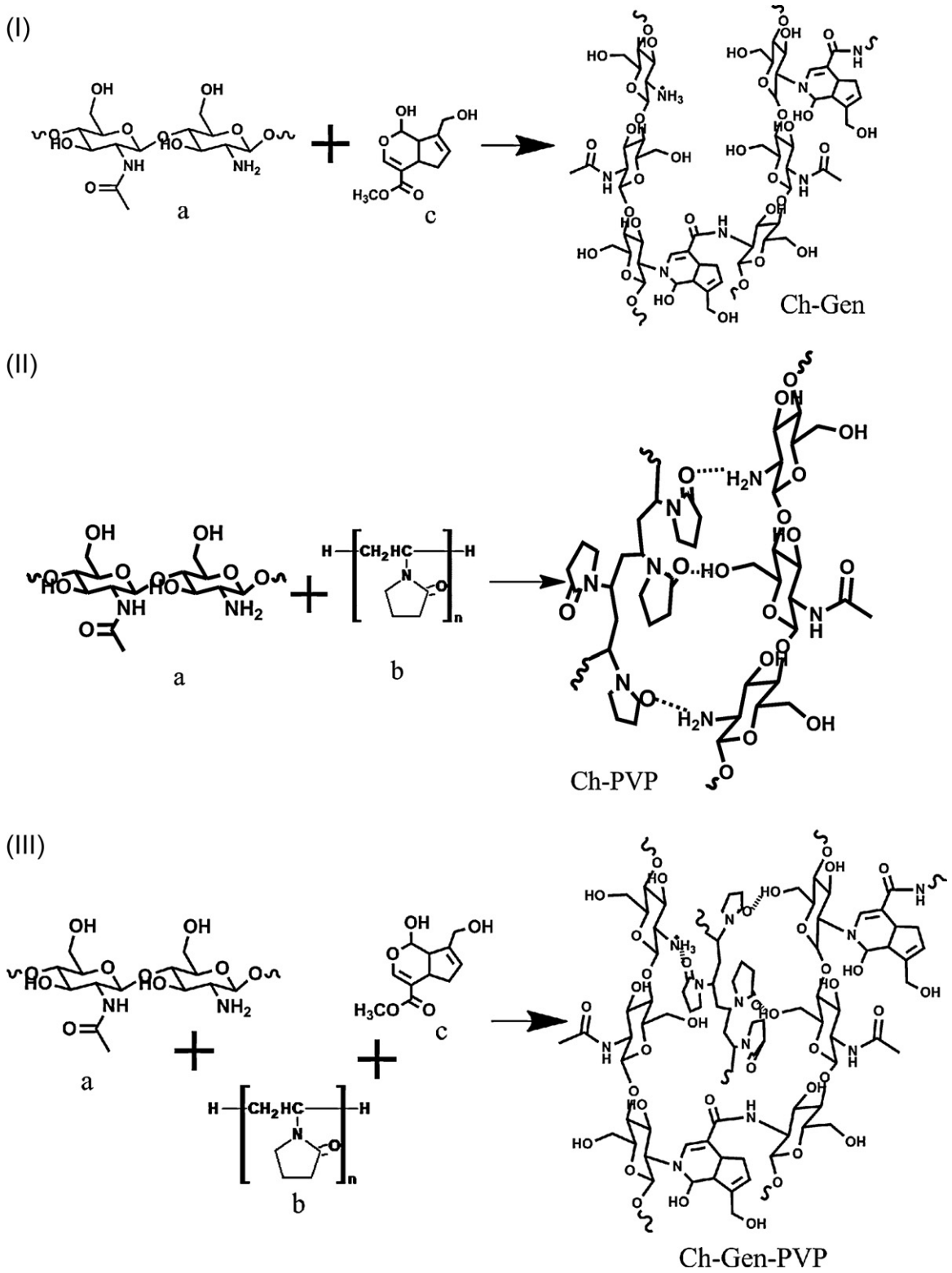

Fig. 1. Reaction scheme between Ch (a) and Gen (c); Ch and PVP (b) and Ch, Gen and PVP.

cross-linking densities were the highest on the basis of ninhidryn assays.

FT-IR spectra of cross-linked films were recorded (Fig. 3A and B).

After the cross-linking reaction, the signal corresponding to the ester group of genipin disappeared, whereas the amide band at 1640 cm^{-1} appeared, thus suggesting that the carboxymethyl group of genipin reacted with the amino groups of chitosan to form a secondary amide. For films prepared in higher concentrations of genipin, adsorption was observed to relatively increase at 1640 cm^{-1} while it decreased at 1567 cm^{-1} [52,8].

Two wide bands can be seen in the IR spectrum in Fig. 3B. At 1653 cm^{-1} the C=O stretching vibration of PVP overlaps with the band corresponding to the C=O vibration of the amide group of the

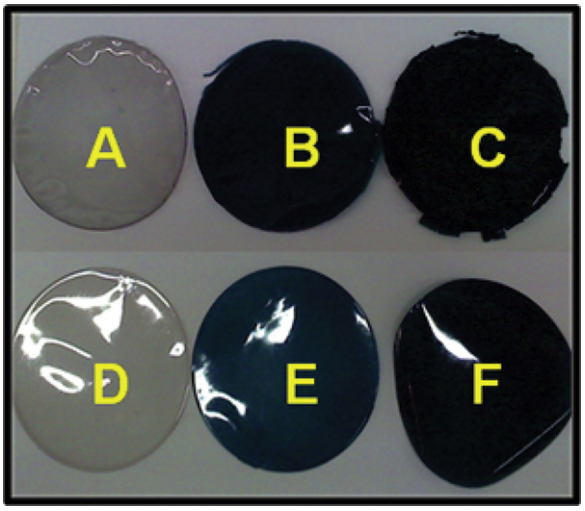

Fig. 2. Photographs of the cross-linked films: Ch–Gen 0.1% (A), Ch–Gen 1% (B), Ch–Gen 3.25% (C) and Ch–Gen 0.1%–PVP (D), Ch–Gen 1%–PVP (E), Ch–Gen 3.25%–PVP (F).

product. At 1559 cm^{-1} there is also an overlapping between the H—N—H vibration of chitosan and the band corresponding to the N—H vibration of the amide group.

Fig. 4 shows the ^{13}C NMR spectrum of Ch–Gen–0.10% cross-linked film swollen in D_2O. ^{13}C NMR signals of original chitosan are reported in the literature [52].

At 23.0 ppm the resonance signal of alkyl group or alicyclic hydrocarbons in the cross-linked chitosan network would be attributed to the Ch–Gen linkage. The resonance signal at 170.5 ppm, ascribed to the ester group of genipin, disappeared in the film spectrum. The resonance which appeared at 181.3 ppm could be attributed to the amide group from the reaction between the amino groups of chitosan and the ester groups of genipin. The upfield chemical shift of chitosan C-1 at 99.3 ppm could result from the ring current of heterocyclic aromatics bound to C-2 [52].

3.2. Swelling studies

Fig. 5(A and B) depicts the degree of film swelling in buffer phosphate pH 7.0.

Maximum swelling was reached with formulations containing a low proportion of genipin. The addition of PVP reduced slightly

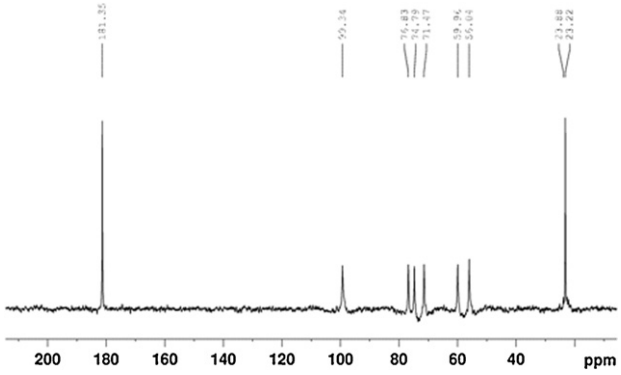

Fig. 4. ^{13}C NMR spectrum of Ch–Gen–0.10% cross-linked film recorded in D_2O.

the extent of film swelling. Such behavior from chitosan/PVP films may be explained by the fact that strong intermolecular interactions between chitosan and PVP molecules result in shorter intermolecular distances forming a more compact network. The effect produced by genipin was clearly seen. These results were consistent with those of the cross-linking degree. Cross-linked networks are more rigid and less expandable. In addition, the swelling rate was faster for Ch–Gen–PVP films than for Ch–Gen films. For instance, Ch–Gen–PVP films achieved maximum/plateau in swelling at approximately 2 min, while chitosan–genipin films did so at about 10 min. PVP is physically entangled in the chitosan network but is more hydrophilic than genipin.

The swelling state of the polymer was reported to be crucial for its bioadhesive behavior [41,37]. Adhesion occurs shortly after the beginning of swelling but will increase with the degree of hydration up to a point where overhydration leads to disentanglement at the polymeric/tissue interface. Genipin–PVP combination could be right for preventing overhydration of the films.

3.3. Thermal analysis

Thermal behavior of the films was analyzed by differential scanning calorimetry (DSC). Chitosan, genipin and PVP possess polar groups, thus water is adsorbed onto the polymers while hydrogen bonds are formed. A weak exothermic peak in DSC at around 80 °C corresponds to the loss of adsorbed water and volatile compounds in the films [53,54]. In addition, DSC shows an exothermic peak at around 160 °C representing the most important loss of absorbed

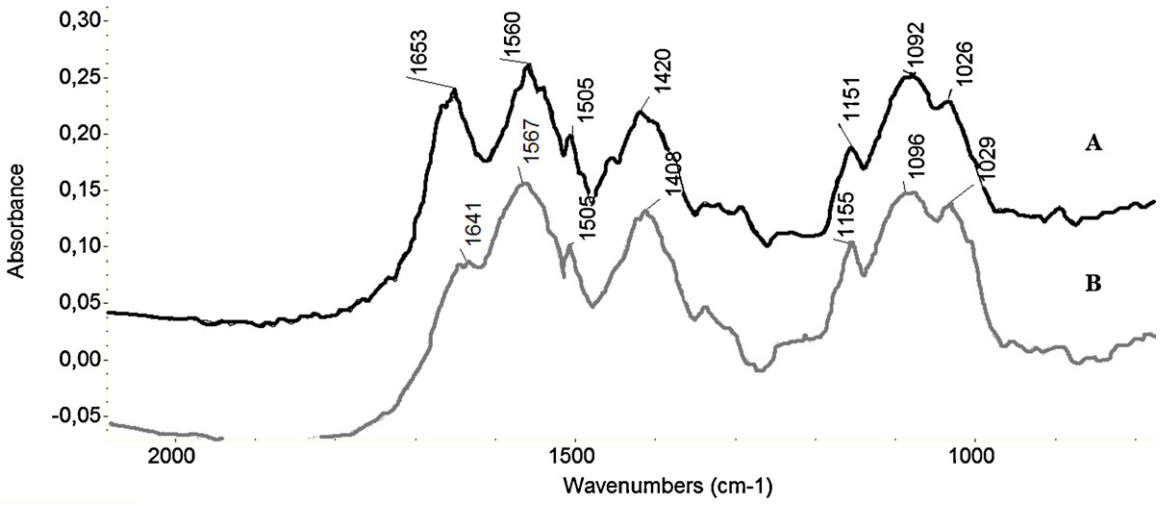

Fig. 3. FT-IR spectra of cross-linked films. Ch–Gen 3.25% (A) and Ch–Gen 3.25%–PVP (B).

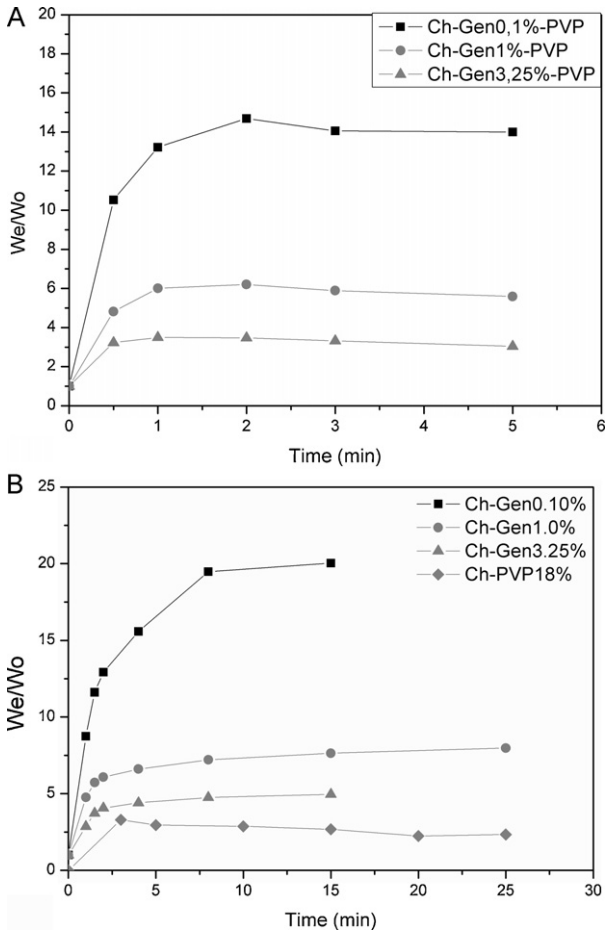

Fig. 5. Swelling studies of cross-linked films in buffer phosphate pH 6.7. Ch–Gen (A) and Ch–Gen–PVP (B).

water temperature of polymeric materials. The onset temperature of films thermo-oxidative degradation is observed at around 300 °C. These peaks have been reported in other studies [55] as degradations, including saccharide rings, dehydratation, depolymerisation and decomposition, and deacetylated and acetylated chitosan units. Fig. 6 compares all decomposition temperatures; it can be seen that the modification of chitosan with genipin and PVP slightly increased the stability of the films relative to pure chitosan (Table 2).

3.4. Mechanical properties

In addition to the important parameter of mucoadhesion strength and residence time of buccal films, the mechanical properties play a crucial role in the physical integrity of the dosage form. Several values can be obtained from a regular stress–strain curve; however, the tensile strength and the elongation at break prove most relevant to the study of buccal films. Desired mechanical properties will vary depending on the formulation goals.

Table 2
Differential scanning calorimetry (DSC) measurements for cross-linked films.

Film	Peak temperature (°C)
Ch	289
Ch–Gen 0.1%	286
Ch–PVP 18%	290
Ch–Gen 0.1%–PVP	295

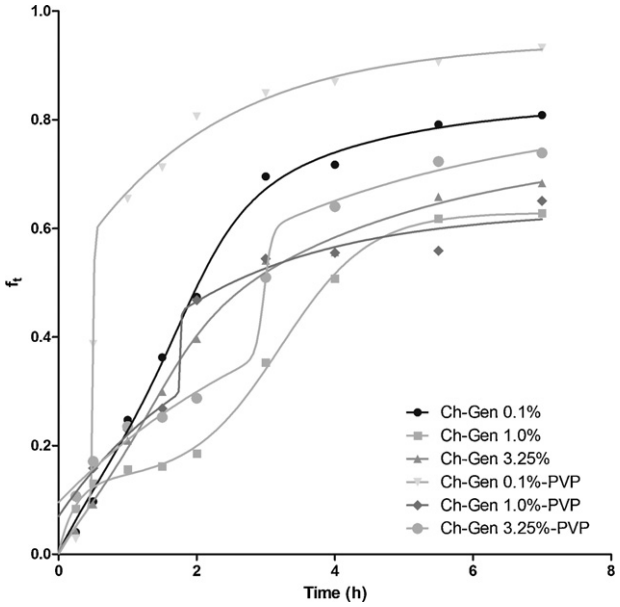

Fig. 6. In vitro release of propranolol hydrochloride from cross-linked films in pH 7.0 buffer phosphate approximated with Gallagher–Corrigan models (–).

A soft and tough polymer is characterized by a moderate tensile strength and elongation at break. Its condition is suggested for a suitable buccal film.

As shown in Table 3, the mechanical performance of films changed with the cross-linking presence. The Ch–PVP 18% film showed same fracture stress and elongation that control film (chitosan without cross-linker). The cross-linked films with small amounts of Gen (0.1% and 1%) were ductile-like and the tensile strength and elongation at break became higher than those of the control film.

Moreover, the addition of PVP to genipin-containing films resulted in a decrease of the elongation at break and an increase of the tensile strength. Analyzing the elongation properties, it was observed that the values of Ch–Gen films with small amounts of genipin are higher than those of the control film. These values increased with increasing genipin content, probably due to the fact that small genipin additions break some intrinsic matrix interactions forming more expansible networks.

When the amount of genipin added in cross-linked films increased to 3.25%, a different trend was observed. The tensile strength and elongation at break of these films drastically decrease. In high cross-linking densities (produced by the large amount of cross-linker) there are restrictions in the movement between the chitosan chains and this becomes in a too brittle and fragile material. Macroscopically, this effect could be seen as the opposite of the plasticizers action.

Table 3
Thickness, elongation at break (%) and tensile strength (MPa) of cross-linked films.

Film	Thickness (μm)	Elongation at break (%)	Tensile strength (MPa)
Ch	64.9	1.54 ± 0.2^{C}	50.2 ± 4.0^{A}
Ch–PVP	67.2	1.76 ± 0.15^{BC}	48.8 ± 2.4^{A}
Ch–Gen 0.10%	54.0	2.76 ± 0.12^{A}	69.4 ± 5.4^{B}
Ch–Gen 0.10%–PVP	60.5	2.46 ± 0.22^{A}	71.8 ± 2.2^{B}
Ch–Gen 1.00%	53.4	2.46 ± 0.32^{A}	79.9 ± 2.9^{BC}
Ch–Gen 1.00%–PVP	58.17	2.28 ± 0.11^{AB}	85.8 ± 3.7^{C}
Ch–Gen 3.25%	50.11	1.81 ± 0.32^{BC}	68.5 ± 6.7^{B}
Ch–Gen 3.25%–PVP	44.87	1.36 ± 0.2^{C}	49.4 ± 4.5^{A}

Any two means in the same column followed by the same letter are not significantly ($P \geq 0.05$) different according to Turkey test.

Table 4
Analysis of Korsmeyer–Peppas and Gallagher–Corrigan models parameters values for films.

Films	Korsmeyer–Peppas model		Gallagher–Corrigan model					
	n	R^2	f_b	k_1	f_{max}	k_2	t_{max}	R^2
Ch–Gen 0.1%	1.15	0.992	0.72	0.44	0.83	2.28	1.82	0.9961
Ch–Gen 1.0%	0.62	0.963	0.16	4.03	0.63	1.55	3.21	0.9984
Ch–Gen 3.25%	0.87	0.997	0.54	0.30	0.76	2.35	1.46	0.9962
Ch–PVP–Gen 0.1%	2.30	0.745	0.71	0.48	0.95	102.9	0.50	0.9969
Ch–PVP–Gen 1.0%	0.53	0.998	0.27	0.45	0.64	135.2	1.76	0.9895
Ch–PVP–Gen 3.25%	0.62	0.992	0.29	0.27	0.83	18.0	2.97	0.9964

In the first moments of the traction test, chains should arrange in order to provide the best possible resistance to tensile stress. If these chains are unable to minimally arrange, the tensile strength will be lower so the material will break.

Probably, this could be the reason why at the time of the tensile test, the sample breaks easily observing low elongation and tensile forces. The same behavior was described in the literature for the elongation at break of soy protein films [37].

It is important to state that, by combining both cross-linkers, it is possible to obtain a soft and tough material.

3.5. In vitro release

The films were loaded with 50 mg of propranolol hydrochloride, a slightly water soluble drug. Fig. 6 shows the release values obtained.

Experimental data were firstly approximated with Korsmeyer–Peppas and Gallagher–Corrigan models (Table 4). Correlation coefficient R^2 was chosen to define the approximation accuracy of an individual model. Acceptable correlation was achieved for all films, with the exception of Ch–Gen 1% and Ch–Gen 0.1%–PVP films. Korsmeyer–Peppas model may be used to describe the Fickian and non-Fickian release behavior of swelling-controlled release systems which swell to a moderate equilibrium degree of swelling and are prepared by incorporation of a drug in a hydrophilic, initially glassy polymer. The diffusional exponent, n, is an important indicator of the different release mechanisms [51,56,57]. If the n value is 0.5, the release mechanism follows Fickian diffusion (indicating diffusion-controlled drug release); $0.5 < n < 1$ (superposition of both phenomena), the value indicates anomalous transport mechanism; $n = 1$ is used for case-II transport (indicating relaxation/erosion-controlled drug release); and $n > 1$ is used for super case-II transport (indicating drug release controlled by polymer relaxation for cross-linked films). As mentioned in Table 4, n values of the films indicate that the drug release of Ch–Gen 0.1% film follows super case II transport and the others follow anomalous transport mechanism.

Ch–Gen 1% and Ch–Gen 0.1%–PVP films fit the Gallagher–Corrigan model [51,58,59]. The kinetic profile of this model is described as initial "burst effect" of a drug non-bound to the drug matrix and following slow release determined by the matrix erosion. One can observe that the model approximates experimental points very well ($R^2 = 0.975$–0.999). The parameters of the first release stage (k_1) are not significantly different from each other. However, f_b values can be found to slightly increase; the second release stage rate constant values (k_2) are highly increased with PVP additions. The time maximum drug release rate (t_{max}) is lowest in most of Ch–PVP films, (Fig. 6). According the above results, the incorporation of PVP affects the process of drug release. In steady state, Ch–Gen–PVP films released higher percentages of drug than Ch–Gen films with equal percentages of genipin. Propranolol hydrochloride was released during 7 h, 80%, 60.5% and 70% of the total for Ch–Gen 0.1%; Ch–Gen 1.0% and Ch–Gen 3.25%, respectively. Addition of 18% PVP increases this release to 87.5%, 67.5 and 75% for Ch–Gen 0.1%–PVP, Ch–Gen 1.0%–PVP and Ch–Gen 3.25%–PVP, respectively.

This behavior could arise from the strong interaction between chitosan and PVP chains by the formation of hydrogen bonds. Moreover, due to the hydrophobicity of propranolol hydrochloride, possible interactions between the drug and the polymeric network are weaker, facilitating their removal from the network. It is clearly observed that in the Ch–Gen 0.1%–PVP film, the drug can easily diffuse inside the film during a particularly short time (approx 3 min).

This film has enough free amino and hydroxyl groups to form a significant amount of hydrogen bond with the solvent and a high degree of swelling. These last factors contribute to eliminating drug from the network.

Ch–Gen 0.1% and Ch–Gen 0.1%–PVP films show burst effect (k_1 value same); by contrast, the k_2 value for the last film is higher than that of Ch–Gen 0.1%. The fastest drug displacement and release for the Ch–Gen 0.1%–PVP film could be attributed to favorable interactions between water and network.

4. Discussion

Fig. 7A exhibits the variation in the swelling with the percentage of genipin of the networks. It is possible to observe that the swelling index is affected by the degree of cross-linking and the incorporation of PVP into the networks. Therefore, the diffusion of water into the polymer network occurs at a lower rate which, in turn, causes an inadequate polymer swelling when density of cross-linking increases. Ch–PVP 18% film was of a rigid nature according to mechanical properties; its swelling index was the lowest [60].

In addition, the percentage of genipin and the incorporation of PVP into the network influence the process of drug release (Fig. 7B).

It is hence interesting to conduct a comparative analysis of release rates and of the swelling index of the different networks. The comparison of the films with 0.1 and 1.0% genipin (Ch–Gen 0.1%, Ch–Gen 1.0% and Ch–Gen 0.1%–PVP, Ch–Gen 1.0%–PVP) shows that the rate of release decreases with the increasing degree of cross-linking. In the Ch–Gen 1.0% film, the diffusion of the drug within the network is hindered and its release is minor.

By increasing the percentage of genipin (3.25%), the degree of cross-linking is higher; Ch–Gen 3.25%–PVP is a particularly rigid network. The swelling index decreases but the rate of propranolol hydrochloride release in the balance increases. The degree of swelling directly relates to the formation of hydrogen bonds between water and amino and hydroxyl groups free of polymeric networks. By increasing the cross-linking, the network has few sites available to interact with water. The genipin is cross-linked covalently to chitosan chains and it can react with itself to form oligomers. These oligomers may then be reacting with chitosan amino groups; the cross-linked network has greater distances between the chains. The diffusion of the drug and its release are favored.

Incorporation of PVP into the chitosan cross-linked films increases the release drug [48]. According to the fitting with the theoretical equations, the incorporation of PVP increases the rate of

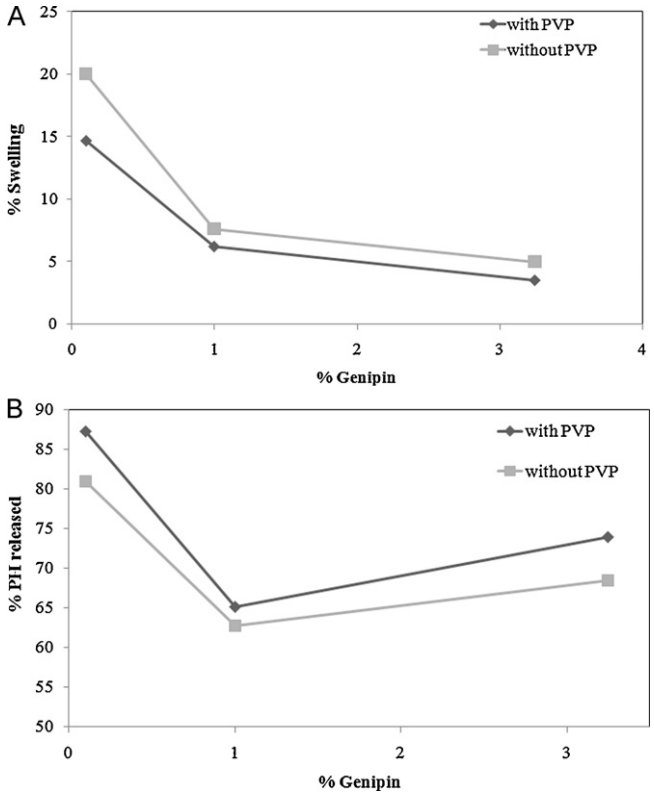

Fig. 7. Analysis of swelling index vs percentage of genipin for the cross-linking films (A). Analysis of drug release with incorporation of PVP into the cross-linked chitosan with genipin (B).

erosion/relaxation process because Ch–Gen–PVP films have a value of k_2 higher than that of Ch–Gen films.

5. Conclusion

In this research the synthesis of cross-linked chitosan films were performed. The material was evaluated as potential buccal drug delivery agent evaluating the effects of physical and chemical cross-linking in mechanical and thermal properties, rate of swelling in water, and controlled release of the products obtained.

According to ours results, the best properties were obtained for the Ch–Gen 0.10% and Ch–Gen 0.10%–PVP films because the drug release was 80% within a period of 7 h, which is an optimal dosing period. In addition, the thermal and mechanical properties of these films were adequate.

Cross-linked films with genipin and PVP could have potential applications in drug release in the oral mucosa. They could represent a friendly material used by patients with oral lesions.

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