Brazilian Journal
of Chemical
Engineering

ISSN 0104-6632
Printed in Brazil
www.abeq.org.br/bjche

Vol. 33, No. 03, pp. 453 - 467, July - September, 2016
dx.doi.org/10.1590/0104-6632.20160333s20150083

INCORPORATION AND RELEASE KINETICS OF ALPHA-BISABOLOL FROM PCL AND CHITOSAN/GUAR GUM MEMBRANES

F. C. Bombaldi de Souza, R. F. Bombaldi de Souza and Â. M. Moraes*

Universidade Estadual de Campinas, (UNICAMP), Faculdade de Engenharia Química, Departamento de Engenharia de Materiais e de Bioprocessos, Av. Albert Einstein 500, Bl. A, Piso Térreo, Cidade Universitária Zeferino Vaz, CEP: 13083-852, Campinas - SP, Brazil.
Phone: (55) (19) 3521-3920
*E-mail: ammoraes@feq.unicamp.br
E-mail: fbombaldi@feq.unicamp.br; rebombaldi@feq.unicamp.br

(Submitted: February 11, 2015 ; Revised: May 21, 2015 ; Accepted: May 26, 2015)

Abstract - Alpha-bisabolol, an anti-inflammatory and antioxidant compound extracted from candeia trees (*Eremanthus erythropappus*), was incorporated into hydrophobic polycaprolactone (PCL) and hydrophilic chitosan/guar gum (Ch-G) membranes aiming at the production of bioactive wound dressings. The incorporation efficiency achieved a maximum of *ca*. 18% (1 gram of alpha-bisabolol per gram of membrane) for Ch-G membranes. For PCL membranes, all of the active compound added was retained (0.2 gram of alpha-bisabolol per gram of membrane). Alpha-bisabolol release in phosphate-buffered saline was relatively slow in both cases, reaching around 6% and 24% after 120 hours respectively for PCL and Ch-G membranes presenting equivalent initial alpha-bisabolol/membrane mass ratios. Both formulations were capable of releasing alpha-bisabolol in the typically recommended topical dose range (from 1 to 10 grams of alpha-bisabolol per gram of vehicle). The extended release periods observed are advantageous, allowing less frequent dressing changes and contributing to turn the treatment more comfortable for the patient.
Keywords: Wound dressing; Membrane; Chitosan; Guar gum; Polycaprolactone; Alpha-bisabolol.

INTRODUCTION

The skin is the largest organ of the human body and acts as an interface with the external environment. This organ presents a complex structure and exerts functions that are crucial for life, such as thermoregulation, immune surveillance, sensitivity and mechanical barrier (Tortora and Derrickson, 2012). Skin lesions may affect this organ at different depths, reaching one or more of its layers and impairing its functions (Hess, 2005).

Wound dressings can be used to aid and enhance the natural healing process of an injury (Franco and Gonçalves, 2008), and the development of dressings with specific properties to treat different types of lesions has advanced significantly in the last decades. Wound dressings are used to protect the lesion from mechanical damage and contamination by microorganisms, as well as to provide an appropriate environment for the healing process, which includes the restoration of the epithelium and the formation of collagen (Weller and Sussman, 2006; Mulder *et al*., 2002). They can be classified according to the role they play in the lesion (barrier, debridement, antibacterial, occlusive, absorbent, adherent), the type of material used in its production (hydrocolloid, alginate, collagen, etc.) and also their physical form (ointment, film, foam, gel) (Boateng *et al*., 2008). Another classification refers to the form of interaction between the dressing and the lesion. According to

*To whom correspondence should be addressed
This is an extended version of the work presented at the 20th Brazilian Congress of Chemical Engineering, COBEQ-2014, Florianópolis, Brazil.

this classification, the materials used as wound dressings are divided into inert or passive, interactive and bioactive (Agrawal *et al*., 2014). Bioactive dressings are capable of interacting with the injured tissue, helping to reduce or eliminate pain and inflammatory processes, stimulating and accelerating the healing process (Weller and Sussman, 2006).

Several types of synthetic and natural polymers may be used for the production of dressings (Sezer and Cevher, 2011). Polymers of natural origin, or biopolymers, can be extracted from different sources, such as vegetables, animals or seaweeds or produced from fermentative pathways and enzymatic processes. Frequently biopolymers have higher biocompatibility and lower immunogenicity when compared to those of synthetic origin for the use in medical applications (Bhardwaj and Kundu, 2010). Natural polymers can mimic many of the characteristics of the extracellular matrix, therefore being able to direct the migration, growth, and organization of cells during the process of regeneration and healing of damaged tissue (Huang and Fu, 2010). The degradation of these polymers depends on enzymatic processes and also on the type of biopolymer used, thus the degradation rate can vary from patient to patient (Cheung *et al*., 2007). Synthetic polymers, on the other hand, are advantageous in comparison to biopolymers because they are more flexible and more easily processed to meet different application requirements. The ability to manipulate the properties of synthetic polymers allows obtaining materials with uniform characteristics and low lot-to-lot variation (Middleton and Tipton, 2000). These polymers are readily adaptable to exert a wide range of functional properties and it is possible to manipulate, for example, their molar mass, mechanical properties and degradability (Bhardwaj and Kundu, 2010). In the case of synthetic polymers, the degradability is related to hydrolysis (mostly of ester bonds), and thus the degradation rate does not vary from patient to patient, except for the occurrence of local inflammation, which may cause pH variations (Cheung *et al*., 2007). Chitosan and guar gum, polymers of natural origin, and polycaprolactone, a polymer of synthetic origin, are examples of materials widely used in the medical field.

Chitosan is a linear semi-crystalline polysaccharide with molecular weight ranging from 300 to 1000 kDa (Rinaudo, 2006; Liu *et al*., 2011). Its chemical structure is composed of a copolymer formed by units of N-acetyl-D-glucosamine (2-acetamido-2-deoxy-D-glucan) and D-glucosamine (2-amino-2-deoxy-D-glucan) joined by glycosidic β (1-4) linkages (Goycoolea *et al*., 2000). Chitosan is not commonly found in nature but can be readily obtained from chitin, a natural polymer mainly extracted from the exoskeleton of crustaceans, through deacetylation (Goycoolea *et al*., 2000). It is a biocompatible, biodegradable and bioadhesive polymer, slightly soluble in acidic solutions and with many important biological properties such as antifungal, antibacterial and haemostatic effect in certain conditions of use (Berger *et al*., 2004; Paul and Sharma, 2004). In addition, this polymer stimulates macrophage function, which accelerates the healing process of injuries (Paul and Sharma, 2004). Chitosan degradation occurs in the body through the action of enzymes, in particular, lysozyme present in human body fluids, leading to the formation of non-toxic oligosaccharides that are incorporated into metabolic routes and naturally eliminated from the organism (Kumar, 2000; Croisier and Jérôme, 2013).

Guar gum is a neutral water-soluble polysaccharide, extracted from the seeds of the plant *Cyamopsis tetragonolobus* (Prabhanjan *et al*., 1989), with mean molar mass varying from 50 to 8000 kDa (Kawamura, 2008). Its molecular structure consists of a linear chain of D-mannopyranose residues to which a D-galactopyranose residue is linked, on average, to every alternate mannose (Prabhanjan *et al*., 1989; Coviello *et al*., 2007). Guar gum is biodegraded if ingested, and its degradation occurs in the human colon by enzymes such as alpha-galactosidase and beta-mannanase, which are produced by colonic bacteria (Gliko-Kabir *et al*., 2000). This biopolymer is employed in various pharmaceutical formulations, including capsules, hydrogels, films and nano- or micro-particles and has been explored as a potential matrix for drug delivery systems (Prabaharan, 2011). It is also used in combination with carboxymethylcellulose as hydrocolloid dressings applicable to partial and full-thickness wounds with low to moderate exudation. However, given that even after a week of use the formation of only limited degradation residues is observed, this type of biomaterial is not recommended for very deep lesions with tunneling (Bower *et al*., 2011). Guar gum can be associated with chitosan to form a physical complex and the synergy between the two biopolymers has been studied and applied in the development of different medical devices (Haupt *et al*., 2006; Randhawa *et al*., 2012).

Polycaprolactone is a biodegradable synthetic polymer obtained from the polymerization of epsilon-caprolactone. It is a linear aliphatic polyester, semi-crystalline and hydrophobic (Pitt, 1990), with average molecular weight generally ranging from 3 to 80 kDa (Woodruff and Hutmacher, 2010). PCL degradation

occurs in two steps: first, by nonenzymatic hydrolysis of ester groups and second, intracellular degradation by macrophage and phagosomes. Since its molar mass can be reduced to 3 kDa or less during the degradation process, as a consequence, PCL is completely absorbed *in vivo* in due time (Woodward *et al*., 1985). Its excellent biocompatibility and ability to be absorbed by the body together with its high permeability to many drugs and bioactive compounds make it suitable for the development of non-removable controlled release devices (Woodruff and Hutmacher, 2010).

Many of the materials used as dressings have intrinsic biological activity themselves and may be formulated to also act as controlled release systems of bioactive compounds incorporated in them. Controlled release in specific body areas is important as it minimizes loss and degradation of the active compound and increases its availability in the treated region (Boateng *et al*., 2008).

Bioactive compounds of natural and synthetic origin are likely to be incorporated into wound dressings. Such compounds include antimicrobial agents, compounds with anti-inflammatory activity, growth factors and vitamins, among others (Boateng *et al*., 2008). Plants produce a wide variety of phytochemicals, which have been used by humans for centuries to treat different kinds of diseases. Due to the widespread development and production of well-characterized synthetic bioactive agents, the use of natural products has been left aside for many years (Cragg and Newman, 2013; Newman and Cragg, 2007; Patra, 2012). However, the development of resistance in various bacterial strains to synthetic antibiotics and antimicrobial agents, as well as concerns regarding their efficacy and safety revived interest in the use of natural compounds as alternatives to synthetic ones (Patra, 2012).

An example of a natural compound useful in the treatment of skin lesions is alpha-bisabolol, a monocyclic unsaturated sesquiterpene alcohol found in plant species such as *Matricaria chamomilla*, *Matricaria recutita*, *Salvia runcinata*, *Salvia stenophylla*, *Vanillosmopsis pohlii*, *Vanillosmopsis arborea*, *Myoporum grassifolium* and *Eremanthus erythropappus*. Its content in these species varies from 50% to 90% (Kamatou and Viljoen, 2010). In Brazil, alpha-(-)-bisabolol is mainly extracted from the bark of candeia trees (*Eremanthus erythropappus*), reaching purity levels equal to or above 95% after processing (Clark *et al*., 2011). This compound has a wide range of relevant biological properties, such as antimicrobial, antifungal, antispasmodic, analgesic, antioxidant and anti-inflammatory effects. It is commonly used in topical formulations in dosages from 0.1 to 1% (1 to 10 mg per gram of vehicle) (Kamatou and Viljoen, 2010; Petronilho *et al*., 2012).

Alpha-bisabolol is a lipophilic compound, practically insoluble in water but soluble in ethanol (Kamatou and Viljoen, 2010). It has a molar weight of 222.37 g/mol, density of about 0.93 g/mL and boiling point of 314.5 °C at 1 atm. This compound exists in the form of four diastereoisomers (Figure 1). The most common form is alpha-(-)-bisabolol or levomenol, which is primarily responsible for the overall biological activity of the compound, while the isomer alpha-(+)-bisabolol is rare in nature. The synthetic compound is usually a mixture of alpha-(±)-bisabolol, containing at least 42.5% of alpha-(-)-bisabolol (Kamatou and Viljoen, 2010; Schilcher *et al*., 2005, Clark *et al*., 2011).

So far, no detailed reports were found in the literature regarding the incorporation of alpha-bisabolol either in hydrophilic or in hydrophobic membranes constituted, respectively, of chitosan combined with guar gum and of polycaprolactone. Hence, the aim of this work was to examine the incorporation of alpha-bisabolol in such matrices and its effects on membrane properties, with a focus on their application as controlled release devices for the therapy of skin lesions. Characteristics such as differences in membrane morphology, color, opacity, Fourier transform infrared (FTIR) spectra, alpha-bisabolol incorporation efficiency and release kinetics were analyzed.

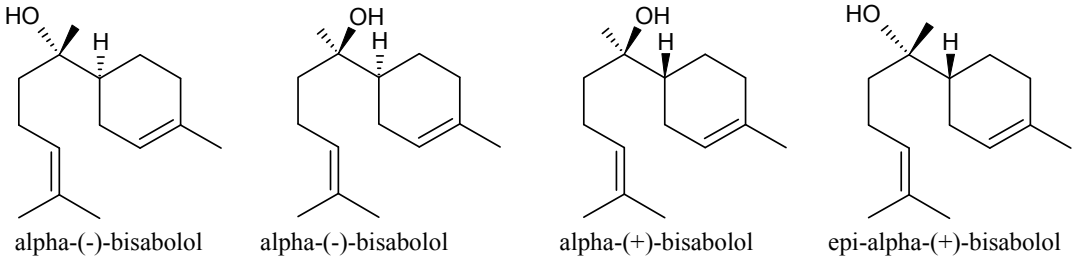

Figure 1: Chemical structure of the four diastereoisomers of alpha-bisabolol. The alpha-(-)-bisabolol is the most common form (adapted from Kamatou and Viljoen, 2010).

MATERIALS AND METHODS

Materials

Membranes were produced using chitosan from shrimp shells (Sigma-Aldrich, C3646, lot number 061M0046V, with a degree of deacetylation of 88%), guar gum (Sigma-Aldrich, G4129, lot number 087K0128), polycaprolactone (Sigma-Aldrich, 440744, lot number MKBJ4388V, with average molar mass of 80 kDa and polydispersity index 1.7), glacial acetic acid, ethanol and chloroform (Synth). Alpha-bisabolol, Albi®, with purity of 96%, was kindly donated by the company Atina (Ativos Naturais). The water used in the tests was distilled and deionized in the Milli-Q® system (Millipore).

Methods

Membranes Preparation and Alpha-Bisabolol Incorporation

Ch-G membranes were prepared according to adaptations of the procedures described by Haupt *et al.* (2006). Solutions of chitosan (aqueous acid solution 2% v/v) and guar gum (aqueous solution) at a concentration of 0.5% (w/v) were used. The volume of each solution was 90 mL and chitosan solution was added at a flow rate of 300 mL/h by the use of a peristaltic pump (Minipuls 3, Gilson) to guar gum solution in a stainless steel jacketed vessel with an internal diameter of 10 cm and a height of 20 cm. The temperature was maintained throughout the process at 25 °C by using a thermostatic bath (214 M2 Quimis). During the addition of the chitosan solution, the system was kept under constant stirring speed of 1000 rpm with the aid of a mechanical stirrer (251 D Quimis) coupled to a marine propeller with 2.5 cm radius. After the addition of chitosan to the guar gum solution was completed, the polymer mixture was deaerated with a vacuum pump (Q-355B2, Quimis) during 120 minutes, transferred to a polystyrene Petri dish of 15 cm diameter and the solvent was evaporated in an oven with air circulation (410D, New Ethics) for 24 hours at 37 °C. The membrane was then washed for 1 minute with 100 mL of 1M NaOH solution 1:1 v/v (water: ethanol) to neutralize the residual acetic acid and then with water (200 mL twice for 30 minutes). Final drying was performed for 24 hours at room temperature.

For the preparation of PCL membranes, polymer pellets were dissolved in 20 mL of chloroform at a concentration of 2% (w/v) under magnetic stirring (Big Squid IKA). The mixture was then deaerated for 10 minutes in a sonicator (3510R-DTH, Bransonic) and transferred to a glass Petri dish of 9 cm diameter. The Petri dish was left at room temperature on a rotatory plate inside a fume hood for 24 hours for complete evaporation of the solvent.

Incorporation of alpha-bisabolol was done by direct addition (DA) of the compound into PCL solution at the proportion of 0.2:1 (w/w) of alpha-bisabolol to polysaccharides. This ratio was chosen to avoid undesired interference on the formation of the polymeric matrix. For Ch-G membranes, alpha-bisabolol was incorporated during membrane swelling resulting from the absorption (AS) of ethanol or hydroethanolic solution (25:75 v/v of water/ethanol) at concentrations varying from 0.075 to 7.5 mg/mL. Samples of 1 cm × 1 cm dimensions previously stored in a desiccator with 22% of moisture content were incubated in the presence of 4 mL of the solution containing the active compound for one hour under mixing at 100 rpm and 25 °C.

Membranes Characterization

Membrane characterization was performed based on procedures described by Bueno and Moraes (2011) and Veiga and Moraes (2011), unless otherwise stated.

Morphology

The morphology of 2 cm x 1 cm samples was evaluated using a scanning electron microscope (LEO 440i, Leica). Samples previously stored in a desiccator for 24 h were fixed on adequate stubs and metalized (mini Sputter coater, SC 7620) by depositing a thin layer of gold (92 Å). For the evaluation of cross section, samples were cryofractured with liquid nitrogen.

Color and Opacity

Color and opacity of the membranes were determined with a colorimeter (ColorQuest II, Hunterlab) operating in the transmittance mode, using the CIELab patterns and the Hunterlab method (Hunter Associates Laboratory, 1997). *Hue* and *Chroma* parameters were calculated using Equations (1) and (2), respectively:

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

$$Chroma = \left[\left(a^*\right)^2 + \left(b^*\right)^2 \right]^{0,5} \quad (2)$$

where a^* and b^* are the color parameters provided by the equipment. The color of the sample was given by the orientation of *Hue* angle in the CIELab diagram (Voss, 1992).

Opacity (Y) of the membranes, in percentage, was calculated by the equipment as a relationship between the opacity of each sample over a black standard (Y_p) and the opacity of each sample over a white standard (Y_b), according to Equation (3).

$$Y = \left(\frac{Y_p}{Y_b} \right) \times 100 \tag{3}$$

Fourier Transform Infrared (FTIR) Spectroscopy

FTIR was performed in order to identify the functional groups present in the samples and evaluate interactions between the polymers in the membranes, as well as to detect possible changes in the structure of the polymeric matrices after incorporating alpha-bisabolol. The data were obtained on a spectrophotometer (Nicolet 6700, ThermoScientific) operating in the attenuated total reflectance (ATR) mode (Smart Omni-Sampler accessory) with wavenumbers ranging from 4000 to 675 cm^{-1}, resolution of 4 cm^{-1} and 32 accumulated scans for reading membrane samples. For powder samples, KBr pellets were used and the range of wavenumbers was adjusted to 4000 to 400 cm^{-1}.

Uptake Capacity and Stability in Ethanol and Hydroethanolic Solution

Uptake capacity of ethanol or hydroethanolic solution (25:75 v/v water/ethanol) was determined using 6 cm x 1 cm membrane samples, in triplicate, with initial mass (M_i) previously determined after storage in a desiccator for 24 hours. Samples were exposed to 10 mL of the tested solution for 1 hour at 25 °C. After this period, the excess of solution was removed with filter paper and samples were weighed (M_f). The uptake capacity of ethanol and hydroethanolic solution, in $g_{solution}/g_{membrane}$, was calculated using Equation (4).

$$U = \frac{M_f - M_i}{M_i} \tag{4}$$

To determine the stability of the material exposed to the mentioned solutions, each sample was immersed for 5 minutes in 20 mL of water for 5 times to remove weakly bonded compounds such as salts and polysaccharides. After that, the sample was dried in an incubator for 24 h at 37 °C, kept in a desiccator for 24 h and then weighed again (M_d). The percentage of mass loss (L) was calculated using Equation (5).

$$L = \frac{M_i - M_d}{M_i} \times 100 \tag{5}$$

Alpha-Bisabolol Incorporation Efficiency

Efficiency of alpha-bisabolol incorporated in the Ch-G membranes by the AS method was determined via quantification of the compound remaining in the ethanol or hydroethanolic solution after the end of the incubation period by spectrophotometry at 208 nm. The same procedure was performed with membranes immersed in solutions without alpha-bisabolol, in order to quantify solvent extractable compounds that were not the test compound and could possibly interfere in absorbance measurements.

The mass of alpha-bisabolol retained in the sample ($M_{c,f}$) was determined by calculating the difference between the mass of compound initially added to the film ($M_{c,i}$) and the mass remaining in the solution. The incorporation efficiency (ε), in percentage, was then calculated using Equation (6).

$$\varepsilon = \frac{M_{C,f}}{M_{C,i}} \times 100 \tag{6}$$

For PCL membranes, the incorporation efficiency by the DA method was also calculated according to Equation (6). Neither evaporation of bioactive compound nor its retention in the Petri dish were observed.

Alpha-Bisabolol Release Kinetics

Alpha-bisabolol release kinetics were evaluated using Ch-G and PCL membrane samples of dimensions equal to 1 cm x 1 cm in triplicate with different alpha-bisabolol/polymer mass ratios.

Samples with initial alpha-bisabolol/polymer mass ratios around 0.2 were weighed and placed in nylon supports at the top of quartz cuvettes containing 3 mL PBS buffer, under mixing at 100 rpm and 37 °C. Preliminary tests were performed to determine the upper limit of alpha-bisabolol concentration in PBS, defined by the concentration above which no alteration was detected in the absorbance of the release solution. To avoid saturation of the PBS solution with alpha-bisabolol, the release medium was replaced at predetermined periods. For the Ch-G membranes with higher alpha-bisabolol/polymer mass ratios, the test was carried out in flasks containing 10 mL of PBS to prevent rapid saturation of the release

medium. Cuvettes and flasks were sealed with parafilm and the incubator environment was saturated with water to minimize loss of the release medium by evaporation. Periodically, the medium was analyzed for alpha-bisabolol concentration by spectrophotometry at 208 nm. For the experiments performed using 3 mL of release medium, the absorbance values were determined by direct measurement in the cuvettes. For Ch-G membranes exposed to 10 mL of PBS, 1 mL aliquots were taken from the bulk solution and, after absorbance measurements, returned to the flasks.

RESULTS AND DISCUSSION

Alpha-Bisabolol Incorporation

Before alpha-bisabolol incorporation, Ch-G and PCL membranes were exposed to ethanol and to a 25:75 v/v water/ethanol solution to determine their uptake capacity and stability in these solvents. The results of these tests, indicated in Table 1, were important for the choice of the most appropriate incorporation method to be used for each type of membrane. PCL samples had a very low uptake capacity of both solutions; therefore, the swelling required for penetration of alpha-bisabolol present in solution and its diffusion through the matrix would not occur. For this reason, the DA method was chosen as the most suitable for incorporating the active compound into PCL membranes, since this approach eliminates the need of matrix swelling for penetration of the compound.

Table 1: Uptake capacity (U) and mass loss (L) in ethanol and water/ethanol solution (25:75 v/v) for membranes exposed to these solvents for one hour.

Sample	Solution	U ($g_{solution}/g_{membrane}$)	L (%)
Ch-G	ethanol	0.35 ± 0.09^a	5.26 ± 0.20^a
	water/ethanol	1.60 ± 0.02^b	7.97 ± 0.41^b
PCL	ethanol	0.07 ± 0.02^c	2.46 ± 0.27^c
	water/ethanol	0.09 ± 0.01^c	2.79 ± 0.31^c

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

Ch-G membranes showed higher uptake capacity of the tested solutions than PCL, and the uptake of the hydroethanolic solution was significantly higher than that observed for ethanol. The swelling of the matrix structure in the hydroethanolic solution was probably promoted by the presence of water molecules, which enabled the separation of the polysaccharide chains, consequently allowing the entry of a great amount of the incorporation solution. The incorporation of alpha-bisabolol through the AS method is more suitable in this case, given that the higher degree of swelling observed in these matrices would allow enhanced penetration of the active compound. If alpha-bisabolol incorporation into Ch-G membranes by direct addition was employed, its limited affinity for the polysaccharide matrix due to their different hydrophilicity character would prevent effective retention of the active compound in the membrane, especially after the washing step.

Despite the increased uptake of the hydroethanolic solution presented by both types of membranes, mass loss in this solution, as well as in ethanol, was not significant, indicating that the two formulations are stable in the tested solvents.

The incorporation of alpha-bisabolol in PCL films by the direct addition method at the initial mass ratio of 200 mg per gram of polysaccharides resulted in an average retention efficiency around 100%. Alpha-bisabolol losses attributed to carrying during chloroform evaporation, to active compound volatilization or even to retention on the Petri dish surfaces were determined to be negligible.

Incorporation efficiencies of alpha-bisabolol in Ch-G membranes through absorption during swelling are shown in Table 2. For Ch-G samples immersed in ethanol, in all cases, low alpha-bisabolol incorporation efficiencies were observed, ranging from 7% to 9% for incorporation solutions with higher concentration (with no significant statistical difference among them) and equal to 0.3% when the solution with lower alpha-bisabolol concentration was used. This result reflects those obtained for the uptake of ethanol (Table 1), which showed the low capacity of the Ch-G membranes to swell in this solvent and, after drying, to retain compounds dissolved therein. Despite the low affinity of the matrix for the solvent and for the active compound itself, the incorporation efficiency obtained by this method was still satisfactory regarding final dosage requirements.

When using water as co-solvent in the incorporation solution, the incorporation efficiency and the corresponding amount of compound retained in the matrices reached values up to twice greater than those observed when using only ethanol (Table 2). This result is due to the fact that the samples' uptake of the hydroethanolic solution is significantly higher than its uptake of ethanol (Table 1). When using the hydroethanolic incorporation solution at a concentration of 1.2 mg/mL of alpha-bisabolol, the amount of compound retained in the membrane (224.90 ± 21.45 mg/g) was equivalent to that obtained for the PCL membrane (200 mg/g).

Table 2: Incorporation efficiencies of alpha-bisabolol to Ch-G membranes by immersion in ethanol and water/ethanol solution (25:75 v/v) (AS method).

Solvent	Initial concentration of alpha-bisabolol (mg/mL)	Alpha-bisabolol added (mg/g)	Alpha-bisabolol retained (mg/g)	Incorporation efficiency (%)
ethanol	0.075	73.2	0.2 ± 0.1^{a}	0.3 ± 0.1^{a}
	1.2	1116.3	96.1 ± 27.7^{b}	9.2 ± 2.7^{b}
	3.0	2857.1	236.6 ± 10.7^{c}	7.3 ± 0.1^{b}
	7.5	7500.0	509.1 ± 28.4^{d}	6.6 ± 0.2^{b}
water/ethanol	0.075	74.1	10.3 ± 1.9^{e}	15.1 ± 2.1^{c}
	1.2	1348.3	224.9 ± 21.5^{e}	16.7 ± 1.3^{c}
	3.0	3000.0	537.3 ± 41.9^{d}	18.1 ± 1.5^{c}
	7.5	6122.5	1076.5 ± 22.1^{f}	17.6 ± 1.2^{c}

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

Even though the incorporation efficiency of alpha-bisabolol in the Ch-G membranes by the AS method was not high, the advantage of this method is that a great amount of the compound may be retained in the membranes, varying with the initial concentration of the incorporation solution used. The remaining solution contains, at the end of the process, a large fraction of the alpha-bisabolol initially solubilized therein, but it can still be reused in new incorporation batches. This strategy would then increase the overall efficiency of the process and the feasibility of this method. Alternatively, it would also be possible to use a technique for the production of polymeric nanocapsules or dense nanoparticles containing alpha-bisabolol, following their incorporation into Ch-G membranes as an attempt to increase the incorporation efficiency of the active agent into the matrices. However, the approach chosen in the present work can be considered to be more attractive because it is technically simpler and less costly.

The PCL membranes incorporating alpha-bisabolol by the DA method and the Ch-G samples retaining the greatest amount of the bioactive compound obtained when using the AS method, i.e., the formulation prepared with alpha-bisabolol solubilized at 7.5 mg/mL in the hydroethanolic solution, were further characterized as follows.

Visual Aspect and Morphology

The typical appearance of Ch-G membranes in which the active compound was incorporated by the AS method (Figure 2B) did not change in comparison to those in which the compound had not been incorporated (Figure 2A), although the amount of alpha-bisabolol retained in the first formulation was highly significant (*ca.* 1 g/g). PCL membranes containing alpha-bisabolol obtained by using the DA method (Figure 2D) were visually more opaque than those in which this compound was not incorporated (Figure 2C).

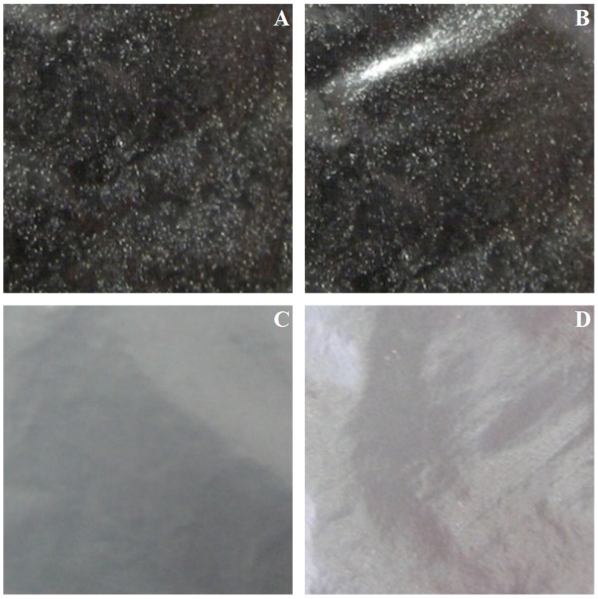

Figure 2: Visual aspects of Ch-G films without alpha-bisabolol (A) and in which the compound was incorporated by the AS method with water/ethanol solution (25:75 v/v) at a concentration of 7.5 mg/mL (B); and PCL films without alpha-bisabolol (C) and in which the compound was incorporated by the DA method using the proportion of 20% (m/m) (D).

In accordance with the visual analysis results, color and opacity parameters of PCL membranes without alpha-bisabolol indicate that this formulation presents greater opacity than the Ch-G formulation also free of the compound (Table 3). *Hue* values show that Ch-G membranes are greenish-yellow while PCL membranes are yellowish-green. However, the *Chroma* parameter indicates that the intensity of

these colors is very low. The results obtained in this work for the color and opacity of Ch-G membranes differ from those reported by Rao *et al.* (2010), who obtained chitosan and guar gum membranes with lower opacity (15.91 ± 0.52%) and yellowish-green color with greater intensity (*Hue* = -57.76 and *Chroma* = 6.79). This difference may be attributed to the different method of preparation of the membranes used by Rao *et al.* (2010), which did not include the neutralization step. In the present work, it was precisely at this step that the color of Ch-G membranes changed to a noticeably yellowish. The existence of the neutralization step in the process of membrane production is important because it avoids potential dissolution of chitosan in aqueous solutions such as body fluids and, moreover, eliminates the irritating effect that the membrane could present when in contact with the lesion due to residual acetic acid. The difference of opacity may be related to the fact that the membranes obtained by Rao *et al.* (2010) had a thickness about 2.5 times lower than those described in the present study (data not shown). Greater film thickness results in higher difficulty of light to pass through it, since the amount of material present therein is increased.

The data in Table 3 also confirm the results regarding the visual analysis of PCL samples containing alpha-bisabolol incorporated by the DA method, since the opacity of the material increased after incorporation of the compound. This increase may have been a result of morphological changes in the membrane due to the introduction of the active compound, which made it less uniform and promoted, as a consequence, more intense light scattering. These changes in membrane morphology are confirmed in Figures 3G and 3H. Also, for these membranes, the value of *Hue* angle underwent little change, but the color of the samples was not altered when compared with that of membranes without the active compound.

For the Ch-G formulation in which alpha-bisabolol was incorporated by the AS method the color and opacity parameters were not significantly different when compared to those of membranes without the compound (Table 3).

Martins *et al*. (2012) and Peng and Li (2014) incorporated lipophilic natural compounds with antioxidant (alpha-tocopherol) and antimicrobial properties (essential oils of lemon, cinnamon and thyme) into hydrophilic chitosan membranes. In contrast to what was observed in the present work for Ch-G membranes, these authors observed an increase in the opacity of the films after the introduction of the active compounds into the matrices. However, the incorporation method used by the authors was the DA strategy that, when used in the present work for introducing alpha-bisabolol into the hydrophobic PCL matrix, also resulted in increased opacity of the membrane. Peng and Li (2014) attributed the increase in opacity to morphological changes resulting from the introduction of the essential oils in the membrane structure, while Martins *et al.* (2012) suggested that alpha-tocopherol addition decreased the transparency of chitosan.

Figures 3A and 3B show that Ch-G samples without alpha-bisabolol have irregular surfaces, but dense and continuous structure. For Ch-G membranes in which alpha-bisabolol was incorporated by immersion in hydroethanolic solution (Figures 3C and 3D), it is possible to observe regions containing structures similar to bubbles on their surface in which the compound was probably located. The cross-sectional analysis also revealed a dense structure, without visible pores.

PCL membranes without alpha-bisabolol (Figures 3E and 3F) also showed bubbles on their surface, similarly to what was reported by Tang *et al.* (2004), who obtained PCL membranes using chloroform as solvent. These bubbles may have been generated on the film surface during membrane casting, due to the rapid evaporation of the solvent, which possibly caused the formation of a thin film that hampered the diffusion of the remaining chloroform out of the matrix, resulting in the accumulation of the solvent under the film. The micrographs of the cross section reveal the formation of multiple lamellae, which corroborates this hypothesis.

Table 3: Color and opacity parameters of membranes without alpha-bisabolol (w/o) and in which alpha-bisabolol was incorporated by the DA method and by immersion in water/ethanol solution (25:75 v/v) (AS method).

Formulation	Ch-G		PCL	
Incorporation method	w/o	AS	w/o	DA
Alpha-bisabolol concentration	0	7.5 mg/mL	0	20%
Opacity (%)	24.83 ± 0.49[a]	24.95 ± 0.78[a]	75.00 ± 2.17[b]	81.63 ± 0.32[c]
Hue	-83.12 ± 0.43[a]	-86.06 ± 0.47[a]	-71.98 ± 0.54[b]	-66.95 ± 0.82[c]
Chroma	1.34 ± 0.01[a]	1.31 ± 0.05[a]	1.90 ± 0.02[b]	1.75 ± 0.04[c]

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

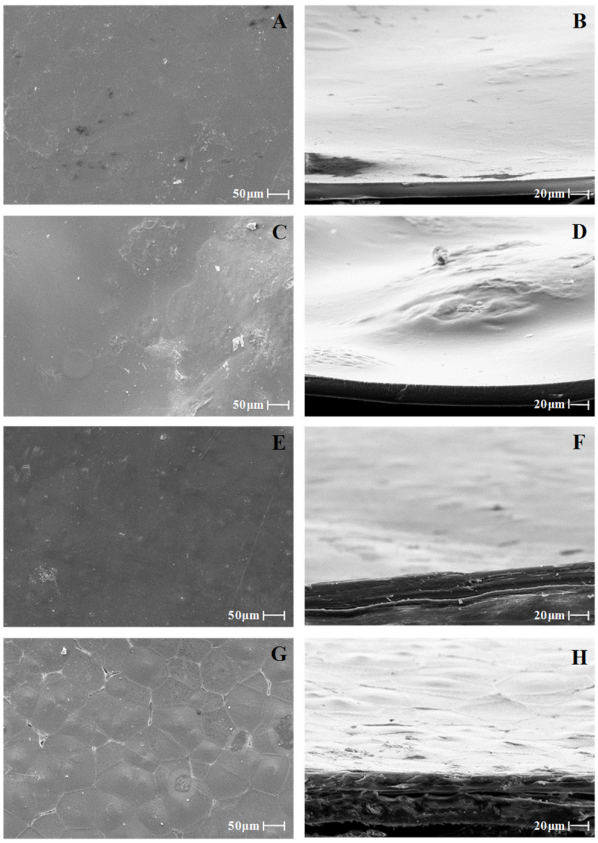

Figure 3: Surface (left) and cross-sectional (right) morphology of Ch-G films without alpha-bisabolol (A, B), in which the compound was incorporated by the AS method with water/ethanol solution (25:75 v/v) at a concentration of 7.5 mg/mL of alpha-bisabolol (C, D); and PCL films without alpha-bisabolol (E, F) and in which the compound was incorporated by the DA method using the proportion of 20% (w/w) (G, H).

PCL membranes containing alpha-bisabolol (Figures 3G and 3H) have interconnected blocks resulting from the exclusion of the active compound during the formation of the polymeric matrix. Its cross-sectional image reveals that alpha-bisabolol is driven to pockets present within the polymeric matrix, where the active compound is probably placed. Yeh *et al.* (2011) produced PCL films with different molar masses using acetone as solvent, a compound in which the polymer is poorly soluble, and reported surface morphology results similar to those obtained in this work. Drawing an analogy between the behavior observed in both studies, it is possible to assume that the polymer matrix is formed by excluding the compound in which its solubility is low, in this case alpha-bisabolol, leading to the formation of the observed polygonal blocks.

FTIR Spectra

Comparative FTIR spectra of the polymeric membranes, the isolated polymers used in their production and alpha-bisabolol are shown in Figures 4 and 5.

The spectra of chitosan and guar gum (Figure 4) show a band between 3600 and 3000 cm^{-1}, related to stretching of hydroxyl groups present in both polymers. Another band is observed between 3000 and 2800 cm^{-1} related to axial vibration of C–H bonds. Several small peaks characteristic of polysaccharides are observed around 1000 cm^{-1}, which are related to C–O, C–C, and C–O–C bonds (Smitha *et al*., 2005; Mudgil *et al*., 2012; Shahid *et al*., 2013).

In addition, the chitosan spectrum also shows peaks at 1643 cm^{-1} and 1581 cm^{-1}, related to the C=O bond of amide I groups still acetylated and to amino groups, respectively (Li *et al*., 2005; Popa *et al*., 2010; Smitha *et al*., 2005), and a further peak at 1380 cm^{-1}, attributed to deformation of $–CH_2$ groups (Li *et al*., 2005; Popa *et al*., 2010; Smitha *et al*., 2005). In the guar gum spectra, the peak at 1650 cm^{-1} can be related to the bending vibration of –OH groups (Wang and Wang, 2009), and the peak at 875 cm^{-1}, which is characteristic of this polymer, refers to galactose and mannose groups (Mudgil *et al*., 2012; Manikoth *et al*., 2012).

The Ch-G membrane spectrum (Figure 4) shows overlapping of peaks related to chitosan amides and guar gum hydroxyls. The resulting peak at 1650cm^{-1} has lower intensity, indicating interaction between the two polymers.

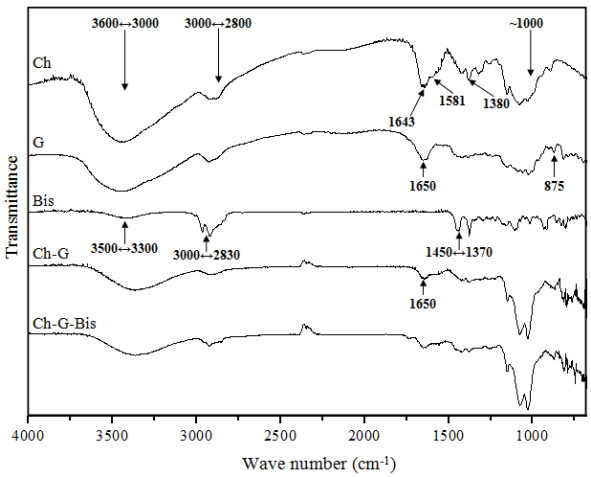

Figure 4: FTIR spectra obtained for Ch-G membranes with or without alpha-bisabolol incorporated by the AS method with a solution of water and ethanol (25:75 v/v) at a concentration of 7.5 mg/mL. The spectra of the isolated components are also shown.

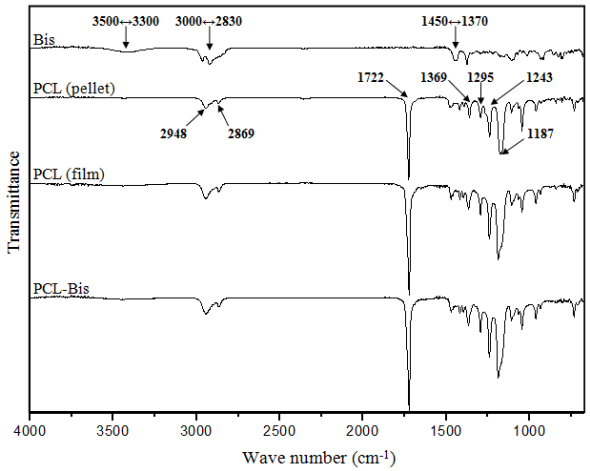

Figure 5: FTIR spectra obtained for PCL membranes with or without alpha-bisabolol incorporated by the DA method. The spectra of the isolated components are also shown.

The alpha-bisabolol spectrum (Figures 4 and 5) presents regions with characteristic peaks, such as the band between 3500 and 3300 cm^{-1}, related to stretching of –OH, the peaks between 3000 and 2830 cm^{-1}, related to axial deformation of C–H bonds and the peaks between 1450 and 1370 cm^{-1}, attributed to the angular deformations of the C–H bonds (Silva, 2009).

The spectrum of the membrane containing alpha-bisabolol is similar to that of the of the membrane without the compound (Figure 4), so it is not possible to confirm, by this technique, the presence of the added compound in the polymer matrix.

The polycaprolactone pellet spectrum (Figure 5) shows peaks at 2948 and 2869 cm^{-1}, related to asymmetrical and symmetrical stretching of $–CH_2$ groups. The intense peak at 1722 cm^{-1} refers to stretching of the carbonyl groups (–C=O), while peaks at 1187 and 1243 cm^{-1} are related to vibrations of ester groups. The peak at 1295 cm^{-1} corresponds to stretching of C–O and C–C bonds and the peak at1369 cm^{-1} is related to vibrations of $–CH_2$ groups (Khatiwala *et al*., 2008; Suganya *et al*., 2011; Martínez-Abad *et al*., 2013).

The PCL membrane spectrum did not change when compared to that of the polymer in pellet form. Similarly, the spectrum of the polymeric membrane containing alpha-bisabolol has the same profile as that observed for the compound-free membrane (Figure 5). This behavior may be attributed to the overlapping of alpha-bisabolol and PCL peaks, which makes difficult the identification of these peaks in the spectrum of the film containing the compound. In addition, another explanation would be that the observed behavior can indicate no clear interaction between the incorporated compound and the polymeric matrix. In fact, the micrographs of these membranes showed exclusion of alpha-bisabolol during formation of the matrix (Figures 3G and 3H).

Alpha-Bisabolol Release Kinetics

The evaluation of alpha-bisabolol release kinetics in a medium that simulates physiological conditions (PBS, pH 7.4, at 37 °C) was performed to verify the ability of the two types of membrane formulations to release the dose of compound required for topical use (1 to 10 mg/g). In this part of the study, Ch-G and PCL membranes with similar alpha-bisabolol/polymer mass ratios were used (225 and 200 mg/g, respectively). For that, the Ch-G formulation containing alpha-bisabolol was obtained by exposure to a hydroalcoholic solution at a concentration of 1.2 mg/mL (AS method), while the PCL membranes were prepared as previously described, incorporating the compound at an initial mass ratio of 0.2:1 alpha-bisabolol/PCL (AD method).

Figure 6 shows alpha-bisabolol release kinetics from the PCL membranes. The release medium was changed at 15 and 48 hours. A two-stage release profile was observed: relatively faster release occurred until 15 hours, followed by a slow release stage. Alpha-bisabolol deposited on the surface of the membrane and weakly associated to the matrix was probably released during the first stage, while the fraction of the compound located in the inner layers of the membrane, or in the pockets (as seen in Figures 3G and 3H) was released in a slow manner due to diffusion through the matrix. In addition to the limitations imposed by the diffusion process during the slow release period, alpha-bisabolol partitioning between the hydrophobic membrane and the hydrophilic release medium should be also considered. Due to its hydrophobic character, alpha-bisabolol tends to accumulate preferentially in the membrane and therefore its release to the medium occurs gradually and slowly. At 120 hours, a total of about 6% of the compound, equivalent to 12 $mg/g_{membrane}$, was released.

Figure 7 shows alpha-bisabolol release kinetics from the Ch-G membranes. In this case, the release medium had to be changed with higher frequency in the beginning of the study to avoid its saturation with free alpha-bisabolol, at 1, 2, 5 and 48 hours. A release profile similar to that of alpha-bisabolol incorporated in PCL membranes was observed, with a faster release period occurring until 15 hours. Proba-

bly, in a way similar to what was observed for PCL membranes, initially the fraction of alpha-bisabolol deposited on the surface of Ch-G membranes was more rapidly released. Also, in this case, the membranes swell in the presence of PBS, what contributes to the burst release kinetic behavior noted.

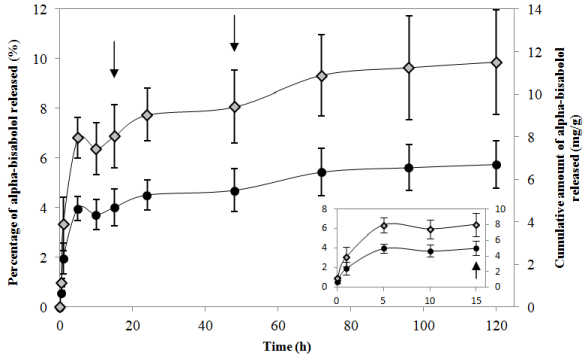

Figure 6: Release of alpha-bisabolol incorporated into PCL membranes by the DA method in terms of mass of compound per mass of membrane (—◇—) and release percentage (—●—). The arrows indicate the moments in which the release medium was changed.

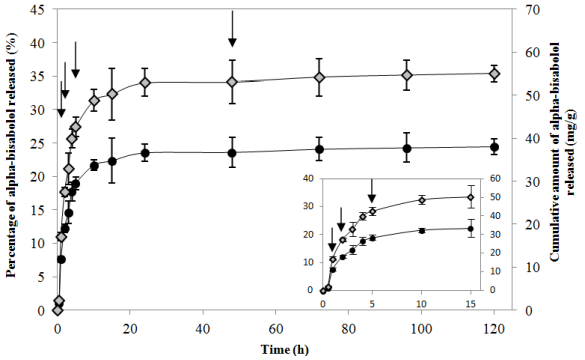

Figure 7: Release of alpha-bisabolol incorporated into Ch-G membranes by the AS method with the compound dissolved at 1.2 mg/mL in hydroethanolic solution. Data are shown in terms of mass of compound per mass of membrane (—◇—) and release percentage (—●—). The arrows indicate the moments in which the release medium was changed.

While the PCL matrix has high affinity for alpha-bisabolol, the hydrophilic Ch-G membrane presents only a limited tendency to interact with it, potentially through weak hydrophobic groups such as $–CH_2$ and $–CH_3$. However, given that the release medium is very hydrophilic, alpha-bisabolol partitioning between the hydrated Ch-G membrane and the aqueous phase is likely to occur to a certain degree, resulting in a significant tendency of the compound to remain embedded in the membrane. Since the interaction of the compound with the Ch-G membrane would not be as intense as that observed for the hydrophobic PCL membrane, higher release of alpha-bisabolol from Ch-G membranes would be expected, as effectively noticed, reaching a cumulative release fraction of 24%, equivalent to 55 mg/$g_{membrane}$ in 120 hours.

Figure 8 shows release kinetic data of Ch-G membranes in which alpha-bisabolol was incorporated by the AS method using hydroethanolic solution at a concentration of the bioactive compound equal to 7.5 mg/mL. The release medium was changed every 1 hour until 4 hours. The rapid alpha-bisabolol release step occurred within the first 10 hours, and around 30% of the compound, equivalent to 300 mg/$g_{membrane}$, was released in 48 hours. The maximum percentage of alpha-bisabolol released was very similar for both types of Ch-G membranes, as well as their release profile with time. Nevertheless, in this case, the cumulative amount of active compound released was about 5.5 times greater than that determined for Ch-G samples containing less alpha-bisabolol.

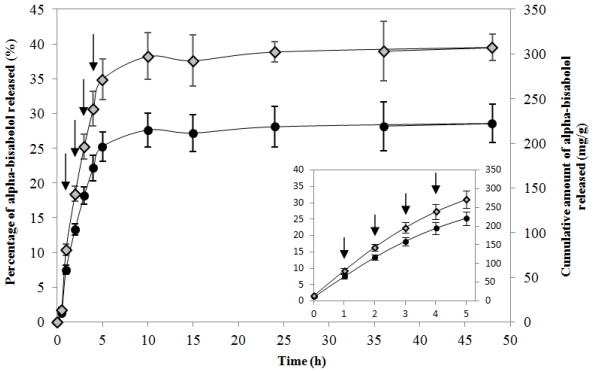

Figure 8: Release of alpha-bisabolol incorporated into Ch-G membranes by the AS method with the compound dissolved at 7.5 mg/mL in hydroethanolic solution. Data are shown in terms of mass of compound per mass of membrane (—◇—) and release percentage (—●—). The arrows indicate the moments in which the release medium was changed.

According to Madhavan (1999), who summarized several data from the literature regarding the safety assessment of alpha-bisabolol, its acute oral values of LD_{50} (dose likely to cause death to 50% of a standard population) are 15.1 mL/kg in mice, and 14.9 and 15.6 mL/kg in male and female rats, respectively, exceeding 5000 mg/kg (Tisserand and Young, 2014) to rats, and not being teratogenic to rats. Also according to Madhavan (1999), alpha-bisabolol was negative in a dermal photosensitization study with guinea pigs and, in addition, a 28-day dermal toxicity

study performed through the application of a solution containing 4% of alpha-bisabolol with 87.5% purity on rats showed that the no-observable-adverse-effect level (NOAEL) was equal to 200 mg/kg/day. More recent data show that alpha-bisabolol is not irritant to rabbits when topically administered at 1% over an area of one square inch (Maurya *et al*., 2014).

Based on the evidence described above, and on the values of the ratio between the mass of the membranes per area (around 6 and 5 mg/cm^2 for PCL and Ch-G membranes, respectively), it is possible to assume that even the cumulative dose of 300 $mg_{alpha\text{-}bisabolol}/g_{membrane}$ administered through the skin would be safe to treat skin lesions. However, potentially the local concentration of alpha-bisabolol at the wound site would be lower due to removal by blood circulation and alpha-bisabolol permeation in the tissue surrounding the lesion. Besides that, swelling of Ch-G membranes might lead to a decrease in the ratio of alpha-bisabolol released per gram of membrane. Ch-G membranes can absorb approximately 100% of their dry weights when immersed in PBS medium for 24 hours (data not shown). Since the degree of swelling may vary with time, variations in the ratio of alpha-bisabolol released per gram of membrane would be expected.

Regarding the burst release observed for all formulations, this effect may have either negative or positive consequences (Huang and Brazel, 2001). High drug concentrations made available quickly from controlled release devices are generally not often desired and may be associated with local or systemic toxicity. However, the burst behavior may be interesting for certain applications in which rapid release or high initial rates of delivery are required. Skin wound treatment is an example of this application type, as an initial burst of the active agent, below the toxic concentration, would provide immediate relief to the patient followed by prolonged release to promote gradual healing. Thus, in the present work, it is reasonable to assume that the burst release would probably be an attractive characteristic of the developed systems, since the cumulative dose of alpha-bisabolol released was considered safe for wound treatment applications. It should be pointed out, though, that even if burst release is desirable, it cannot be controlled (Huang and Brazel, 2001). Thereby, alternatives to avoid this behavior could be used, such as the encapsulation of the active compound in slow release particles followed by their incorporation into the membranes, as well as membrane surface coating with another polymer, which would increase the resistance to diffusion (Allison, 2008). Moreover, in a practical application, the previous surface extraction method could be used. This method comprises the extraction of the drug for a short period of time *in vitro*, by immerging the dressing in appropriate release media, before using it in an *in vivo* application (Huang and Brazel, 2001).

CONCLUSIONS

Alpha-bisabolol incorporation into PCL membranes made them more opaque, but did not change the Ch-G membranes visual aspect. Thus, regarding this property, the last formulation would be more appropriate as a wound dressing since it is more translucent and allows better visual monitoring of the wound site.

For both formulations, alpha-bisabolol release showed an initial burst followed by a long slow release period. A greater amount of alpha-bisabolol was released from Ch-G when compared to PCL membranes. Both formulations were able to release the dosage of the compound commonly used in topical formulations (1 to 10 $mg/g_{vehicle}$), and the final cumulative doses reached up to 300 mg of alpha-bisabolol per gram of dry membrane. Even though the amount of alpha-bisabolol released was higher than the common topical dose, the use of this compound for the treatment of skin lesions may be considered to be safe.

The slow release may be advantageous since the dressings obtained could be used as reservoirs of the active compound for long periods, requiring less frequent changes and thus providing more comfort to the patient. Because a significant fraction of alpha-bisabolol remains in the matrices and since the active compound has antimicrobial activity, the membranes could also act as barriers against the penetration of microorganisms to open lesions.

When released to the wound site, alpha-bisabolol, which is also an antioxidant and anti-inflammatory agent, would reduce the incidence of infection and inflammation. Considering the overall results, the use of Ch-G membranes would be more appropriate for lesions in the early stages of recovery, while PCL membranes would be suitable for the treatment of lesions in late stages of healing, since the occurrence of infections and inflammations in this last condition is reduced. Furthermore, late stage healing wounds are generally low exuding, which also makes the application of PCL membranes more appropriate in this case, as they are hydrophobic and thus cannot absorb large amounts of exudate.

ACKNOWLEDGEMENTS

The authors would like to acknowledge the support of this research from *Coordenação de Aperfeiçoamento de Pessoal de Nível Superior* (CAPES, Brazil), *Conselho Nacional de Desenvolvimento Científico e Tecnológico* (CNPq, Brazil), to the *Laboratório de Recursos Analíticos e de Calibração* (LRAC/FEQ/UNICAMP, Brazil) and to Atina (*Ativos Naturais*, Pouso Alegre, MG, Brazil).

REFERENCES

Agrawal, P., Soni, S., Mittal, G. and Bhatnagar, A., Role of polymeric biomaterials as wound healing agents. International Journal of Lower Extremity Wounds, v. 13, p. 180-190 (2014).

Allison, S. D., Analysis of initial burst in PLGA microparticles. Expert Opinion on Drug Delivery, v. 5, p. 615-628 (2008).

Berger, J., Reist, M., Mayer, J. M., Felt, O. and Gurny, R., Structure and interactions in chitosan hydrogels formed by complexation or aggregation for biomedical applications. European Journal of Pharmaceutics and Biopharmaceutics, v. 57, p. 35-52 (2004).

Bhardwaj, N. and Kundu, S. C., Electrospinning: A fascinating fiber fabrication technique. Biotechnology Advances, v. 28, p. 325-347 (2010).

Boateng, J. S., Matthews, K. H., Stevens, H. N. E. and Eccleston G. M., Wound healing dressings and drug delivery systems - a review. Journal of Pharmaceutical Sciences, v. 97, p. 2892-2923 (2008).

Bower, K. A., Mulder, G. D., Reineke, A. and Guide, S. V., Dermatologic Conditions and Symptom Control. In: Wolfe, J., Hinds, P., Sourkes, B. Textbook of Interdisciplinary Pediatric Palliative Care. Chapter 35, Elsevier Health Sciences, (2011).

Bueno, C. Z. and Moraes, A. M., Development of porous lamellar chitosan-alginate membranes: Effect of different surfactants on biomaterial properties. Journal of Applied Polymer Science, v. 122, p. 624-631 (2011).

Cheung, H. Y., Lau, K., Lu, T. and Hui, D., A critical review on polymer-based bio-engineered materials for scaffold development. Composites, Part B, v. 38, p. 291-300 (2007).

Clark, A., Khweiss, N., Salazar, L. and Verdadero, L., Promoting sustainability in the value chain of natural bisabolol, a Brazilian rainforest product. School of International and Public Affairs, SIPA at Columbia University (2011).

Coviello, T., Matricardi, P., Marianecci, C. and Alhaique, F., Polysaccharide hydrogels for modified release formulation. Journal of Controlled Release, v. 119, p. 5-24 (2007).

Cragg, G. M. and Newman, D. J., Natural products: A continuing source of novel drug leads. Biochimica et Biophysica Acta, v. 1830, p. 3670-3695 (2013).

Croisier, F. and Jérôme, C., Chitosan-based biomaterials for tissue engineering. European Polymer Journal, v. 49, p. 780-792 (2013).

Franco, D. and Gonçalves, L. F., Feridas cutâneas: A escolha do curativo adequado. Revista do Colégio Brasileiro de Cirurgiões, v. 35, p. 203-206 (2008).

Gliko-Kabir, I., Yagen, B., Baluom, M. and Rubinstein, A., Phosphated crosslinked guar for colon-specific drug delivery II. *In vitro* and *in vivo* evaluation in the rat. Journal of Controlled Release, v. 63, p. 129-134 (2000).

Goycoolea, F. M., Arguelles-Monal, W., Peniche, C. and Higuera-Ciapara, I., Chitin and chitosan. Novel Macromolecules in Food Systems, p. 265-308 (2000).

Haupt, S., Zioni, T., Gati, I., Kleinstern, J. and Rubinstein, A., Luminal delivery and dosing considerations of local celecoxib administration to colorectal cancer. European Journal of Pharmaceutical Sciences, v. 28, p. 204-211 (2006).

Hess, C. T., Wound Care. 5th Ed., Lippincott William and Wilkins (2005).

Huang, S. and Fu, X., Naturally derived materials-based cell and drug delivery systems in skin regeneration. Journal of Controlled Release, v. 142, p. 149-159 (2010).

Huang, X. and Brazel, C. S., On the importance and mechanisms of burst release in matrix-controlled drug delivery systems. Journal of Controlled Release, v. 73, p. 121-136 (2001).

Hunter Associates Laboratory, Universal Software, Version 3.2, Reston (1997).

Kamatou, G. P. P. and Viljoen, A. M., A review of the application and pharmacological properties of alpha-bisabolol and alpha-bisabolol-rich oils. Journal of the American Oil Chemists' Society, v. 87, p. 1-7 (2010).

Kawamura, Y., Guar gum, chemical technical assessment. 69th Joint FAO/WHO Expert Committee on Food Additives, p. 1-4 (2008).

Khatiwala, V. K., Shekhar, N., Aggarwal, S. and Mandal, U. K., Biodegradation of poly(*e*-caprolactone) (PCL) film by *Alcaligenes faecalis*. Journal of Polymers and the Environment, v. 16, p. 61-67 (2008).

Kumar, M. N. V. R., A review of chitin and chitosan

applications. Reactive & Functional Polymers, v. 46, p. 1-27 (2000).

Li, Z., Ramay, H. R., Hauch, K. D., Xiao, D. and Zhang, M., Chitosan-alginate hybrid scaffolds for bone tissue engineering. Biomaterials, v. 26, p. 3919-3928 (2005).

Liu, X., Ma, L., Mao, Z. and Gao, C., Chitosan-Based Biomaterials for Tissue Repair and Regeneration. Chitosan for Biomaterials II. In: Jayakumar, R., Prabaharan, M., Muzzarelli, (Eds.), Advances in Polymer Science. Heidelberg, Springer Berlin, p. 81-127 (2011).

Manikoth, R., Kanungo, I., Fathima, N. N. and Rao, J. R., Dielectric behaviour and pore size distribution of collagen-guar gum composites: Effect of guar gum. Carbohydrate Polymers, v. 88, p. 628-637 (2012).

Martínez-Abad, A., Sánchez, G., Fuster, V., Lagaron, J. M. and Ocio, M. J., Antibacterial performance of solvent cast polycaprolactone (PCL) films containing essential oils. Food Control, v. 34, p. 214-220 (2013).

Martins, J. T., Cerqueira, M. A. and Vicente, A. A., Influence of α-tocopherol on physicochemical properties of chitosan-based films. Food Hydrocolloids, v. 27, p. 220-227 (2012).

Maurya, A. K., Singh, M., Dubey, V., Srivastava, S., Luqman, S. and Bawankule, D. U., Alpha-(-)-bisabolol reduces pro-inflammatory cytokine production and ameliorates skin inflammation. Current Pharmaceutical Biotechnology, v. 15, p. 173-181 (2014).

Middleton, J. C. and Tipton, A. J., Synthetic biodegradable polymers as orthopedic devices. Biomaterials, v. 21, p. 2335-2346 (2000).

Mudgil, D., Barak, S. and Khatkar, B. S., Effect of enzymatic depolymerization on physicochemical and rheological properties of guar gum. Carbohydrate Polymers, v. 90, p. 224-228 (2012).

Mulder, M., Small, N., Botma, Y, Ziady, L. and Mackenzie, J., Basic Principles of Wound Care. Maskew Miller Longman (2002).

Newman, D. J and Cragg, G. M., Natural products as sources of new drugs over the last 25 years. Journal of Natural Products, v. 70, p. 461-477 (2007).

Patra, A. K., Dietary Phytochemicals and Microbes. Springer Science and Business Media, (2012).

Paul, W. and Sharma, C. P., Chitosan and alginate wound dressings: A short review. Trends in Biomaterials & Artificial Organs, v. 18, p. 18-23 (2004).

Peng, Y. and Li, Y., Combined effects of two kinds of essential oils on physical, mechanical and structural properties of chitosan films. Food Hydrocolloids, v. 36, p. 287-293 (2014).

Petronilho, S., Maraschin, M., Coimbra, M. A. and Rocha, S. M., *In vitro* and *in vivo* studies of natural products: A challenge for their valuation. The case study of chamomile (*Matricaria recutita L.*). Industrial Crops and Products, v. 40, p. 1-12 (2012).

Pitt, C. G., Poly-ε-Caprolactone and its Copolymers. In: Chasin, M., Langer, R., (Eds.), Biodegradable Polymers as Drug Delivery Systems. New York: Marcel Dekker, p. 71-120 (1990).

Popa, N., Novac, O., Profire, L., Lupusoru, C. E. and Popa, M. I., Hydrogels based on chitosan-xanthan for controlled release of theophylline. Journal of Materials Science, v. 21, p. 1241-1248 (2010).

Prabaharan, M., Prospective of guar gum and its derivatives as controlled drug delivery systems. International Journal of Biological Macromolecules, v. 49, p. 117-124 (2011).

Prabhanjan, H., Gharia, M. M. and Srivastava, H. C., Guar gum derivatives. Part I: preparation and properties. Carbohydrate Polymers, v. 11, p. 279-292 (1989).

Randhawa, R., Bassi, P. and Kaur, G., *In vitro*, *in vivo* evaluation of inter polymer complexes between carboxymethyl fenugreek gum and chitosan or carboxymethyl guar gum and chitosan for colon delivery of tamoxifen. Asian Pacific Journal of Tropical Disease, p. 202-207 (2012).

Rao, M. S., Kanatt, S. R., Chawla, S. P. and Sharma, A., Chitosan and guar gum composite films: Preparation, physical, mechanical and antimicrobial properties. Carbohydrate Polymers, v. 82, p. 1243-1247 (2010).

Rinaudo, M., Chitin and chitosan: Properties and applications. Progress in Polymer Science, v. 31, p. 603-632 (2006).

Schilcher, H., Imming, P. and Goeters, S., Active chemical constituents of *Matricaria chamomilla L. syn. Chamomilla recutita* (*L.*) *Rauschert*. In: Chamomile Industrial Profiles, Chapter 4, CRC Press (2005).

Sezer, A. D. and Cevher, E., Biopolymers as Wound Healing Materials -Challenges and New Strategies. In: Pignatello, R. Biomaterials Application for Nanomedicine. Chapter 19, In Tech (2011).

Shahid, M., Bukhari, S. A., Gul, Y., Munir, H., Anjum, F., Zuber, M., Jamil, T. and Zia, K. M., Graft polymerization of guar gum with acryl amide irradiated by microwaves for colonic drug delivery. International Journal of Biological Macromolecules, v. 62, p. 172-179 (2013).

Silva, A. P., Síntese e avaliação da atividade antitumoral de tiossemicarbazonas derivadas do alfa-

(-)-bisabolol. Master Thesis, Department of Chemistry, State University of Maringá – Maringá (2009). (In Portuguese).

Smitha, B., Sridhar, S. and Khan, A. A., Chitosan-sodium alginate polyion complexes as fuel cell membranes. European Polymer Journal, v. 41, p. 1859-1866 (2005).

Suganya, S., Ram, T. S., Lakshmi, B. S. and Giridev, V. R., Herbal drug incorporated antibacterial nanofibrous mat fabricated by electrospinning: An excellent matrix for wound dressings. Journal of Applied Polymer Science, v. 121, p. 2893-2899 (2011).

Tang, Z. G., Black, R. A., Curran, J. M., Hunt, J. A., Rhodes, N. P. and Williams, D. F., Surface properties and biocompatibility of solvent-cast poly[ε-caprolactone] films. Biomaterials, v. 25, p. 4741-4748 (2004).

Tisserand, R., Young, R., Constituent Profiles. In: Essential Oil Safety - A Guide for Health Care Professionals. Chapter 14, Churchill Livingstone Elsevier (2014).

Tortora, G. J. and Derrickson, B., Principles of Anatomy and Physiology. John Wiley & Sons, 13th Ed. (2012).

Veiga, I. G. and Moraes, A. M., Study of the swelling and stability properties of chitosan-xanthan membranes. Journal of Applied Science, v. 124, p. 154-160 (2011).

Voss, D. H., Relating colorimeter measurement of plant color to the Royal Horticultural Society colour chart. HortScience, v. 27, p. 1256-1260 (1992).

Wang, W. and Wang, A., Preparation, characterization and properties of superabsorbent nanocomposites based on natural guar gum and modified rectorite. Carbohydrate Polymers, v. 77, p. 891-897 (2009).

Weller, C. and Sussman, G., Wound dressings update. Journal of Research in Pharmacy Practice, v. 36, p. 318-324 (2006).

Woodruff, M. A. and Hutmacher, D. W., The return of a forgotten polymer -Polycaprolactone in the 21st Century. Progress in Polymer Science, v. 35, p. 1217-1256 (2010).

Woodward, S. C., Brewer, P. S., Moatamed, F., Schindler, A. and Pitt, C. G., The intracellular degradation of poly-epsilon-caprolactone. Journal of Biomedical Materials Research, v. 19, p. 437-444 (1985).

Yeh, C. C., Chen, C. N., Li, Y. T., Chang, C. W., Cheng, M. Y. and Chang, H. I., The effect of polymer molecular weight and UV radiation on physical properties and bioactivities of PCL films. Cellular Polymers, v. 30, p. 227-242 (2011).