# Preparation and Characterization of Scleroglucan Drug Delivery Films: The Effect of Freeze-Thaw Cycling

**Nora J. François, Marta E. Daraio**

*Grupo de Aplicaciones de Materiales Biocompatibles, Departamento de Química, Facultad de Ingeniería, Universidad de Buenos Aires, C1063ACV, Buenos Aires, Argentina*

Received 31 July 2008; accepted 9 November 2008
DOI 10.1002/app.29651
Published online 12 February 2009 in Wiley InterScience (www.interscience.wiley.com).

**ABSTRACT:** This study examined the effect of the freeze-thaw process on the physical properties of films prepared from scleroglucan (Scl) hydrogels, suitable for drug delivery applications. Films made from Scl, using glycerol as plasticizer, were prepared from hydrogels by two procedures: a room temperature drying (RTD) method and a freeze-thaw cyclic process, before the application of RTD, which results in a reinforced physically cross-linked network. Films were characterized by studies of water vapor transmission (WVT), swelling, tensile tests, ESEM microscopy, FTIR, and drug release measurements. These determinations showed significant differences between films obtained by both treatments. The films prepared through freeze-thaw cycles showed an important increase of the tensile strength with respect to those corresponding to films only air dried and a decreasing swelling degree in direct relationship to the number of freeze-thaw cycles. A model drug, Theophylline, was included in these biocompatible films for *in vitro* drug release measurements, using a flat Franz cell. The physical differences observed between Scl films prepared with both methods can be explained proposing that the number of crosslinking points by hydrogen bonding increase when increasing the number of freezing and thawing cycles used for film preparation. © 2009 Wiley Periodicals, Inc. J Appl Polym Sci 112: 1994–2000, 2009

**Key words:** scleroglucan; films; freeze-thaw; drug delivery systems; swelling

## INTRODUCTION

Hydrogels have become increasingly important materials for industrial, pharmaceutical, and biomedical applications because of their water imbibing property, their biocompatibility, and also because they exhibit characteristics comparable with natural tissue. They are used in a variety of applications including artificial skin,[1] controlled release drug delivery systems,[2] contact lenses,[3] etc. The most important properties of hydrogels relevant to their biomedical applications, especially for use as drug carriers and tissue engineering matrices, have been covered in previous studies.[4,5]

Scleroglucan (Scl) is a hydrogel-forming non-ionic polysaccharide, produced by fungi. In an aqueous solution, this polysaccharide adopts a stable triple-stranded helical conformation held together by hydrogen bonds. In previous works, we studied Scl hydrogels as matrices for controlled release.[6–10]

The interest in biodegradable materials and polymer films is currently increasing. Coatings and films play an important role in many fields of application (drug delivery coatings, thin films for food protection, polymer films as drug delivery systems for wound healing, etc).[11–15] For certain applications, as in the field of transdermal drug delivery, a biocompatible and moderately high water vapor permeable film may well be desirable.[16]

Particularly, for biological applications, it is central that the methods of preparation of polymer films be free of impurities, such as residual crosslinking drugs. The main crosslinking-agent free preparation method for poly (vinyl alcohol) (PVA) membranes is a freezing-thawing process.[17,18] These hydrogels prepared by exposing aqueous PVA solutions to repeated cycles of freezing and thawing are materials insoluble in water, because of the formation of crystallites[19] that operate as crosslinks. Other authors come to the conclusion that the freezing-thawing cyclic treatment of PVA solutions results in chemical crosslinking arising from the creation of free radicals.[20]

The previously mentioned applications of biocompatible polymer films were the motivation to investigate the preparation and characterization of films obtained from Scl hydrogels, reinforced trough a freezing and thawing procedure.

*Correspondence to:* M. E. Daraio (medit@fi.uba.ar).
Contract grant sponsor: University of Buenos Aires; contract grant number: UBACyT I032.

In this work, two different strategies to prepare films from the hydrogel have been applied:

(i) a room temperature air drying method, and
(ii) a freezing and thawing cyclic process, before the application of room temperature drying (RTD).

The aim of this work was to investigate the effect of freeze-thaw cycles on the production of Scl films with improved physical properties, suitable for controlled drug release or other biomedical applications such as in the field of wound healing. Prepared materials were characterized by studies of water vapor transmission (WVT), swelling, mechanical strength, ESEM microscopy, and drug release tests.

To our knowledge, this is the first time a film structure obtained from Scl hydrogels by using a freeze-thaw process has been studied.

## EXPERIMENTAL

### Materials

Scl of molecular weight (Mw) $4.5 \times 10^5$ provided by CarboMer was used at 1% w/w in all preparations. Glycerol analytical grade (Cicarelli, Argentina) was used at 2% w/w as plasticizer to improve the physical properties of the films by decreasing brittleness. Water was purified using a Millipore simplicity system. Theophylline (Th, $C_7H_8N_4O_2$, $M_w = 180.17$), in compliance with the British Pharmacopoeia standards, was purchased from Droguería Saporiti (Buenos Aires, Argentina) and used at 0.2% w/w.

Th-release assessments of the films were performed with a flat ground joint type Franz cell (PermeGear). A membrane of cellulose (average pore diameter = 48 Å; Mw cut-off = 12,000; Arthur Thomas) was placed between the upper section of the Franz cell (holding the Scl film) and the lower receptor compartment containing distilled water.

The water vapor permeation cells were acrylic cups with an internal diameter of 5.0 cm and an external diameter of 8.5 cm. The film is fixed with an acrylic ring shaped cover fastened with four screws. The cells were 3.5 cm deep and contained $CaCl_2$ [0% relative humidity (RH), 0 Pa water vapor partial pressure].

## METHODS

### Preparation of scleroglucan hydrogels

Scl concentration was kept constant in all experiments (1% w/w) by using 0.10 g Scl/10 g of total system (water and Scl). The necessary amount of polymer powder was dispersed in water containing 2% w/w glycerol, and in the case of films for drug release testing, 0.2% w/w of dissolved Th. These dispersions were kept at constant temperature under magnetic stirring for 96 h, to obtain proper swelling of the polymer and homogeneous gel formation. Well-defined stirring conditions were kept constant for each gel preparation.

### Preparation of polymer films

#### Room temperature drying

After obtaining the hydrogel, a certain amount was poured and spread onto a level circular (5 cm diameter) polypropylene plate. The quantity poured onto the plate was calculated to obtain 306 mg $cm^{-2}$. The material was initially dried at 50°C during 1 h and then allowed to dry at room temperature (about 25°C) in contact with ambient air for a week. Translucent films were obtained.

#### Freeze-thaw cycling (FT)

Freshly prepared hydrogels poured onto the plates were placed at −20°C for 24 h. After the freezing process, they were thawed at 25°C for 1 h. This freezing and thawing cycle was repeated for an additional 4 to 8 times. Finally, the material was dried at 50°C during 1 h and allowed to dry at room temperature for a week.

### Films thickness measurements

Thickness of films was measured at five different positions and to the nearest 0.001 mm using a digital thickness meter (Schwyz, Type II) with 10 mm diameter ceramic contact faces.

### Water vapor transmission

WVT through the films was determined using a modified ASTM E96-00 method.[21] Transmission tests were conducted to compare properties between both materials obtained by different methods of preparation of films.

A film sample was placed between the water vapor permeation cell and its acrylic ring shaped cover. A 10 mm air gap was left between the film and the $CaCl_2$ layer. The cells were stored in an isolated chamber that maintained a constant temperature of 27°C and RH of 74%, which were measured regularly. Water vapor transport was determined from the weight gain of the cell. All tests were conducted at least in duplicate.

The WVT rate (g $m^{-2}$ $s^{-1}$) is the steady water vapor flow in unit time through unit area of a body, normal to specific parallel surfaces, under specific conditions of temperature and humidity at each surface. Sufficient time (24 h) was allowed before measurements to ensure a stable WVT rate. After steady

state conditions were reached, changes in weight were recorded daily (at the nearest 0.1 mg) over an 8 days period and plotted as a function of time. WVT was calculated from the slope of the straight line divided by the exposed area of the film (7.07 $\times 10^{-4}$ $m^2$).

Permeance (g $m^{-2}$ $s^{-1}$ $Pa^{-1}$) is the time rate of WVT through unit area induced by unit vapor pressure difference between two specific surfaces, under specified temperature and humidity conditions. Permeance was calculated as:

$$\mathrm{WVT}/S(R_e - R_p)$$

where $S$ is the saturation vapor pressure of water at test temperature (3565 Pa in our experiments), $R_e$ is the RH at the test chamber (74%) expressed as a fraction and $R_p$ is the RH inside the permeation cell (0%) expressed as a fraction.

### Swelling

The swelling experiments were conducted by immersion of the film sample contained in a stainless steel basket, in purified water at 25°C. The amount of water absorbed was determined by weighing, after wiping, at various time intervals. Swollen films were weighed with an electronic balance (ACCULAB, capacity 210 g) to the nearest 0.1 mg.

The films were characterized by the swelling degree's variation in time ($Q_t$) determined as

$$Q_t = (m_t - m_0)/m_0$$

where $Q_t$ is the swelling degree at time $t$, $m_t$ is the mass of the swollen film at time $t$, $m_0$ is the mass of the dry sample at time 0 and ($m_t - m_0$) is the weight of the solvent absorbed by the film at time $t$.

### Mechanical tests

Tensile tests were performed using an Instrom 1125 tensile tester instrument according to the ASTM D638:03 standard method.[22] The samples were equilibrated at 23°C and 50% RH. Each test strip was placed in pneumatic grips, with initial grip separation of 30 mm and cross-head speed of 100 mm $min^{-1}$. Samples were measured by quadruplicate.

### Environmental scanning electronic microscopy (ESEM)

Micrographs of Scl films with and without freezing-thawing cycles were obtained at 20°C using an environmental scanning electron microscope 2010 (FEI Company, Hillsboro, OR) running in the so-called environmental wet mode. The sample chamber was kept at a constant pressure of 10–20 Torr (1 Torr = 133 N $m^{-2}$) displayed at the bottom of each micrograph. The electron beam had a voltage of 20 kV.

### Fourier transform infrared spectroscopy (FTIR)

Fourier transform infrared transmission spectroscopy was carried out on film samples using a Nicolet SIOP FTIR spectrometer, with KBr plates to support the films.

### Drug release measurements

Theophylline was chosen for the drug release studies as a model drug because it is stable in water solutions (water solubility at 25°C = 8.3 mg $g^{-1}$) and it is easily detected by its UV absorption (271 nm).

In release experiments, the film containing the drug was placed in the upper compartment of the Franz Cell, fitted with a membrane between the donor and the lower compartment, which was initially filled with pure water. The transference area of the film sample was 4.91 $cm^2$. The pore size of the hydrophilic cellulose membrane was 48 Å. Taking into account the drug size (Th radius: 3.8 Å[23]) and the Scl Mw, the membrane pores did not introduce a rate limiting step in the release experiments. The kinetic experiments were performed under thermostatic control (25°C) and constant stirring of the receptor solution. Measurements of drug concentration were performed by taking samples (0.20 mL) with a precision syringe at fixed times from the sampling port of the receptor compartment, and measuring Th absorbance in a Shimadzu UV-2401 spectrophotometer (Shimadzu, Kyoto, Japan). After each aliquot was taken, the volume (20 mL) in the receptor was made up with distilled water, assuring a constant volume in the lower compartment and a full contact between the Scl film supported by the membrane and the receptor liquid. Data reproducibility was assessed by running the experiments in duplicate or triplicate.

For each experiment, the cumulative concentration of drug released was calculated from a calibration plot (measured molar absorption coefficient of Th at 271 nm in water = 1.0 $\times 10^4$ $M^{-1}$ $cm^{-1}$). For every set of experimental conditions, mean values from replicated experiments were used. The fraction of drug released was calculated as the ratio of the measured concentration and the highest Th concentration attainable in the receptor compartment. This last value was computed taking into account the Th mass available in the release area of the tested film. As the FT process produces films with a smaller area than the RTD one, the total area of each film and the transfer area (given by the dimension of the Franz cell) were considered in the calculation of the highest Th concentration attainable in the receptor

**TABLE I**
**Water Vapor Transmission and Permeance of Scleroglucan Films (Average and Standard Deviation are Reported)**

| Physical property | RTD[a] | FT[b] 4 cycles | FT[b] 8 cycles |
|---|---|---|---|
| WVT (g $s^{-1}$ $m^{-2}$) | $(1.38 \pm 0.04) \times 10^{-2}$ | $(1.36 \pm 0.03) \times 10^{-2}$ | $(1.24 \pm 0.01) \times 10^{-2}$ |
| Permeance (g $s^{-1}$ $m^{-2}$ $Pa^{-1}$) | $(5.21 \pm 0.15) \times 10^{-6}$ | $(5.17 \pm 0.12) \times 10^{-6}$ | $(4.71 \pm 0.02) \times 10^{-6}$ |

The average thickness of films is 0.090 ± 0.010 mm; Temperature: 27°C; RH: 74%.
[a] Room temperature drying method.
[b] Freeze-thaw process.

compartment. Graphs of the fraction of Th released as a function of time were plotted.

## RESULTS AND DISCUSSION

### Water vapor transmission

The weight gain of the permeation cells as a function of time showed linear behavior. The slope of each curve was calculated by linear regression and the coefficient $R^2$ was over 0.997 in all cases.

Table I shows WVT and Permeance data for Scl films, obtained by both methods. A one-way ANOVA analysis indicates that the method of preparation affected WVT and Permeance, when 8 cycles were applied but not when 4 cycles were used. Both properties were significantly reduced (at 95% confidence level) by 8 cycles of freezing and thawing. For all samples, Permeance values are in the range of 4.7–5.2 $\times$ $10^{-6}$ g $s^{-1}$ $m^{-2}$ $Pa^{-1}$. Values for permeability calculated as the product of permeance and thickness[21] are in the range of 4.3–5 $\times$ $10^{-10}$ g $s^{-1}$ $m^{-1}$ $Pa^{-1}$, comparable with those obtained by Flores et al.,[14] for tapioca-starch films. In the case of Scl films, the rather high transmission of water vapor is favored due to profuse hydrophilic groups in the biopolymer structure. It is important to remark that the FT method of preparation only decreases the moisture permeability in ~ 10% while resulting in a reinforced physically cross-linked network. Additional crosslinking probably increases the tortuosity of inner channels connecting the pores, accounting for a slight decrease in moisture permeability.

### Swelling

Swelling measurements are a relatively simple method to characterize polymer networks. A decreased swelling degree can be helpful to deduce the existence of a higher number of crosslinking points in physical gels. It can also affect drug release from the matrix when it is in contact with a fluid.

The effect of both preparation methods (RTD and FT) on the swelling characteristics of Scl films is shown in Figure 1. By increasing the number of the FT cycles, the water uptake is reduced. This fact points to the attainment of a denser structure, due to an increase of the physical crosslinking points in the

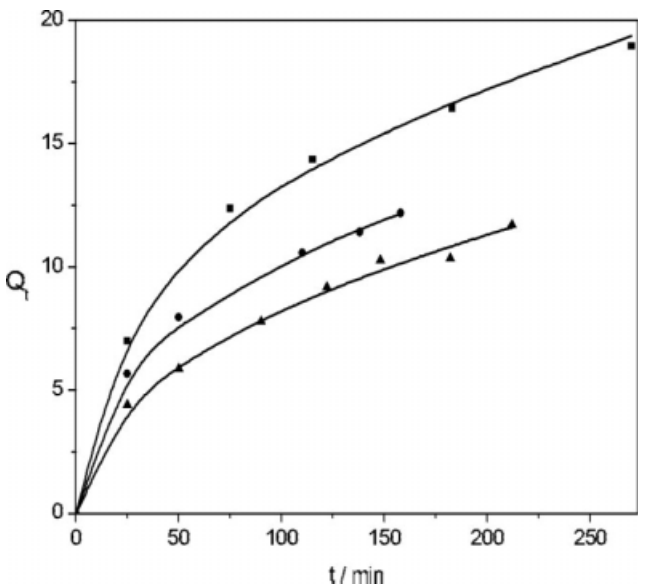


**Figure 1** Effect of different number of freezing and thawing cycles ($n_{FT}$) on the swelling curves of Scl films in water: ■: $n_{FT} = 0$; ●: $n_{FT} = 4$; ▲: $n_{FT} = 8$.

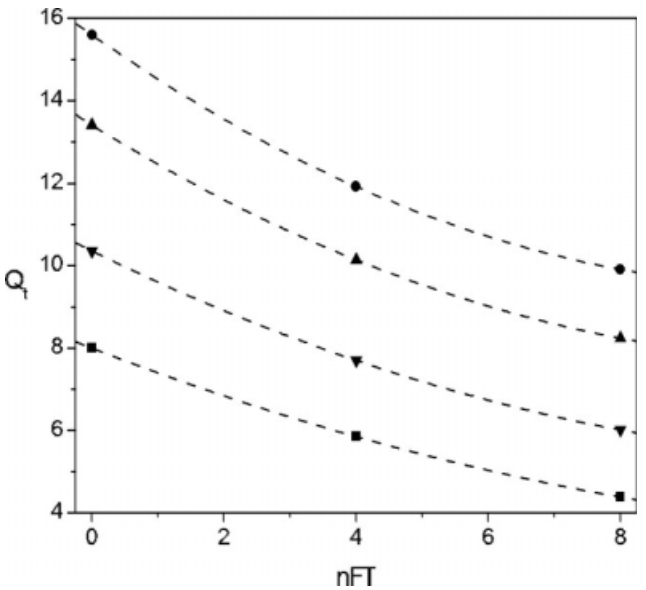


**Figure 2** Swelling degree ($Q_t$) at diverse times as a function of the number of freezing and thawing cycles ($n_{FT}$) for the Scl films mentioned in Figure 1. ■: 25 min; ▼: 50 min; ▲: 100 min; ●: 150 min. The dashed curves show a second degree polynomial fit.

**TABLE II**
**Tensile Strength and Elongation at Break of Scleroglucan Films**

| RTD[a] | | FT[b] 6 cycles | |
|---|---|---|---|
| Tensile strength $\times 10^{-6}$ (Pa) | Elongation at break (%) | Tensile strength $\times 10^{-6}$ (Pa) | Elongation at break (%) |
| Mean ± SD* | Mean ± SD* | Mean ± SD* | Mean ± SD* |
| 1.1 ± 0.1 | 89 ± 18 | 2.0 ± 0.3 | 81 ± 6 |

Temperature: 23°C; RH: 50%.
[a] Room temperature drying method.
[b] Freeze-thaw process.
* Standard deviation for four replicates.

network. Analyzing the swelling degree at individual times (see Fig. 2), a second degree polynomial dependence of swelling level on the number of FT cycles can be deduced.

### Mechanical properties

Table II shows the results of films mechanical properties, without or with 6 freezing-thawing cycles. The tensile strength of FT treated films was higher than the value observed for room temperature dried films, at the same RH. This result is to be expected if FT cycles induce crosslinking of polymer chains, leading to a more organized structure. It has been stated that lowering the temperature of a polymer solution is conductive to a system with a higher structure organization through a disorder-to-order conformational transition, and it is the association of such ordered regions of the polymer chains, which is the origin of the crosslinking development.[24] This effect increases structural integrity and slightly reduces the extensibility (percentage of elongation at break) of the studied Scl films.

### ESEM

The advantage of ESEM compared with traditional scanning electron microscopy is that the imaging of a sample is performed in a water vapor environment. This technique allows the imaging of surfaces of practically any specimen wet or dry, insulating or conducting, without any previous treatment of the sample.[25] Hydrophilic samples remain intact and the observed topography represents the actual surface structure of the material.

Micrographs of Figures 3 and 4 show a porous morphology for Scl films obtained without or with FT cycles, respectively, with a smaller pore size when the sample was freeze-thawed for 8 cycles. Moreover, this treatment leads to a more compact structure with a thicker pore wall. These observations point to the existence of reinforced physical crosslinking as a consequence of FT cycles in the preparation of the film.

Figure 5 shows the FTIR spectrum of Scl RTD and FT films. FTIR is an appropriate technique to determinate the occurrence of interactions between various groups in polymeric materials because is sensitive to inter e intramolecular interactions.[26,27] Figure 5 shows infrared spectra for RTD and FT films in the 4000–

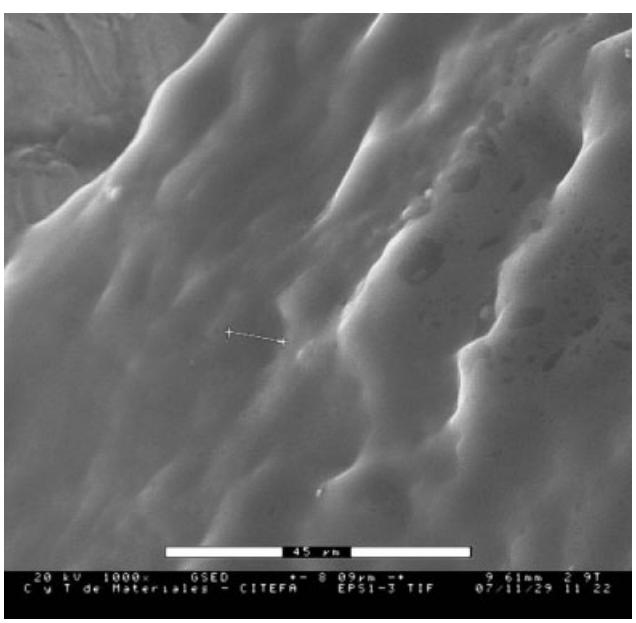


**Figure 3** Environmental scanning electron micrograph of cross section of Scl film, observed at the edge: air dried (0 FT cycles), measured pore size on the micrograph = 8.09 µm. Scale bar shows 45 µm.

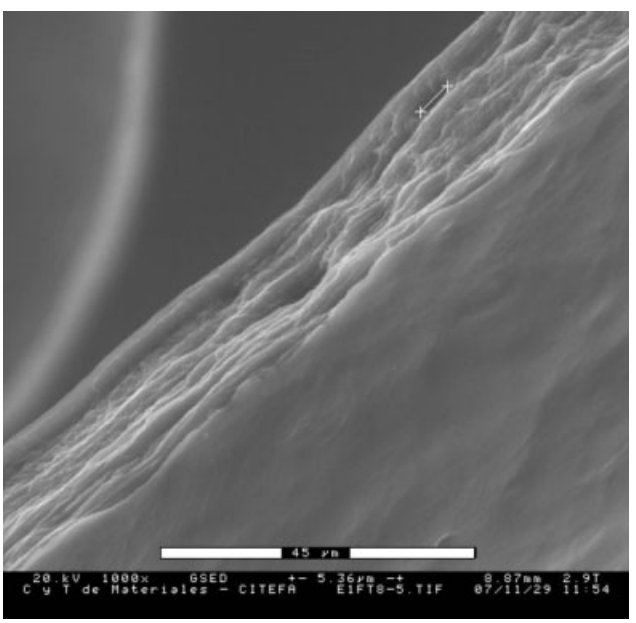


**Figure 4** Environmental scanning electron micrograph of cross section of Scl film, observed at the edge: 8 FT cycles, measured pore size on the micrograph = 5.36 µm. Scale bar shows 45 µm.

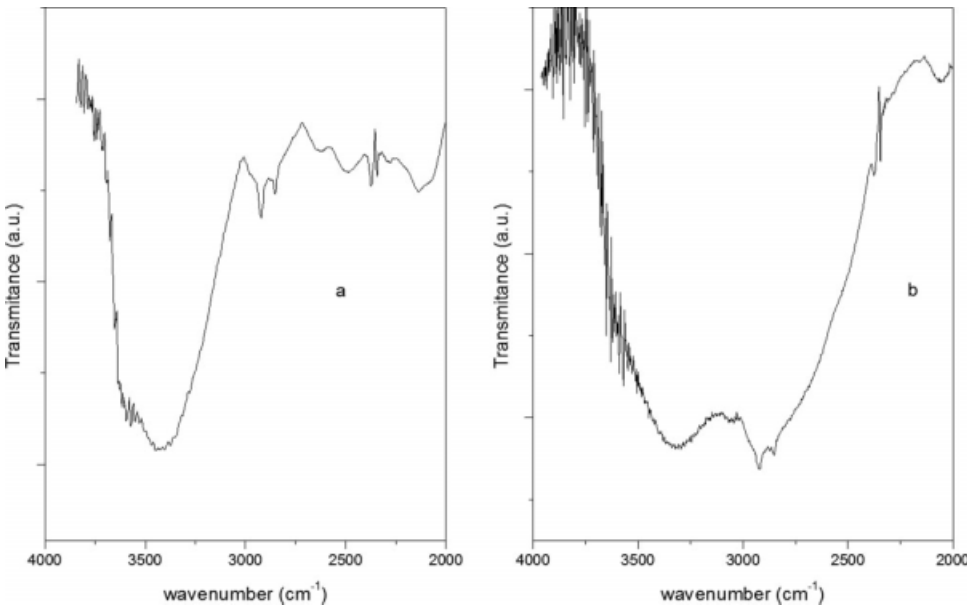


**Figure 5** FTIR spectra in the 2000–4000 $cm^{-1}$ region for Scl samples: (a) RTD film and (b) FT film with 8 cycles.

2000 $cm^{-1}$ range, enclosing the region sensitive to the hydrogen bonding formation. The spectrum of RTD film shows a shoulder at 3600 $cm^{-1}$ from the absorption of free hydroxyl groups and a broad band centered at 3400 $cm^{-1}$ related to the absorption of hydrogen-bonded hydroxyl groups. In the case of FT film, the band from hydrogen-bonded hydroxyl groups shifts to lower wavenunber and adopts a wider shape than in RTD film. This broad band can be assigned to a sum of contributions of hydrogen-bonded O—H groups, from dimers to polymer chains involving a high number of OH-groups. The wide band at 3340 $cm^{-1}$ in the FT spectrum can be related to hydrogen-bonded hydroxyl groups, intermolecular H-bridge between OH groups.[28] These spectra point to an increased number of intra e intermolecular hydrogen bonds resulting from FT process, leading to a higher crosslinking density.

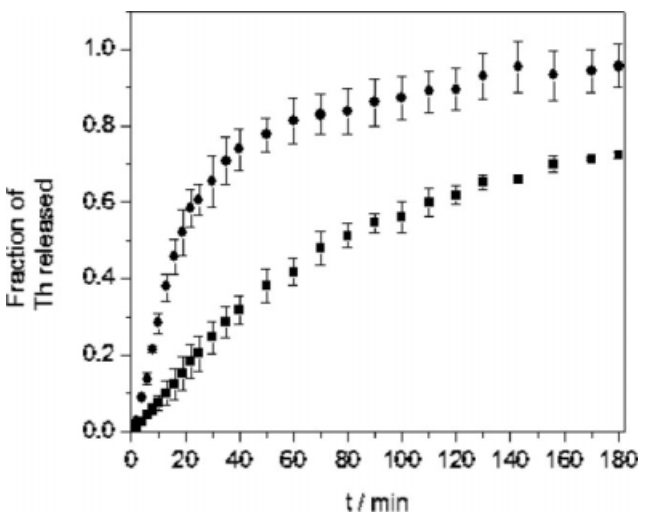


**Figure 6** Fraction of Th released as a function of release time: (■) FT films; (●) RTD films with 8 cycles. Error bars correspond to mean ± standard error of the mean.

### Drug release

Figure 6 shows the effect of freezing and thawing cycles on the drug release behavior of Scl films. Experiments were carried out in duplicate or triplicate and the obtained values lay within 6–10% of the mean. FT treatment causes a significant modification in the Theophylline release pattern, leading to a lengthened drug release period. This effect could be assigned to an increased chain entanglement and degree of crosslinking, taking into account that increasing crosslinking density implies shrinking the mesh size of the material for drug diffusion.[29] In the same manner, a considerable decrease in the Theophylline release rate was found from PVA matrix by increasing the crosslinker (glutaraldehyde) content,[30] which was assigned to an increased chain entanglement.

A prolonged drug release can be achieved by using FT cycles in the preparation of the drug loaded film, leading to a wider time window for drug delivery.

## CONCLUSIONS

The objective of the present study was to investigate the effect of the freeze-thaw process on the physical properties of films prepared from Scl hydrogels. The structural characterization as well as the kinetics of swelling and drug release was studied as a function

of the number of freeze-thaw cycles. It was found that the films prepared through freeze-thaw cycles had an increased tensile strength, a decreased swelling degree, and a longer drug release time with respect to those corresponding to films prepared using the air dried method, and those measurements are related to the number of freeze-thaw cycles used. To explain these observations, it was proposed that the number of physical crosslinking points increases with the freezing and thawing cycles during the preparing of the film. In conclusion, by using repetitive freeze-thaw cycles in the preparation protocol, films with enhanced mechanical properties can be produced, and a controlled drug release may be attained.

The research of the influence of different variables on the drug release behavior from these films is taking place in our laboratory.

The authors acknowledge Grupo de Materiales CITEFA, for ESEM microscopy measurements.

## References

1. Ostrovidova, G. U.; Makeev, A. V.; Shamtsiam, M. M. Mater Sci Eng 2003, 23, 545.
2. Vinogradov, S. V.; Broncch, T. K.; Kabanov, A. V. Adv Drug Deliv Rev 2002, 54, 135.
3. Fung, L. K.; Saltzmann, W. M. Adv Drug Deliv Rev 1997, 26, 209.
4. Hoffman, A. S. Adv Drug Deliv Rev 2002, 43, 3.
5. Peppas, N. A.; Zach Hilt, J.; Khademhosseini, A.; Langer, R. Adv Mater 2006, 18, 1345.
6. Daraio, M.; François, N.; Bernik, D. Drug Deliv 2003, 10, 79.
7. François, N.; Rojas, A. M.; Daraio, M. E.; Bernik, D. J. Control Release 2003, 90, 355.
8. François, N.; Rojas, A. M.; Daraio, M. E. Polym Int 2005, 54, 1613.
9. François, N. J.; Allo, S.; Jacobo, S. J.; Daraio, M. E. J Appl Polym Sci 2007, 105, 647.
10. Viñarta, S. C.; François, N. J.; Daraio, M. E.; Figueroa, L. I. C.; Fariña, J. I. Int J Biol Macromol 2007, 41, 314.
11. Buonocuore, G. G.; Del Nobile, M. A.; Panizza, A.; Corbo, M. R.; Nicolais, L. J Control Release 2003, 90, 97.
12. Sanjurjo, K.; Flores, S.; Gerschenson, L.; Jagus, R. Food Res Int 2006, 39, 749.
13. Chang, Y. P.; Abd Karim, A.; Seow, C. C. Food Hydrocolloids 2006, 20, 1.
14. Flores, S.; Famá, L.; Rojas, A. M.; Goyanes, S.; Gerschenson, L. Food Res Int 2007, 40, 257.
15. Schmidt, R. Topical Delivery of α1-Antichymotrypsin for Wound Healing, Thesis; Ludwig-Maximilians-Universität München, Munich, Gremany, 2005.
16. Nicoli, S.; Colombo, P.; Santi, P. The AAPS J 2005, 7, 20. Available at: http://www.aapsj.org.
17. Hassan, C. M.; Peppas, N. A. Adv Polym Sci 2000, 153, 37.
18. Nugent, M. J. D.; Hanley, A.; Tomkins, P. T.; Higginbotham, C. L. J Mater Sci Mater Med 2005, 16, 1149.
19. Stauffer, S. R.; Peppas, N. A. Polymer 1992, 33, 3932.
20. Hernandez, R.; Lopez, D.; Mijangos, C.; Guenet, J.-M. Polymer 2002, 43, 5661.
21. ASTM Standard E96-00. Standard Test Methods for Water Vapor Transmission of Materials; American Society for Testing and Materials: Philadelphia, 2000.
22. ASTM Standard D638-03. Standard Test Method for Tensile Properties of Plastics; American Society for Testing and Materials: Philadelphia, 2003.
23. Amsden, B. Macromolecules 1998, 31, 8382.
24. Lapasin, R.; Pricl, S. Rheology of Industrial Polysaccharides. Theory and Applications; Blackie Academic & Professional, Chapman & Hall: Glasgow, 1995, 375.
25. Micic, M.; Radotic, K.; Benitez, I.; Ruano, M.; Jeremic, M.; Moy, V.; Mabrouki, M.; Leblanc, R. Biophys Chem 2001, 94, 257.
26. Huang, M.; Kuo, S.; Wu, H.; Chang, F.; Fang, S. Polymer 2002, 43, 2479.
27. Kuo, S.; Lin, C.; Chang, F. Polymer 2002, 43, 3943.
28. Heinze, T.; Liebert, T.; Koschella, A. Esterification of Polysaccharides; Springer Berlin: Heidelberg, 2006, 17.
29. Korsmeyer, R. W.; Peppas, N. A. J Memb Sci 1981, 9, 211.
30. Varshosaz, J.; Koopaie, N. Iran Polym J 2002, 11, 123.