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# Polysaccharide-based membranes loaded with erythromycin for application as wound dressings

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**ABSTRACT:** In this study, the antibiotic erythromycin (Ery) was incorporated into chitosan (Ch)–alginate (A) and Ch–xanthan (X) membranes with the aim of using them as bioactive wound dressings. Drug incorporation was performed by direct addition (DA) to the polysaccharide mixture and by membrane impregnation in solution (IS). A higher incorporation efficiency was obtained for DA, but higher amounts of drug were loaded into membranes by the IS method (maxima ≈ 2.1 and 0.7 g/g for Ch–X and Ch–A, respectively) because the initial concentration of drug could be higher than that in the DA method. Ery release in phosphate-buffered saline was slow, reaching about 12 and 32 mg of drug/g of membrane in 60 h for Ch–X and 4 and 16 mg/g for Ch–A by the DA and IS methods, respectively. With formulations prepared with IS, the required therapeutic dosage was reached within 60 h, whereas for those incorporating the drug by DA, prolonged use would be required. Both membrane types behaved as drug reservoirs, providing continuous antibiotic release to the wound site. Formulations with higher drug contents showed effective antibacterial activity against two species of bacteria commonly found in skin lesions, *Staphylococcus aureus* and *Pseudomonas aeruginosa*, and were thus potentially capable of protecting the wound site from bacterial attack. © 2016 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2016**, *133*, 43428.

**KEYWORDS:** drug-delivery systems; films; membranes; polysaccharides; properties and characterization

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## INTRODUCTION

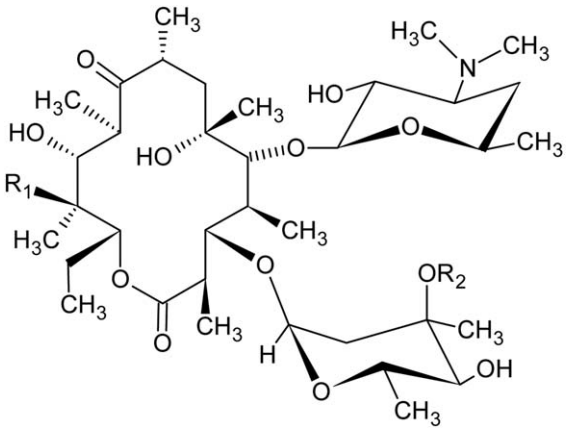
Healthy human skin acts as a protective barrier against pathogens; however, skin lesions may affect the functions of this organ and expose the wound to bacterial attack.[1,2]

The wound provides a moist and nutritious microenvironment, which supports bacterial colonization and proliferation.[2] Colonization of the wound by a relatively small amount of bacteria does not imply the occurrence of an infection because the presence of these microorganisms can facilitate healing through the production of proteolytic enzymes, which aid in the debridement process.[3] However, the presence of high concentrations of bacteria on the wound site may impair healing because of the intense and inefficient inflammatory response.[2] Moreover, as a result of infection, collagen synthesis is retarded, and epithelial cell migration is inhibited.[4] *Staphylococcus aureus* and *Pseudomonas aeruginosa* are the bacteria that are predominantly found in the wound bed.[2] Superficially infected lesions can be treated with topical antibiotics and dressings impregnated with these compounds;[3] however, many commercially available materials are ineffective against bacterial biofilms.[5]

Erythromycin (Ery) is a medium-spectrum antibiotic of the macrolide family and is produced by *Streptomyces erythreus*.[6] Susceptible microorganisms include aerobic Gram-positive bacteria, Gram-negative cocci, spirochetes, actinomycetes, rickettsia, *Chlamydia trachomatis*, *Mycoplasma pneumoniae*, *Legionella pneumophila*, nontuberculous mycobacteria, and some anaerobic bacteria.[7] This antibiotic consists of a 13-carbon ring with two sugars attached by glycosidic linkages[8,9] (Figure 1) and has a molecular weight of 733.9 Da. It is slightly soluble in water and very soluble in organic solvents such as ethanol[10]; it is useful in the therapy of pneumonia, diphtheria, whooping cough, urethritis, and erythrasma and acne treatments.[7] For topical administration, the common dosage is 20 mg/g of vehicle.[11]

The incorporation of Ery into wound dressings is of interest if prolonged protection and drug delivery are simultaneously desired, mostly for extensive and painful lesions of difficult manipulation. These systems show improved therapy efficiency by means of an appropriate release profile; this leads to drug availability at the wound site for long periods and, thereby, reduces the need for repeated administrations.[12]

The main purpose of using a wound dressing is to provide a suitable environment at the wound site so that healing is fast



| Erythromycin | $R_1$ | $R_2$ |
|---|---|---|
| A | OH | $CH_3$ |
| B | H | $CH_3$ |
| C | OH | H |

**Figure 1.** Chemical structure of Ery (adapted from Pendela *et al.*[9]).

and free of complications. Natural and synthetic polymers are commonly used for the production of wound dressings because of their biodegradability and biocompatibility.[13] Natural polymers frequently used for this application include chitosan (Ch), xanthan (X), and alginate (A), which can be combined to give materials with improved properties over those made of the isolated polymers.[14–18]

Ch is a semicrystalline linear polysaccharide[19] consisting of *N*-acetyl-D-glucosamine (2-acetamido-2-deoxy-D-glucan) and D-glucosamine (2-amino-2-deoxy-D-glucan) units joined by glycosidic $\beta$-(1–4) linkages [Figure 2(A)].[20] It is obtained by the deacetylation of chitin, a polymer found in the exoskeleton of crustaceans and insects and the cell walls of some fungi.[20] At low pH values, the amino groups of Ch are protonated, and the polymer is able to interact with negatively charged species, such as anionic polymers, to form polyelectrolyte complexes (PECs).[21] Ch presents intrinsic properties of high relevance for wound-dressing applications.[21–23]

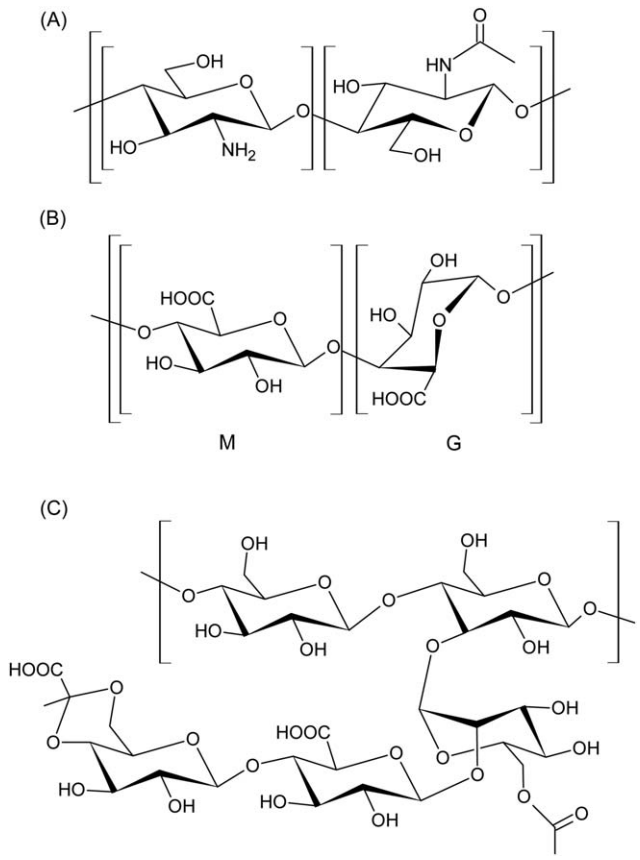



**Figure 2.** Chemical structures of the (A) Ch, (B) A, and (C) xanthan gum (adapted from Croisier and Jérôme,[31] Lee and Mooney,[25] and Hamman[30]).

Alginate is an anionic linear polysaccharide typically extracted from brown algae. Its structure is composed of two repeating subunits called *(1,4)-β-D-mannuronate* (M block) and *(1,4)-α-L-guluronate* [G block; Figure 2(B)].[24] Because of its gel-forming ability and positive effects on wounds, this compound has been widely applied in the medical field.[25]

Ch–A PECs are formed by electrostatic interactions between amino groups of Ch and carboxyl groups of A. They can present distinct forms, such as three-dimensional matrices, nanotubes, nanoparticles/microparticles, fibers, or gels, depending on the preparation process.[26] The formation of a complex between the two polymers may occur in the absence[27,28] or presence of a crosslinking agent. For this last case, either Ch and/or A chains may be crosslinked, and organic or ionic crosslinkers, such as calcium ions for A, can be used.[29] However, $Ca^{+2}$ ions exert an inhibitory effect on PEC gelation at low concentrations, and their use may result in a decreased dispersion stability.[29] In this study, an approach that consisted of the addition of $Ca^{+2}$ ions in two different steps was used to promote uniformity and strength of the gel through the prevention of the fast gelation of the complex.

In addition to the biodegradable and biocompatible characteristics observed for the isolated polymers, Ch–A PECs also present enhanced mechanical properties, stability under a greater range of pH conditions, and the ability to absorb relatively high proportions of aqueous solutions.[14,30] On the other hand, the microbicidal activity exhibited by Ch is not maintained after PEC formation because of the unavailability of free amino groups to interact with negatively charged molecules on the bacterial surface.[31] Thus, although the resulting biomaterial has no intrinsic antibacterial activity, it works effectively as a physical barrier against bacterial attack and allows gas exchange; it is, therefore, useful for wound dressings.[32]

Similarly to A, xanthan gum is an anionic polysaccharide but with a branched structure consisting of repeated pentasaccharide blocks formed by one glucuronic acid, two glucose, and two mannose units. Its main chain consists of ß-D-glucose units linked at the 1 and 4 positions [Figure 2(C)].[33] It is industrially produced by *Xanthomonas campestris* in culture and is nontoxic and nonsensitizing. This biopolymer is frequently used in the food industry as an emulsifier and a stabilizer[33,34] and also for controlled release purposes.[35]

Interactions between Ch amino groups and X carboxyl groups also result in the formation of PECs,[26] which are already used for the immobilization of enzymes[36] and probiotic bacteria[37] and to produce microspheres,[38] tablets,[39–41] microcapsules,[42] membranes,[16,17] controlled release systems,[35,39,43,44] and scaffolds for regenerative medicine applications.[45,46]

The incorporation of bioactive compounds into matrices used as wound dressings can be accomplished by different approaches, such as the addition of the compound directly to the polymeric solution [direct addition (DA)] or its impregnation in the matrix after its preparation with aqueous or organic solvents [impregnation in solution (IS)]. DA is the most commonly used method, as the active compound is easily incorporated into the device, and this favors homogeneous distribution along the polymeric film. A disadvantage of this method is the possibility of degradation of the compound because the evaporation of the solvent used in the production of the device requires exposure to heat or a vacuum for long periods. The IS method is advantageous because it allows a range of different compounds to be incorporated into the membrane after its preparation and, in addition, prevents the degradation of the active compound by avoiding exposure to the harsh conditions frequently used during membrane production. However, this method has disadvantages, such as a low incorporation yield and the heterogeneous dispersion of the compound within the polymeric matrix.[13,47]

In this context, the aim of this study was to incorporate Ery into polymeric membranes consisting of dried Ch–A or Ch–X PECs with two different incorporation methods, DA and IS, to obtain controlled release systems capable of acting efficiently in the topical therapy of superficially infected skin lesions. Different release profiles were expected from Ch–A and Ch–X matrices because of the structural differences in the polyanions (linear for A and branched for xanthan gum) and PECs (crosslinked structure of Ch–A) and also because of the different drug concentrations available through each incorporation method.

## EXPERIMENTAL

### Materials

Ch-based PEC membranes were prepared with the following reagents, which were analytical grade: Ch from shrimp shells (C3646, lot number 061M0046V, deacetylation degree = 88%), sodium alginate of low viscosity obtained from brown algae (A2158, lot number 090M0092V), xanthan gum (G1253, lot number 108K0038), and Ery (E5389, CAS number 114-07-8) from Sigma–Aldrich; ethanol and glacial acetic acid from Synth; and calcium chloride dihydrate and sodium hydroxide from Merck. The water used throughout this study was distilled and deionized in a Milli-Q system (Millipore). The molecular weights of Ch and A were determined by viscometry by Bueno *et al.*[18] and were $1.26 \times 10^6$ and $4.69 \times 10^4$ g/mol, respectively. The same method was used to determine the molecular weight of xanthan gum with polysaccharide solutions prepared at 0.0125, 0.025, 0.05, 0.075, and 0.1% w/v with 0.01*M* NaCl and constants of the Mark–Houwink–Sakurada equation obtained from Tinland and Rinaudo.[48] The intrinsic viscosity calculated for this polymer was 802.84 mL/g at 25 °C; this resulted in a molecular weight of $7.57 \times 10^5$ g/mol.

### Membrane Preparation and Ery Incorporation

The membranes were prepared on the basis of procedures described by Rodrigues *et al.*[14] and Bueno and Moraes[15] for Ch–A and Veiga and Moraes[16] for Ch–X formulation.

To obtain the Ch–A membranes, solutions of Ch at 1% w/v (prepared with an acetic acid aqueous solution at 2% v/v) and A (aqueous solution) at a concentration of 0.5% w/v were used. First, 90 mL of Ch solution was added at a rate of 200 mL/h to 180 mL of A solution in a jacketed, stainless steel reactor with an internal diameter of 10 cm and a height of 20 cm with a peristaltic pump (Minipuls 3, Gilson). The temperature was maintained at 25 °C throughout the process with a thermostatic bath (214 M2, Quimis). A mechanical stirrer (251 D, Quimis) with inclined blades with a radius of 2.1 cm was used for stirring at 500 rpm. After the addition of Ch solution, the mixing rate was raised to 1000 rpm for 10 min. Then, 16.8 mL of a 1*M* NaOH aqueous solution was added to correct the pH to 5.3, and the system was kept under agitation at 1000 rpm for 10 min more. Finally, 3.6 mL of a 2% w/v $CaCl_2$ aqueous solution was added to crosslink the carboxyl groups of the A that were not complexed with Ch amino groups and the system was kept under stirring for additional 10 min. After this step, the polymer mixture was degassed in a vacuum pump (Q-355b, Quimis) for 2 h and transferred to two polystyrene Petri dishes 15 cm in diameter, and the solvent was evaporated in an oven with air circulation (410D, New Ethics) for 24 h at 37 °C. Then, the membranes were washed for 30 min with 150 mL of 2% w/v $CaCl_2$ and subsequently with water (200 mL twice for 30 min each time). Final drying was done at room temperature.

To obtain Ch–X membranes, solutions of Ch (in 2% v/v aqueous acetic acid solution) and X (aqueous solution), both at a concentration of 0.5% w/v, were used. The volume of each solution was 90 mL. The same equipment was used, except for the stirring system, which in this case consisted of a mechanical stirrer with a marine propeller with a radius of 2.5 cm, to improve mixing. The procedure basically consisted of the formation of Ch–X PEC through the mixture of the polymers in the reactor. This was followed by its deaeration (2 h), molding into a Petri dish, evaporation of the solvent (37 °C, 24 h), washing (twice with 200 mL of deionized water for 30 min each time), and subsequent drying (37 °C, 24 h).

The incorporation of Ery was carried out by DA or IS. DA of the antibiotic was performed by the placement of the drug into the reactor at ratios of 20, 40, and 60 mg of antibiotic/g of polymer right after the polysaccharides were mixed. These ratios

were chosen on the basis of the concentration of drug used in topical formulations, which was 20 mg/g of vehicle. The effects of higher concentrations were also analyzed to ensure that we obtained doses of Ery equivalent to those normally used in topical formulations after the partial release of the compound with respect to the occurrence of possible losses during processing and device usage.

Incorporation by impregnation was performed by the immersion of $1 \times 1\,cm^2$ membrane samples that were previously stored in a desiccator (at 22% relative humidity) in 4 mL of an ethanolic solution containing Ery at concentrations of 1, 3, and 5 mg/mL for 1 h at 25 °C under agitation at 100 rpm.

### Membrane Characterization

The characterization of the membranes was performed on the basis of procedures described by Bueno and Moraes[15] and Veiga and Moraes,[16] unless otherwise stated.

**Morphology.** The morphologies of the surfaces and cross sections of the membranes were assessed with scanning electron microscopy (LEO 440i, Leica). Samples with dimensions of $2 \times 1\,cm^2$ and previously stored in desiccator for 24 h were metalized with a thin layer of gold (92 Å, Mini Sputter Coater, SC 7620). To evaluate the cross sections, the samples were fractured after immersion for approximately 10 s in liquid nitrogen stored at −196 °C.

**Color and Opacity.** Color and opacity were measured with a colorimeter (ColorQuest II, Hunterlab) operating in transmittance mode with CIELAB standards and the Hunterlab method.[49] Hue and chroma were calculated with eqs. (1) and (2), respectively:

$$\text{Hue} = \tan^{-1}(b*/a*) \quad (1)$$

$$\text{Chroma} = [(a*)^2 + (b*)^2]^{0.5} \quad (2)$$

where $a^*$ and $b^*$ are color parameters provided by the equipment. The color of the sample was determined according to hue angle in the CIELAB diagram.[50]

The opacity ($Y$) of the sample was calculated by the equipment's software as the ratio of the opacity of the film compared to a black standard ($Y_b$) and the opacity of the film compared to a white standard ($Y_w$) according to eq. (3):

$$Y = (Y_b/Y_w) \times 100 \quad (3)$$

**Fourier Transform Infrared Spectroscopy.** IR spectroscopy analysis was performed to assess the existing functional groups and to evaluate possible interactions between the polymers in the membranes and changes in their structures originating from the incorporation of the active compound into the polymeric matrix.

The spectra were obtained on a spectrophotometer (Nicolet 6700, Thermo Scientific) operating in attenuated total reflectance mode (Smart Omni-Sampler accessory) with wave numbers ranging from 4000 to 675 $cm^{-1}$. KBr pellets were used when examining the powder samples with wave numbers ranging from 4000 to 400 $cm^{-1}$. In both cases, a resolution of 4 $cm^{-1}$ and 32 accumulated scans were used to analyze the materials.

**Uptake Capacity and Stability in Ethanol.** The capacity of the membranes to absorb ethanol was determined with of $6 \times 1\,cm^2$ samples in triplicate; the samples had an initial mass denoted as $M_i$ and were previously stored in a desiccator for 24 h. The samples were exposed to 10 mL of ethanol for 1 h at 25 °C. After this step, excess ethanol was gently removed with filter paper, and the samples were weighed ($M_f$). The uptake capacity of ethanol ($U$; g of ethanol/g of membrane) was calculated according to eq. (4):

$$U = (M_f - M_i)/M_i \quad (4)$$

To determine the weight loss of the material exposed to ethanol, each sample was immersed for 5 min in 20 mL of water five times to remove weakly bounded compounds such as ions and polysaccharides. Then, the samples were dried for 24 h at 37 °C, kept in desiccator for 24 h, and weighed again ($M_d$). The weight loss ($L$; in %) was determined with eq. (5):

$$L = (M_i - M_d)/M_i \times 100 \quad (5)$$

**Ery Incorporation Efficiency.** The efficiency of Ery incorporation into the Ch–A and Ch–X membranes by the DA method was determined by the analysis of the washing solution containing the remaining residue of drug removed from the membrane during the washing step. For this analysis, the solution was evaporated at 37 °C for 20 h, and the residual powder was recovered and resolubilized in ethanol. The obtained ethanolic solution was filtered with a membrane with a 0.45-$\mu$m pore size. Ery was then quantified by spectrophotometry at 205 nm with a calibration curve prepared with known amounts of drug. To quantify the loss of antibiotic in the molding plates, the plates were washed with ethanol, and the ethanolic solution was analyzed analogously.

This procedure was also performed for membranes without Ery to quantify extractable compounds in ethanol that were not the compound of interest. The quantification of the antibiotic retained in the samples ($M_{c,m}$) was taken as the difference between the masses added to the membrane ($M_{c,i}$) and lost during the washing procedure or retained on the polystyrene plates. The incorporation efficiency ($\varepsilon$) was then calculated by eq. (6):

$$\varepsilon = (M_{c,m}/M_{c,i}) \times 100\% \quad (6)$$

To calculate the incorporation efficiency when the IS method was used, the amount of Ery remaining in the ethanolic solution after the incubation of the membranes was determined by spectrophotometry at 205 nm. This procedure was also carried out for samples exposed to ethanol not containing Ery. $M_{c,m}$ was determined as the difference between $M_{c,i}$ and the mass remaining in the incorporation solution. The incorporation efficiency was also calculated by eq. (6).

**Ery Release Kinetics.** To assess the antibiotic release kinetics, three $1 \times 1\,cm^2$ membrane samples were weighed and placed into vials containing 3 mL of phosphate-buffered saline under stirring at 100 rpm and 37 °C. Each set of samples was analyzed at predetermined periods of time. The contents of the vials were analyzed to determine the concentration of the active compound by spectrophotometry with the method described by Ford *et al.*[51]

**Table I.** Incorporation Efficiencies of Ery by the DA Method for the Ch–A and Ch–X Membranes

| Formulation | Ery added (mg/g) | Ery retained (mg/g) | Incorporation efficiency (%) |
|---|---|---|---|
| Ch-A | 20 | $8.1 \pm 2.0^{a}$ | $41.9 \pm 3.0^{a}$ |
| | 40 | $18.1 \pm 3.2^{b}$ | $47.7 \pm 6.0^{a,b}$ |
| | 60 | $25.5 \pm 2.8^{c}$ | $44.1 \pm 1.9^{a}$ |
| Ch-X | 20 | $10.5 \pm 2.0^{a}$ | $49.9 \pm 2.2^{b}$ |
| | 40 | $22.4 \pm 3.6^{b,c}$ | $52.3 \pm 3.2^{b}$ |
| | 60 | $34.8 \pm 5.1^{d}$ | $53.6 \pm 4.3^{b}$ |

The same letter in the same column indicates no significant difference between the mean values (Tukey test, $p < 0.05$).

**Antibacterial Activity.** The formation of a growth inhibition zone by membrane samples ($1 \times 1\,cm^2$) with or without Ery was evaluated by the placement of the material on the surface of solid Mueller–Hinton agar previously inoculated with *P. aeruginosa* ($5 \times 10^7$ cfu/mL) or *S. aureus* ($1 \times 10^9$ cfu/mL). After incubation at 37 °C for 24 h, the inhibition zone of each formulation was assessed.

## RESULTS AND DISCUSSION

### Ery Incorporation Efficiency

Tables I and II show the results of the incorporation efficiencies obtained with the DA and IS methods, respectively. When the DA method was used, satisfactory incorporation efficiency values were observed for both formulations; these ranged from 42 to 54%. For the IS method, the incorporation efficiencies were lower and ranged from about 9 to 20%.

Greater efficiencies were not obtained for the DA method, possibly because of the fact that part of the Ery particles added to the mixture were weakly bound to the matrix and were probably just deposited over the surfaces facing the plate and the air. This might have happened because of the lack of affinity between the hydrophobic compound and the hydrophilic matrices. As a result, a fraction of the particles may have been lixiviated during the washing of the membranes, and this resulted in a loss of Ery. The washing process is an important step because it eliminates residual acetic acid present in the matrix. If this step is not performed, the dissolution of Ch in aqueous solutions and irritation caused by this solvent when in contact with the skin or mucosa may occur. The Ery molecules that remained in the membranes were probably those capable of settling in the inner layers or underlying surface of these devices.

As an attempt to further increase the incorporation efficiency with the DA method, the drug could be directly added to the solution of one of the polysaccharides before the mixing process. Thus, it would be expected that the drug would be entrapped mostly in the PEC and predominantly on the surfaces of the membrane. However, the entrapment of the Ery molecules in the inner structure of the PECs formed could be a disadvantage of this approach, as drug release from the matrix could decrease to undesirably low levels because of hampered mass transfer.

The low efficiency observed for the IS method was related to the low absorption of ethanol by the membranes ($0.6 \pm 0.1$ g of ethanol/g of membrane for Ch–A and $0.7 \pm 0.1$ g of ethanol/g of membrane for Ch–X). Because ethanol acts as a vehicle for the incorporation of the compound, it would be expected a smaller swelling of the membranes in the presence of ethanol would result in a smaller amount of drug entrapped in the polymeric matrices. As a positive outcome, low ethanol absorption also resulted in a low weight loss of the membranes when they were exposed to it ($4.2 \pm 0.5\%$ for Ch–A and $8.2 \pm 0.6\%$ for Ch–X); this means that they were stable in the presence of this solvent.

Despite the higher incorporation efficiency obtained through the DA method, a significantly lower amount of Ery was retained in the membranes (maximum = 35 mg/g for Ch–X) in relation to the IS method (ca. 2.1 g/g). It is important to emphasize that the same amount of drug could not be added for both methods, as each of them had specific restrictions. By the DA method, it was not possible to initially add a large amount of drug to the membrane because this could affect the formation and stability of the polymeric complex and also because of the very limited solubility of the drug in aqueous solutions. By the IS method, on the other hand, a large initial amount of drug could be used in the incorporation solution; this resulted in greater retention. The use of comparable initial amounts of Ery for both methods would result in small retention of the drug in the matrix for IS because, in this case, the incorporation efficiency is very low.

The DA method is advantageous because, after the polymeric mixture obtained in the reactor is degassed, molded, dried, and washed, the resulting device is already loaded with the drug.

**Table II.** Incorporation Efficiencies of Ery by the IS Method for Ch–A and Ch–X Membranes

| Formulation | Initial concentration of Ery (mg/mL) | Ery added (mg/g) | Ery retained (mg/g) | Incorporation efficiency (%) |
|---|---|---|---|---|
| Ch-A | 1 | $802.7 \pm 57.9^{a}$ | $82.7 \pm 18.4^{a}$ | $10.3 \pm 2.4^{a,c}$ |
| | 3 | $2178.3 \pm 176.5^{b}$ | $430.3 \pm 65.7^{b}$ | $19.8 \pm 2.8^{b}$ |
| | 5 | $3868.8 \pm 677.4^{c}$ | $673.8 \pm 134.8^{c}$ | $17.4 \pm 0.4^{b}$ |
| Ch-X | 1 | $1684.3 \pm 212.2^{d}$ | $143.5 \pm 7.1^{d}$ | $8.6 \pm 0.7^{c}$ |
| | 3 | $5339.1 \pm 1549.6^{c}$ | $837.7 \pm 190.5^{c}$ | $16.6 \pm 5.2^{a,b}$ |
| | 5 | $10{,}818.7 \pm 413.5^{e}$ | $2127.9 \pm 130.4^{e}$ | $9.7 \pm 2.0^{a,c}$ |

The same letter in the same column indicates no significant difference between the mean values (Tukey test, $p < 0.05$).

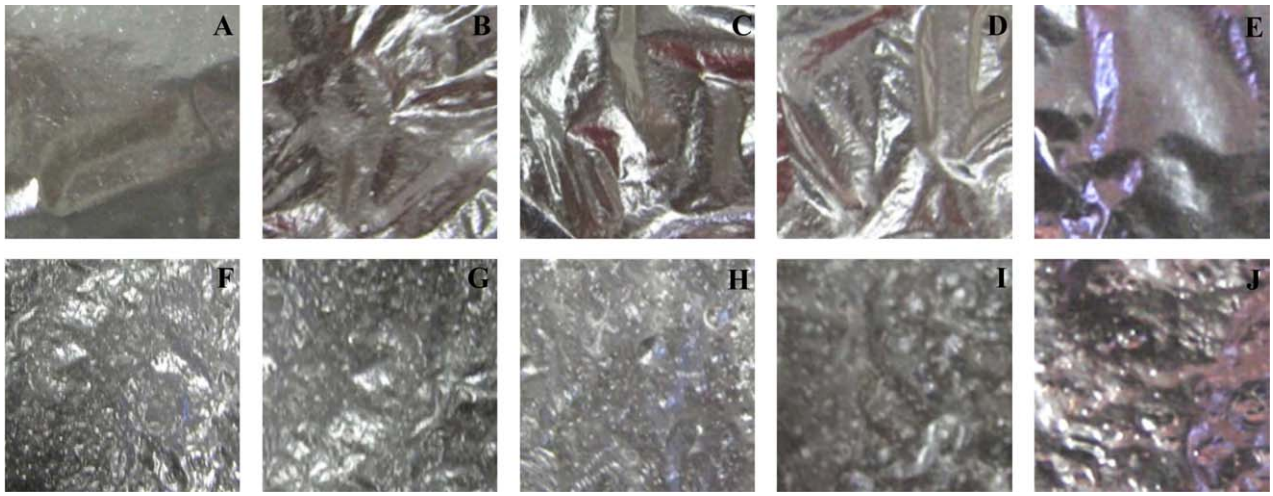



**Figure 3.** Visual aspect of the Ch–A (top) and Ch–X films (bottom): (A,F) without Ery, (B,G) with the compound incorporated by the DA method with the proportion of 20 mg/g, (C,H) with the compound incorporated by the DA method with the proportion of 40 mg/g, (D,I) with the compound incorporated by the DA method with the proportion of 60 mg/g, and (E,J) with the compound incorporated by the IS method with the solution at a concentration of 5 mg/mL. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Thus, it is possible to eliminate two processing steps that are required when the incorporation is performed by the IS method: the immersion of the membranes into the solution containing the compound and their subsequent drying. If large-scale production of these devices is considered, the elimination of these steps would be attractive, as it would probably imply a lower energy demand and also a faster production cycles. The IS method, on the other hand, allows one to obtain membranes with significantly higher amounts of drug incorporated. However, the limitations of this method include, in addition to the extra processing steps, a low incorporation efficiency; this implies the need of a large amount of material to be initially added so that satisfactory amounts can be retained in the membranes. A possible way to circumvent this limitation would be to recycle the drug remaining in the incorporation solution in subsequent processes and, thus, increase the overall efficiency of this procedure. However, the use of this strategy would imply the introduction of one more step to the production process.

The samples obtained by the DA method were further characterized, and because of the high amount of drug required to perform incorporation by the IS method, additional characterization was restricted to the samples entrapping the highest quantity of Ery, that is, those in which the concentration of the incorporation solution was equal to 5 mg/mL.

### Visual Aspect, Morphology, Color, and Opacity of the Membranes

Figure 3 shows the visual aspect of the Ch–A and Ch–X membranes with or without Ery. The Ch–A membranes presented smoother surfaces and were less opaque than the Ch–X membranes. In addition, the Ch–X membranes showed visible polymeric fibers throughout their structure. Within the same group, no significant change in the aspect of the formulations prepared in the absence or presence of different proportions of the antibiotic was observed.

Table III provides information about the opacity and color parameters of the membranes. In accordance with visual analysis, the Ch–X membranes had greater opacity than Ch–A. Although it was not directly detectable through photographic analysis, the opacity of the Ch–X samples containing antibiotic incorporated by the DA method was lower when compared to the opacity of the Ch–X membranes in which the compound was absent. For Ch–A membranes containing Ery obtained through the DA method, a small decrease in the opacity was observed when the amount of Ery incorporated into the matrices was increased. For both the Ch–A and Ch–X formulations, the samples containing Ery obtained by the IS method showed greater opacities when compared to the membranes without the compound. The variation of opacity observed after the incorporation of the antibiotic may have been due to light scattering and the increased heterogeneity of the polysaccharide matrix network because of the presence of drug particles with relatively high molar masses within the structure. The first effect was probably predominant in samples obtained by the IS method, whereas the second one was prevalent in samples produced through the DA method. In fact, for the IS method, the drug remained predominantly on the membrane surface, in contrast to what was observed for the other method (as shown in Figures 4 and 5), which favored light scattering. Bierhalz *et al.*[52] incorporated the antimycotic agent natamycin, a hydrophobic compound, into A and pectin films with the DA method. In contrast to what was observed in this study, Bierhalz *et al.*[52] observed an increase in the opacity of the films containing natamycin when compared to that free of the compound; this was attributed to the low solubility of the antimycotic in the matrix. The same behavior was observed by Yang and Paulson,[53] who also added hydrophobic compounds (lipids) into films consisting of gellan gum. In the last case, Yang and Paulson[53] attributed the increased opacity to light scattering caused by the presence of lipid droplets dispersed throughout the matrix.

**Table III.** Color and Opacity Parameters of Membranes without Ery and Membranes in Which the Compound Was Incorporated by DA into the Polymeric Mixture or by IS (Ethanol)

| Formulation | Incorporation method | Ery added | Opacity (%) | Hue | Chroma |
|---|---|---|---|---|---|
| Ch–A | Without Ery | 0 | $16.77 \pm 0.23^{a}$ | $-81.72 \pm 0.47^{a}$ | $1.27 \pm 0.02^{a}$ |
| | DA | 20 mg/g | $12.43 \pm 1.65^{b}$ | $-78.80 \pm 0.53^{b}$ | $3.21 \pm 0.05^{b}$ |
| | | 40 mg/g | $9.90 \pm 0.89^{b}$ | $-80.64 \pm 0.53^{a,b}$ | $2.40 \pm 0.22^{c}$ |
| | | 60 mg/g | $5.33 \pm 0.80^{c}$ | $-79.25 \pm 0.34^{b}$ | $1.82 \pm 0.07^{d}$ |
| | IS | 5 mg/mL | $24.85 \pm 0.70^{d}$ | $-82.97 \pm 0.36^{a}$ | $1.82 \pm 0.07^{d}$ |
| Ch–X | Without Ery | 0 | $46.13 \pm 0.42^{e}$ | $-48.01 \pm 5.26^{c}$ | $0.31 \pm 0.04^{e}$ |
| | DA | 20 mg/g | $17.23 \pm 0.64^{a,f}$ | $-52.17 \pm 6.21^{c}$ | $0.28 \pm 0.02^{e}$ |
| | | 40 mg/g | $18.90 \pm 1.75^{a}$ | $-46.45 \pm 4.92^{c}$ | $0.31 \pm 0.04^{e}$ |
| | | 60 mg/g | $15.77 \pm 0.20^{f}$ | $-48.26 \pm 4.11^{c}$ | $0.33 \pm 0.03^{e}$ |
| | IS | 5 mg/mL | $53.95 \pm 0.78^{g}$ | $-47.71 \pm 3.81^{c}$ | $0.31 \pm 0.02^{e}$ |

The same letter in the same column indicates no significant difference between the mean values (Tukey test, $p < 0.05$).

The hue values showed that the Ch–A formulation were greenish yellow, whereas the Ch–X membranes were yellowish green (Table III). However, the intensity of these colors was very low, as indicated by the chroma values (Table III). For all of the formulations containing Ery, regardless of the method of incorporation used, both the hue and chroma values remained very similar to those observed for membranes without the antibiotic; this indicated that adequate dispersion of the drug in the matrix was attained and the incorporation of the compound into the polymeric mixture did not have a significant effect on their color.

Because one of the desirable characteristics for wound dressings is translucency, which would allow monitoring of the wound-healing process without the need for removing the dressing, the Ch–A membrane would probably be more suitable for this application because it had a smoother surface and lower opacity than the Ch–X formulation.

The analysis of the surface and cross-sectional morphology of the membranes by scanning electron microscopy showed that the Ch–A membranes had a slightly rough surface (not noticeable to the naked eye) and also that this biomaterial presented lamellae along its thickness [Figure 4(A,B)]. The Ch–X membranes presented wavy surfaces and many fibers scattered throughout their structure [Figure 5(A,B)]. The presence of lamellae in the Ch–X formulation was also observed, but they were less compact when compared to those of the Ch–A formulation [Figure 5(B)].

The hypothesis to explain the formation of the lamellae was based on the interactions that stabilized the final complex structure. According to Lankalapalli and Kolapalli,[54] the formation of PECs involves three steps. The first step is the primary complex formation, driven by electrostatic interactions. The second step involves the formation of new bonds and/or the correction of the distortions of the polymer chains; this leads to a secondary arrangement of the structure. Finally, intercomplex aggregation occurs because of interactions among secondary complexes, mainly through hydrophobic interactions. The presence of —CH groups in the structures of all polysaccharides provides them with some hydrophobic character.[55] The lamellae were probably a result of the spacing between secondary complexes as a consequence of the hydrophobic interactions, and they occurred both for Ch–A and Ch–X. The lamellae of the Ch–A complexes were more compact because the A structure was linear, and the polymer chains were crosslinked with calcium; this led to their approximation.

Heterogeneous distributions of the antibiotic on the surfaces of the membranes of all of the formulations containing the active compound were observed.

For Ch–A formulations into which Ery was incorporated by the DA method, the antibiotic was present in its typical form of crystals. There was no significant difference between the surfaces of the samples in which different amounts of the compound were incorporated. The cross-sectional analysis showed that Ery remained in a greater proportion in the lower and upper edges of the matrices, that is, at the first underlying layer of the surface, and there was a small amount of the compound distributed throughout the thickness of the membranes. This behavior was attributed to the exclusion of the compound out of the aqueous phase during the drying process, as mentioned previously [Figure 4(A–H)]. For the Ch–X formulation, the antibiotic was distributed within the matrix, and the presence of beads on the membrane surface was observed; this suggested that the compound was also located at the first underlying layer of the membrane surface, possibly because the branched structure of X in Ch–X may have facilitated the entrapment of Ery within the PEC network [Figure 5(A–H)]. For both formulations, no significant difference in the micrographs of the samples containing different proportions of Ery was observed.

For the membranes to which Ery was incorporated by the IS method [Figures 4 and 5(I,J)], antibiotic agglomerates heterogeneously distributed were observed on their surface. In addition, the drug presented a different aspect (small elongated platelike

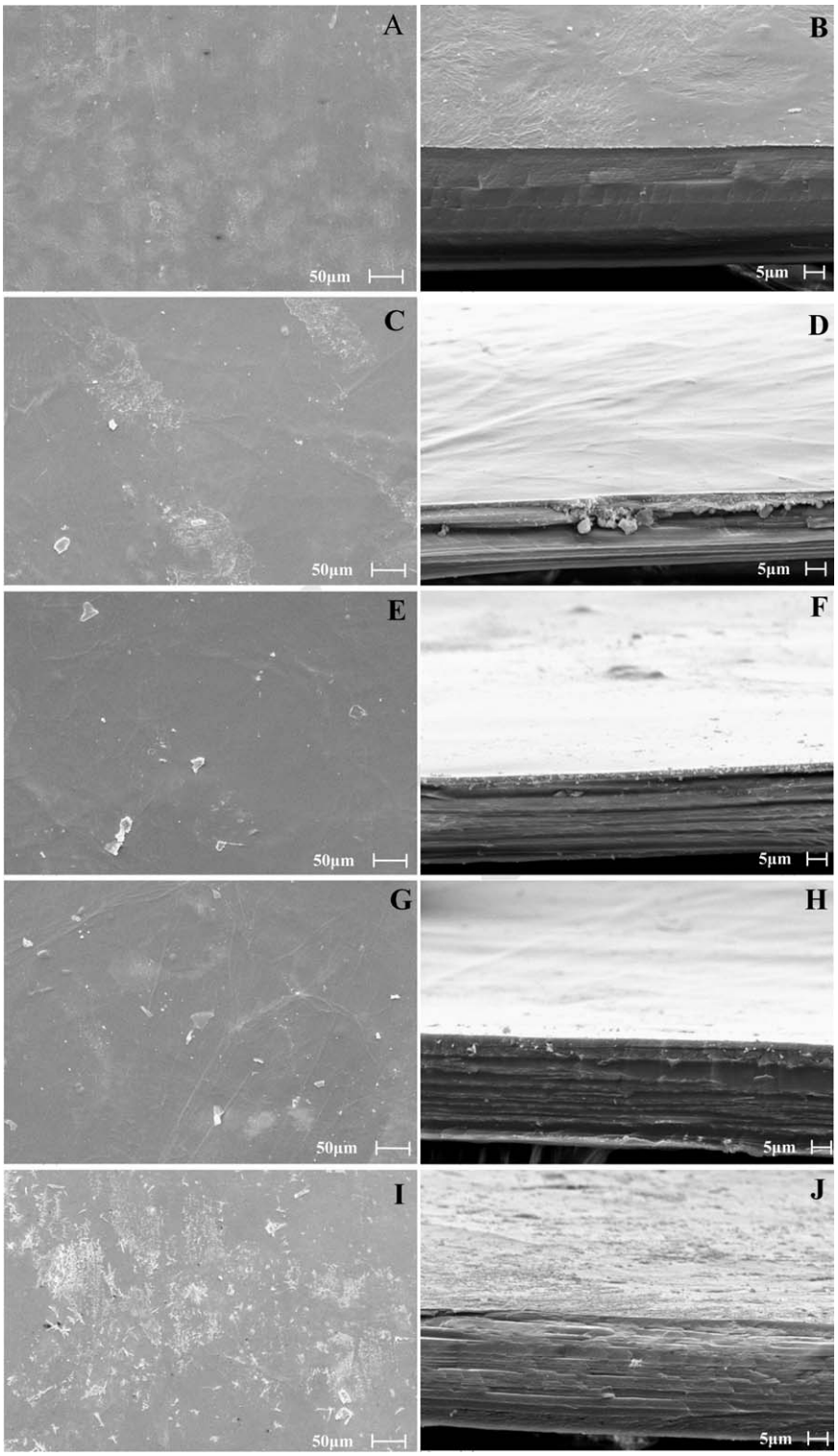



**Figure 4.** Surface (left) and cross-sectional morphologies (right) of the Ch–A films: (A,B) without Ery, (C,D) with the compound incorporated by the DA method with a proportion of 20 mg/g, (E,F) with the compound incorporated by the DA method with a proportion of 40 mg/g, (G,H) with the compound incorporated by the DA method with a proportion of 60 mg/g, and (I,J) with the compound incorporated by the IS method with the solution at a concentration of 5 mg/mL.

crystals); this was a result of the drug polymorphism in the presence of ethanol, as also reported by Mirza *et al.*[56] They observed different configurations of the drug crystals after exposure to acetone, methyl ethyl ketone, ethanol, and isopropyl alcohol. It was not possible to observe the presence of the drug within the polymeric matrix when the cross sections of the

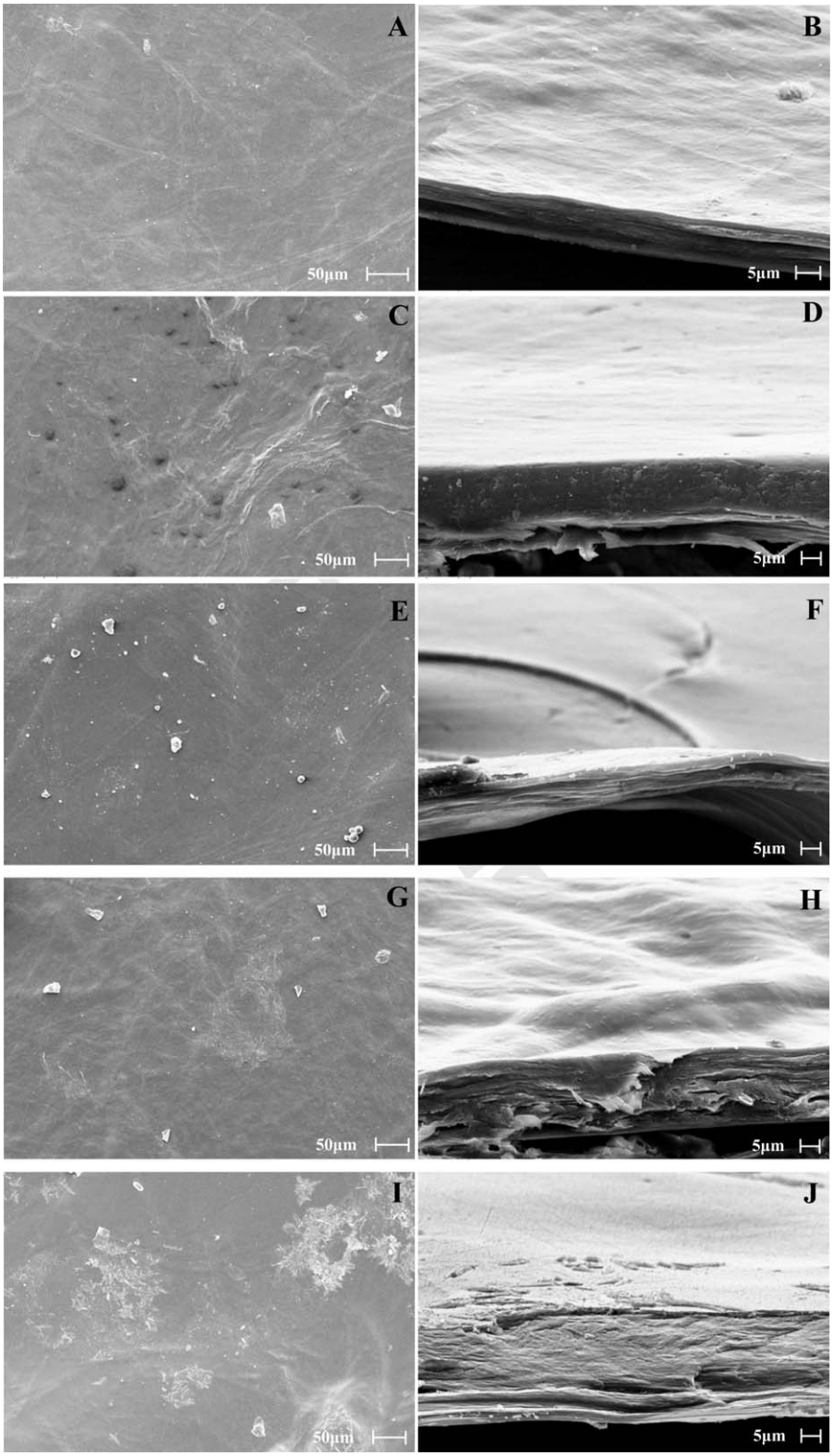



**Figure 5.** Surface (left) and cross-sectional morphologies (right) of the Ch–X films: (A,B) without Ery, (C,D) with the compound incorporated by the DA method with a proportion of 20 mg/g, (E,F) with the compound incorporated by the DA method with a proportion of 40 mg/g, (G,H) with the compound incorporated by the DA method with a proportion of 60 mg/g, and (I,J) with the compound incorporated by the IS method with the solution at a concentration of 5 mg/mL.

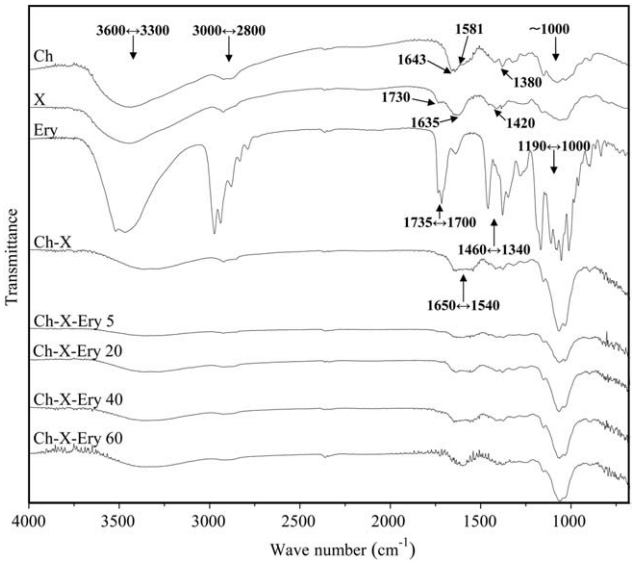



**Figure 6.** Fourier transform infrared spectra obtained for the Ch–X membranes without or with Ery incorporated by the IS method with the solution at a concentration of 5 mg/mL (Ch–X–Ery 5) and by the DA method with proportions of 20 (Ch–X–Ery 20), 40 (Ch–X–Ery 40), and 60 mg/g (Ch–X–Ery 60). The spectra of the isolated components are also shown.

samples were analyzed; this was possibly due to the low absorption of ethanol by these membranes, which led to the low incorporation of Ery and its preferential deposition on the surface.

**Fourier Transform Infrared Spectra**

Figures 6 and 7 show the Fourier transform infrared spectra for the Ch–X and Ch–A formulations and their isolated components. We observed that the Ch, X, and A spectra presented absorption bands between 3600 and 3000 $cm^{-1}$, which were related to the stretching of the hydroxyl groups present in the structure of all three polymers. Another band appeared between 3000 and 2800 $cm^{-1}$; this was related to axial vibrations of the —C—H groups. At wave numbers near 1000 $cm^{-1}$, characteristic polysaccharide peaks were observed; these were attributed to vibrations of —C—O, —C—C, and —C—O—C groups.[57–59]

In the Ch spectrum, we also observed a peak at 1643 $cm^{-1}$ related to the —C═O bond of amide I groups still acetylated, a peak at 1581 $cm^{-1}$ related to the amino groups, and a peak at 1380 $cm^{-1}$, which refers to the deformation of —$CH_2$ groups.[57,58,60]

X presented peaks at 1730 and 1635 $cm^{-1}$, which were characteristic of acetate, pyruvate, and glucuronate groups,[58,59] as shown in the spectrum in Figure 6. Another peak was observed at 1420 $cm^{-1}$, which was a result of the deflection angle of —C—H groups.[61] In the A spectrum, shown in Figure 7, we observed a peak at 1600 $cm^{-1}$, relative to the asymmetrical vibrations of carbonyl groups (—C═O),[62] and also a peak at 1415 $cm^{-1}$, which referred to the stretching of carboxylate salts.[57]

The spectrum of Ery presented sets of peaks in several wavenumber ranges. In the range 1735–1700 $cm^{-1}$, there were peaks related to the angular deformation of the ketone group, and in the range 1460–1340 $cm^{-1}$, peaks related to the angular deformation of —$CH_2$ and —$CH_3$ groups were observed. Ether group deformations were indicated by peaks in the range 1190–1000 $cm^{-1}$.[63] In addition to these peaks, Ery also showed the characteristic bands of vibrations of the —O—H and —C—H groups in the ranges 3600–3000 and 3000–2800 $cm^{-1}$, respectively, as observed for the isolated polysaccharides.

In the Ch–X membrane spectrum, shown in Figure 6, there was an absorption band between 1650 and 1540 $cm^{-1}$. This band was attributed to the overlapping of the peaks related to the amino groups of Ch and the glucuronate groups of X; this could indicate possible interactions between these groups. Popa *et al.*[58] reported the appearance of a peak at 1663 $cm^{-1}$ in the spectrum of Ch–X hydrogels, and this band was absent in the spectrum of isolated Ch or X. The formation of this unique band was attributed to the possible collapse of the —C—O bonds of carboxyl groups after complexation of the biopolymers. Similar behavior was observed in this study.

The spectra of Ch–X membranes in which Ery was incorporated, either by the DA or IS method, were highly similar to the profile observed for the membrane without Ery. Therefore, it was not possible to identify by Fourier transform infrared spectroscopy, operating in attenuated total reflectance mode, the presence of the active compound in the Ch–X membranes. Although it was not detected in this assay, the scanning electron microscopy images shown in Figure 5 clearly demonstrated the presence of Ery in the membranes. Thus, it was assumed that there was an overlap in the peaks found in the spectrum of Ery with those present in the spectrum of the film that impaired their identification.

In the spectrum of the Ch–A membrane, shown in Figure 7, there were two absorption peaks at 1600 and 1420 $cm^{-1}$. Similar results were obtained by Li *et al.*[60] for Ch–A scaffolds

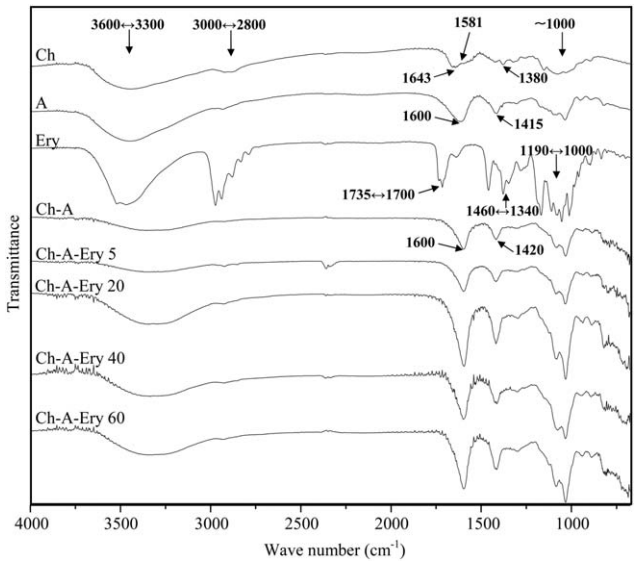



**Figure 7.** Fourier transform infrared spectra obtained for the Ch–A membranes without or with Ery incorporated by the IS method with the solution at a concentration of 5 mg/mL (Ch–A–Ery 5) and by the DA method with proportions of 20 (Ch–A–Ery 20), 40 (Ch–A–Ery 40), and 60 mg/g (Ch–A–Ery 60). The spectra of the isolated components are also shown.

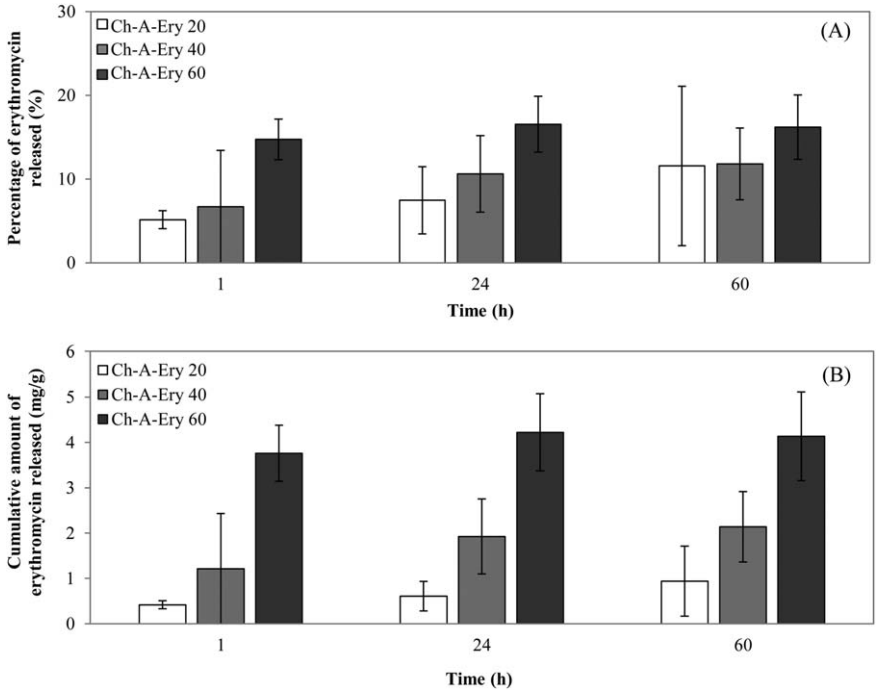



**Figure 8.** Ery release as a function of time for the Ch–A films in which the compound was incorporated by the DA method with proportions of 20, 40, and 60 mg/g in terms of (A) the percentage and (B) the mass of the compound per mass of the membrane.

crosslinked with calcium. They attributed the presence of the peak in the range of 1600 $cm^{-1}$ to the interaction of the amino groups of Ch with the carboxyl groups of A, which thereby formed the polymeric network.

The analysis of the spectra of the Ch–A membranes containing Ery showed that no significant alteration in the profile was observed in comparison to that of the Ch–A membrane spectrum without Ery; this was analogous to the results of the Ch–X membranes.

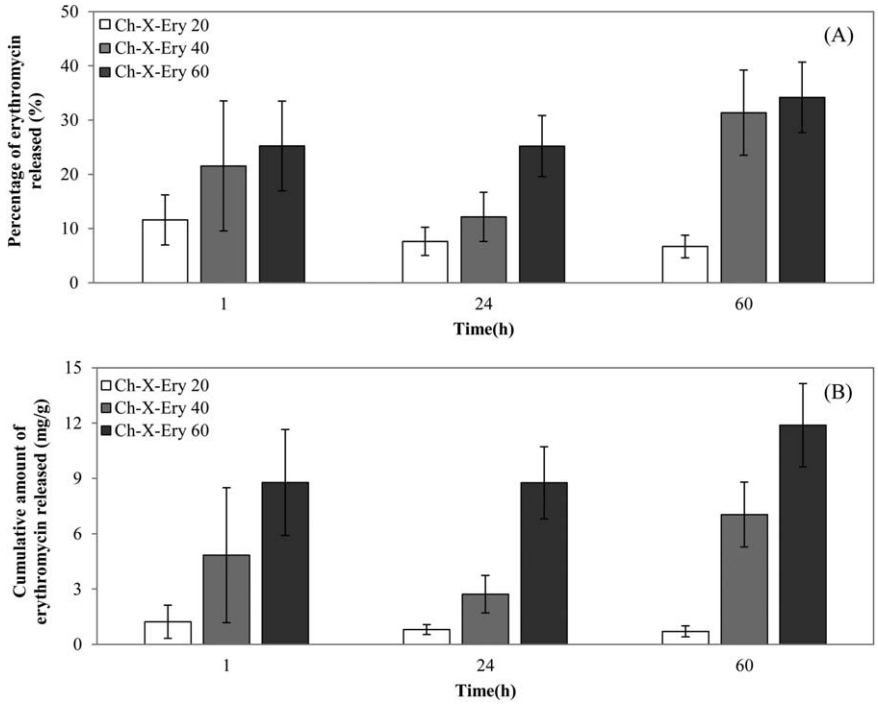



**Figure 9.** Ery release as a function of time for Ch–X films in which the compound was incorporated by the DA method with proportions of 20, 40, and 60 mg/g in terms of the (A) percentage and (B) mass of the compound per mass of the membrane.

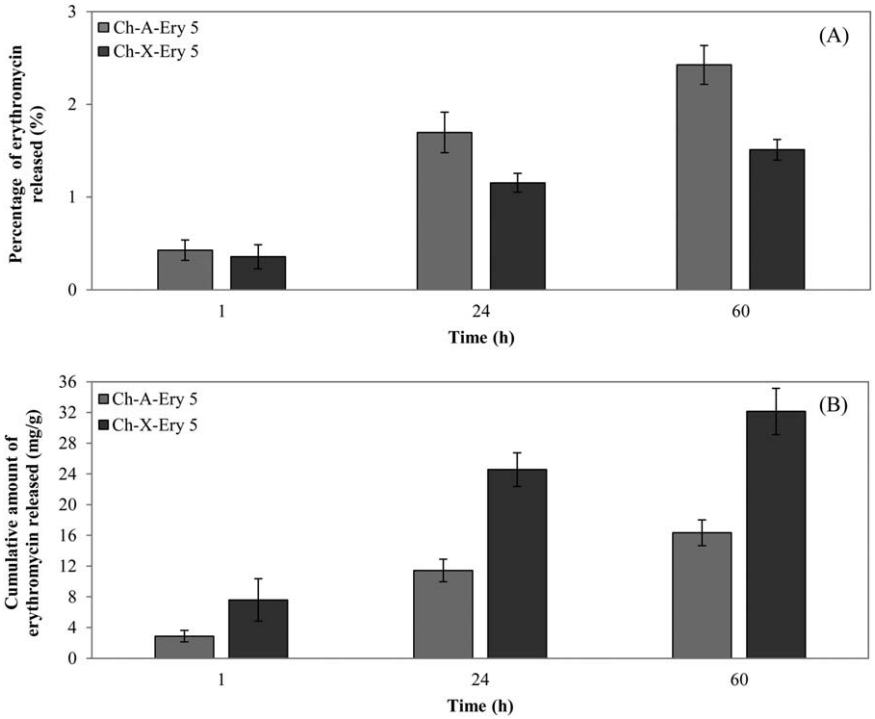



**Figure 10.** Ery release as a function of time for the Ch–A and Ch–X films in which the compound was incorporated by the IS method with the solution at a concentration of 5 mg/mL in terms of the (A) percentage and (B) mass of the compound per mass of the membrane.

### Ery Release Kinetics

Figures 8 and 9 show the release kinetics for the Ch–A and Ch–X membranes, respectively, into which Ery was incorporated in different proportions with the DA method. For both cases, the amount of antibiotic released did not represent a large portion of that initially incorporated; this was attributed to the lack of affinity of the compound for the aqueous medium. This indicated that the driving force to release the compound out of the matrix was very small, and probably, the amount of Ery initially released corresponded to the molecules deposited onto the surface of the membrane. The maximum percentages of released drug reached about 12, 12, and 17% at 60 h [Figure 8(A)], equivalent to approximately 0.9, 2.1, and 4.2 mg of Ery/g of membrane [Figure 8(B)], for the Ch–A formulation. The values were 7, 31, and 34% also at 60 h [Figure 9(A)], equivalent to approximately 0.7, 7.0, and 11.9 mg of Ery/g of membrane [Figure 9(B)] for the Ch–X formulation to which 20, 40, and 60 mg of antibiotic/g of membrane were added, respectively.

**Table IV.** Ery Released Per Unit of Area of Ch–A and Ch–X Films in Which the Compound Was Incorporated by DA to the Polymeric Mixture or by IS (Ethanol)

| Formulation | Incorporation method | Ery added | Ery released per unit area (mg/cm$^2$) |
|---|---|---|---|
| Ch-A | DA | 20 mg/g | 0.0039 |
| | | 40 mg/g | 0.0090 |
| | | 60 mg/g | 0.0174 |
| | IS | 5 mg/mL | 0.0686 |
| Ch-X | DA | 20 mg/g | 0.0017 |
| | | 40 mg/g | 0.0169 |
| | | 60 mg/g | 0.0285 |
| | IS | 5 mg/mL | 0.0771 |

The greater amount of Ery released by the Ch–X membranes when compared to the corresponding Ch–A formulations at the same releasing times could have been related to the branched structure of X present in those formulations. The branches enabled a larger retention of water by these membranes when compared to Ch–A, and the resulting swelling of the structure facilitated the mass transfer of the antibiotic throughout the matrix.

Figure 10 shows the release kinetics for the Ch–A and Ch–X membranes to which Ery was incorporated by the IS method with the drug solution at 5 mg/mL. Because the Ch–X formulation contained a higher initial amount of antibiotic retained in its structure, a larger amount was released, up to 32 mg of drug/g of membrane after 60 h [Figure 10(B)] when compared to the Ch–A formulation. For the same period, the Ch–A formulation released approximately half this amount. Although the total amount of Ery released was high compared to the values attained for the formulations obtained by the DA method, the equivalent percentages of released compound were only 1.5 and 2.4% for formulations Ch–X and Ch–A, respectively [Figure 10(A)].

According to the kinetics profile, drug release occurred slowly, probably because of the relatively large size of the Ery molecule; this prevented its rapid diffusion throughout the matrix.

The results attained were analyzed with the goal of reaching the required antibiotic dosage *in vivo* at a wound site normally treated with a commercially available Ery-containing ointment. For that, the concept of standardizing the amount of dermatological

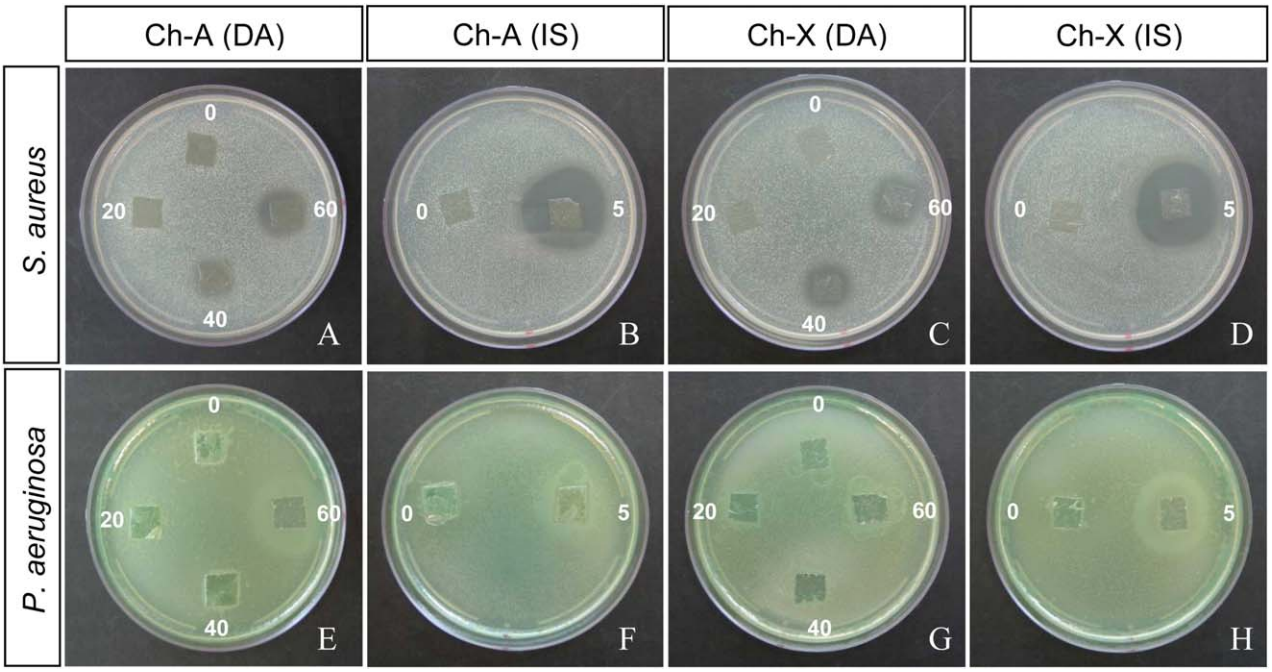



**Figure 11.** Culture plates inoculated with *S. aureus* and *P. aeruginosa* containing the Ch–A and Ch–X films without Ery (0; control) and films in which the compound was incorporated by the DA method with proportions of 20, 40, and 60 mg/g or in which the compound was incorporated by the IS method with the solution at a concentration of 5 mg/mL. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

ointment applied on a given surface of the body defined by Finlay *et al.*[64] was used. They specified the fingertip unit as the amount of ointment applied from the distal skin crease to the tip of the index finger of an adult, corresponding to an average of 0.5 g of ointment. In addition, the area covered by this amount of ointment is approximately 286 $cm^2$.[65] Because Ery ointments contain 20 mg of antibiotic/g of vehicle, 1 fingertip unit of ointment has 10 mg of antibiotic, and with the standard area mentioned previously, it corresponds to a coverage of about 0.035 mg of Ery/$cm^2$ of skin.

Given that the mass of $10 \times 10$ $mm^2$ membranes is on average, 0.042 g for Ch–A and 0.024 g for Ch–X formulations, it was possible to estimate the mass of Ery released per area covered by the membranes. Thus, the maximum amount of released Ery for each formulation at 60 h was calculated and is shown in Table IV.

From Table IV, we concluded that membranes obtained by the IS method released a dosage of Ery higher than that commonly used in topical formulations. The membranes obtained with the DA method did not reach this dosage within 60 h; however,

**Table V.** Mean Diameters of the Inhibition Zone in Culture Plates Inoculated with *S. aureus* and *P. aeruginosa* Containing Ch–A and Ch–X Membranes without Ery and Membranes in Which the Compound Was Incorporated by DA to the Polymeric Mixture or by IS (Ethanol)

| | | | Inhibition zone diameter (mm) | |
|---|---|---|---|---|
| Formulation | Incorporation method | Ery added | *S. aureus* | *P. aeruginosa* |
| Ch-A | Without Ery | 0 | $0^a$ | $0^a$ |
| | DA | 20 mg/g | $0^a$ | $0^a$ |
| | | 40 mg/g | $14 \pm 2^b$ | $11 \pm 2^b$ |
| | | 60 mg/g | $20 \pm 1^c$ | $25 \pm 0^c$ |
| | IS | 5 mg/mL | $30 \pm 1^d$ | $24 \pm 1^c$ |
| Ch-X | Without Ery | 0 | $0^a$ | $0^a$ |
| | DA | 20 mg/g | $0^a$ | $13 \pm 1^b$ |
| | | 40 mg/g | $17 \pm 1^b$ | $13 \pm 2^b$ |
| | | 60 mg/g | $18 \pm 3^{b,c}$ | $21 \pm 1^d$ |
| | IS | 5 mg/mL | $29 \pm 1^d$ | $26 \pm 1^c$ |

The same letter in the same column indicates no significant difference between the mean values (Tukey test, $p < 0.05$).

prolonged use of these devices would probably allow them to achieve the required Ery release level. This type of drug administration is interesting because it provides a continuous dosing of the antibiotic at the wound site and prevents peaks of dosage that may be harmful to the patient.

**Antibacterial Activity**

The antibacterial activities of the membranes against Gram-positive (*S. aureus*) and Gram-negative (*P. aeruginosa*) bacteria were examined by the determination of the inhibition zone around the samples.

The results show that membranes containing higher amounts of Ery presented satisfactory antimicrobial activities against both bacteria because growth inhibition zones were observed (Figure 11). Halo formation was observed for the samples obtained by the DA method to which 40 and 60 mg/g of drug were added in an *S. aureus* culture [Table V and Figure 11(A,C)], and it was satisfactory only for 60 mg/g in the *P. aeruginosa* culture [Table V and Figure 11(E,G)]. The inhibition zones of the samples obtained by the IS method were similar to those for Ch–X and Ch–A for both bacteria, and they were larger than the halo observed for the samples obtained by the DA method because of a larger amount of drug was retained by the matrices processed by impregnation [Table V and Figure 11(B,D,F,H)].

These results agree with those shown in Table IV, which indicates that significantly more drug per unit area was released from both membrane formulations incorporating Ery by IS than from their DA counterparts after exposure to phosphate-buffered saline. As a result, in a practical application, the first ones would probably be more effective in protecting the lesion bed from bacterial growth.

## CONCLUSIONS

The incorporation of Ery into Ch–A and Ch–X membranes was successfully performed by direct drug addition or by its impregnation in the dressings, although a higher efficiency was observed with the DA method (with a maximum of 54% for Ch–X). By the IS approach, however, greater amounts of the active compound were retained in the matrices (with a maximum of 2.1 g/g for Ch–X). In all cases, the drug was heterogeneously distributed throughout the membranes, but this did not significantly affect their macroscopic aspect.

The relatively large size of Ery molecules was a factor that contributed to its slow release from the Ch–A and Ch–X matrices. The maximum proportions of antibiotic released in phosphate-buffered saline reached about 12 and 32 mg/g in 60 h for preparations obtained with the DA and IS methods, respectively, both for the Ch–X formulation.

When the IS method was used, the amount of drug released by both formulations exceeded the therapeutic dosage within the period analyzed. In the case of devices in which the DA method was used to incorporate Ery, the time to achieve this dosing was higher; thus, the obtained membranes could function as a drug reservoir and act as a release agent for long periods, and prolonged use of these devices could be effective for obtaining the required dosage for the treatment of skin lesions. Prolonged release is advantageous because it would allow less frequent dressing changes, favor wound healing, and provide more comfort to the patient. With the release period analyzed in this study, the Ch–X formulation to which 60 mg of Ery/g of membrane was added by the DA method would be the most appropriate membrane for the suggested application, as the amount of antibiotic released was the closest to the therapeutic dosage.

Membranes containing higher amounts of Ery showed antibacterial activities against *S. aureus* and *P. aeruginosa*, both bacteria commonly found in skin lesions. Therefore, these membranes could effectively inhibit bacterial proliferation and protect the wound site from bacterial attack.

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## REFERENCES

1. Gawkrodger, D. J. Dermatology: An Illustrated Colour Text; Churchill Livingstone Elsevier: London, **2002**.
2. Seth, A. K.; Geringer, M. R.; Hong, S. J.; Leung, K. P.; Mustoe, T. A.; Galiano, R. D. *J. Surg. Res.* **2012**, *178*, 330.
3. Broussard, K. C.; Powers, J. G. *Am. J. Clin. Dermatol.* **2013**, *14*, 449.
4. Boateng, J. S.; Matthews, K. H.; Stevens, H. N. E.; Eccleston, G. M. *J. Pharm. Sci.* **2008**, *97*, 2892.
5. Percival, S. L.; Hill, K. E.; Williams, D. W.; Hooper, S. J.; Thomas, D. W.; Costerton, J. W. *Wound Repair Regen.* **2012**, *20*, 647.
6. Kirst, H. A. In Macrolide Antibiotics; Schönfeld, W., Kirst, H. A., Eds.; Birkhäuser Verlag: Basel, Switzerland, **2002**; Chapter 1, p 1.
7. Constant, A. B. L. In Antibióticos e Quimioterápicos; Auto, H. F., Constant, J. M. C., Constant, A. B. L., Eds.; Edufal: Maceió, Brazil, **1982**; Vol. 5, p 151.
8. Majer, J. In Antibiotics: Isolation, Separation and Purification; Weinstein, M. J., Wagman, G. H., Eds.; Elsevier: Amsterdam, **1978**; Vol. *15*, p 275.
9. Pendela, M.; Béni, S.; Haghedooren, E.; Van den Bossche, L.; Noszál, B.; Van Schepdael, A.; Hoogmartens, J.; Adams, E. *Anal. Bioanal. Chem.* **2012**, *402*, 781.
10. World Health Organization. International Pharmacopoeia, 4th ed.; **2006**; Vol. *1*, p 398, Who Press: Geneva, Switzerland.
11. Hamilton, R. J. Tarascon Pocket Pharmacopoeia; Jones & Bartlett Learning: Burlington, MA, **2012**.
12. Duarte, A. R. C.; Costa, M. S.; Aguiar-Ricardo, A.; Simplício, A. L.; Cardoso, M. M.; Duarte, C. M. M. *Int. J. Pharm.* **2006**, *308*, 168.

13. Dias, A. M. A.; Braga, M. E. M.; Seabra, I. J.; Ferreira, P.; Gil, M. H.; Sousa, H. C. *Int. J. Pharm.* **2011**, *408*, 9.
14. Rodrigues, A. P.; Sanchez, E. M. S.; da Costa, A. C.; Moraes, A. M. *J. Appl. Polym. Sci.* **2008**, *109*, 2703.
15. Bueno, C. Z.; Moraes, A. M. *J. Appl. Polym. Sci.* **2011**, *122*, 624.
16. Veiga, I. G.; Moraes, A. M. *J. Appl. Polym. Sci.* **2011**, *124*, 154.
17. Bellini, M. Z.; Pires, A. L. R.; Vasconcelos, M. O.; Moraes, A. M. *J. Appl. Polym. Sci.* **2012**, *125*, E421.
18. Bueno, C. Z.; Dias, A. M. A.; Sousa, H. J. C.; Braga, M. E. M.; Moraes, A. M. *Mater. Sci. Eng. C* **2014**, *44*, 117.
19. Rinaudo, M. *Prog. Polym. Sci.* **2006**, *31*, 603.
20. Goycoolea, F. M.; Arguelles-Monal, W.; Peniche, C.; Higuera-Ciapara, I. *Dev. Food Sci.* **2000**, *41*, 265.
21. Dash, M.; Chiellini, F.; Ottenbrite, R. M.; Chiellini, E. *Prog. Polym. Sci.* **2011**, *36*, 981.
22. Berger, J.; Reist, M.; Mayer, J. M.; Felt, O.; Gurny, R. *Eur. J. Pharm. Biopharm.* **2004**, *57*, 35.
23. Paul, W.; Sharma, C. P. *Trends Biomater. Artif. Organs* **2004**, *18*, 18.
24. Gombotz, W. R.; Wee, S. F. *Adv. Drug Delivery Rev.* **1998**, *31*, 267.
25. Lee, K. Y.; Mooney, D. J. *Prog. Polym. Sci.* **2012**, *37*, 106.
26. Luo, Y.; Wang, Q. *Int. J. Biol. Macromol.* **2014**, *64*, 353.
27. Saether, H. V.; Holme, H. K.; Maurstad, G.; Smidsrød, O.; Stokke, B. T. *Carbohydr. Polym.* **2008**, *74*, 813.
28. Sibaja, B.; Culbertson, E.; Marshall, P.; Boy, R.; Broughton, R. M.; Solano, A. A.; Esquivel, M.; Parker, J.; Fuente, L. D. L.; Auad, M. L. *Carbohydr. Polym.* **2015**, *134*, 598.
29. Turkoglu, T.; Tascioglu, S. *J. Appl. Polym. Sci.* **2014**, *131*, 40019.
30. Hamman, J. H. *Mar. Drugs* **2010**, *8*, 1305.
31. Croisier, F.; Jérôme, C. *Eur. Polym. J.* **2013**, *49*, 780.
32. Caetano, G. F.; Frade, M. A.; Andrade, T. A.; Leite, M. N.; Bueno, C. Z.; Moraes, A. M.; Ribeiro-Paes, J. T. *J. Biomed. Mater. Res. B* **2015**, *103*, 1013.
33. García-Ochoa, F.; Santos, V. E.; Casas, J. A.; Gómez, E. *Biotechnol. Adv.* **2000**, *18*, 549.
34. Coviello, T.; Matricardi, P.; Marianecci, C.; Alhaique, F. J. *Controlled Release* **2007**, *119*, 5.
35. Santos, H.; Veiga, F.; Pina, M. E. *Eur. J. Pharm. Sci.* **2004**, *21*, 271.
36. Liu, H.; Nakagawa, K.; Kato, D.; Chaudhary, D.; Tadé, M. O. *Mater. Chem. Phys.* **2011**, *129*, 488.
37. Argin, S.; Kofinas, P.; Lo, Y. M. *Food Hydrocolloids* **2014**, *40*, 138.
38. Chellat, F.; Tabrizian, M.; Dumitriu, S.; Chornet, E.; Rivard, C. H.; Yahia, L. *J. Biomed. Mater. Res.* **2000**, *53*, 592.
39. Caddeo, C.; Nácher, A.; Díez-Sales, O.; Merino-Sanjuán, M.; Fadda, A. M.; Manconi, M. *J. Microencapsul.* **2014**, *31*, 694.
40. Li, L.; Wang, L.; Li, J.; Jiang, S.; Wang, Y.; Zhang, X.; Ding, J.; Yu, T.; Mao, S. *Int. J. Pharm.* **2014**, *476*, 253.
41. Varshosaz, J.; Tavakoli, N.; Fatemeh, M.; Hassami, E. G. *Farmacia* **2015**, *63*, 65.
42. Argin-Soysal, S.; Kofinas, P.; Lo, Y. L. *Food Hydrocolloids* **2009**, *23*, 202.
43. Dehghan, M. H. G.; Kazi, M. *Iran. J. Pharm. Res.* **2014**, *13*, 769.
44. Kulkarni, N.; Wakte, P.; Naik, J. *Int. J. Pharm. Invest.* **2015**, *5*, 73.
45. Bellini, M. Z.; Caliari-Oliveira, C.; Mizukami, A.; Swiech, K.; Covas, D. T.; Donadi, E. A.; Oliva-Neto, P.; Moraes, Â. M. *J. Biomater. Appl.* **2015**, *29*, 1155.
46. Horn, M. M.; Martins, V. C. A.; Plepis, A. M. G. *Int. J. Biol. Macromol.* **2015**, *80*, 225.
47. Davies, O. R.; Lewis, A. L.; Whitaker, M. J.; Tai, H.; Shakesheff, K. M.; How, S. M. *Adv. Drug Delivery Rev.* **2008**, *60*, 373.
48. Tinland, B.; Rinaudo, M. *Macromolecules* **1989**, *22*, 1863.
49. Universal Software, Version 3.2; Hunter Associates Laboratory: Reston, VA, **1997**.
50. Voss, D. H. *HortScience* **1992**, *27*, 1256.
51. Ford, J. H.; Prescott, G. C.; Iiinjiaiv, J. W.; Carou, E. L. *Anal. Chem.* **1953**, *25*, 1195.
52. Bierhalz, A. C. K.; Silva, M. A.; Kieckbusch, T. G. *J. Food Eng.* **2012**, *110*, 18.
53. Yang, L.; Paulson, A. T. *Food Res. Int.* **2000**, *33*, 571.
54. Lankalapalli, S.; Kolapalli, V. R. M. *Indian J. Pharm. Sci.* **2009**, *71*, 481.
55. Rinaudo, M. *Polym. Int.* **2008**, *57*, 397.
56. Mirza, S.; Miroshnyk, I.; Heinamaki, J.; Christiansen, L.; Karjalainen, M.; Yliruusi, J. *J. Pharm. Sci.* **2003**, *5*, 1.
57. Smitha, B.; Sridhar, S.; Khan, A. A. *Eur. Polym. J.* **2005**, *41*, 1859.
58. Popa, N.; Novac, O.; Profire, L.; Lupusoru, C. E.; Popa, M. I. *J. Mater. Sci.* **2010**, *21*, 1241.
59. Lungan, M. A.; Popa, M.; Desbrieres, J.; Racovita, S.; Vasiliu, S. *Carbohydr. Polym.* **2014**, *104*, 213.
60. Li, Z.; Ramay, H. R.; Hauch, K. D.; Xiao, D.; Zhang, M. *Biomaterials* **2005**, *26*, 3919.
61. Gunasekar, V.; Reshma, K. R.; Treesa, G.; Gowdhaman, D.; Ponnusami, V. *Carbohydr. Polym.* **2014**, *102*, 669.
62. Lawrie, G.; Keen, I.; Drew, B.; Chandler-Temple, A.; Rintoul, L.; Fredericks, P.; Grøndahl, L. *Biomacromolecules* **2007**, *8*, 2533.
63. Sotiro, K. R. Master's Thesis, Universidade Estadual Paulista, Câmpus de Araraquara, **2007**.
64. Finlay, A. Y.; Edwardm, P. H.; Harding, K. G. *Lancet* **1989**, *2*, 155.
65. Long, C. C.; Finlay, A. Y. *Clin. Exp. Dermatol.* **1991**, *16*, 444.