Journal of Applied Polymer Science

Cyclodextrin Functionalization of Several Cellulosic Substrates for Prolonged Release of Antibacterial Agents

Oriol Cusola,[1,2] Nicolas Tabary,[1,3] Mohamed Naceur Belgacem,[1] Julien Bras[1]

[1]Laboratory of Pulp and Paper Science (LGP2), 461, rue de la papeterie, St-Martin-d'Héres Cedex, 38402, France

[2]Textile and Paper Engineering Department, Universitat Politécnica de Catalunya (UPC-BarcelonaTech), Colom 11, E-08222 Terrassa, Spain

[3]Unité Matériaux et Transformation (UMET), Ingénierie des Systmes Polymres, Université de Lille 1, F-59655 Villeneuve D'Ascq, France

Correspondence to: O. Cusola (E-mail: oriol.cusola@etp.upc.edu.)

ABSTRACT: Several cellulosic substrates have been surface-functionalized with cyclomaltoheptaose (β-cyclodextrin, β-CD) using citric acid as a crosslinker agent to obtain new surface-modified materials able to release antiseptic molecules over a prolonged period, in view of their use in medical domain. Three different commercial cellulosic substrates were used, namely: (i) an uncoated paper, (ii) a crepe paper, and (iii) a medical bandage. They were successfully grafted by a crosslinked polymer consisting on β-CD molecules as assessed by scanning electron microscopy and Fourier transform infrared spectroscopy analysis. Several time–temperature kinetic cycles were performed to reach the optimum curing parameters. The grafted and nongrafted samples were loaded with chlorhexidine digluconate (digCHX), a widely used antiseptic agent. The drug-delivery kinetics of the encapsulated digCHX was carried out by immersing the sample under investigation into an aqueous medium, and the quantity of the released digCHX was measured, as a function of time, by UV spectroscopy. The optimal grafting conditions were established on the basis of the highest weight gain. These samples did not give the best release performance. Nevertheless, several grafted substrates were able to uptake an appreciable amount of active molecules and release them over a prolonged time of about 20 days. © 2012 Wiley Periodicals, Inc. J. Appl. Polym. Sci. 129: 604–613, 2013

KEYWORDS: functionalization of polymers; grafting; drug delivery systems; cellulose and other wood products; fibers

Received 19 July 2012; accepted 19 October 2012; published online 13 November 2012

DOI: 10.1002/app.38748

INTRODUCTION

Current investigations tend to search new functionalities for papers and cellulose-based substrates. Deactivation of pathogen substances,[1] liberation of flavoring agents, or liberation of fragrances,[2] or cellulosic fabrics for insecticide delivery[3] are examples of new functionalities for papers. Papers and paper-based cellulosic substrates with enhanced functionalities apart from conventional ones (printing or packaging) are called specialty or active papers. The main sectors where active papers are reaching high interest are packaging and medical. The objective of surface functionalization is to provide new properties for cellulose-based materials. From this point of view, the use of cyclodextrins seems to be a good method to functionalize cellulosic substrates.[4,5]

β-Cyclodextrin (β-CD) produced by enzymatic degradation of starch is a cyclic oligosaccharide consisting of seven D-glucose units linked by α-(1→4) glycosidic bonds.[6–11] More generally, β-CDs contain a lipophilic central cavity and a hydrophilic outer surface. Due to the chair conformation of the glucopyranose units, the cyclodextrins are shaped like a truncated cone rather than perfect cylinders. β-CDs are able to form host–guest complexes with hydrophobic molecules given the unique nature imparted by their structure.[6,9,11] As a result, these molecules have found a number of applications in a wide range of fields thanks to their encapsulation ability.[12–14] In addition to the above-mentioned pharmaceutical applications for drug release, β-CDs can be used in environmental protection; they can effectively immobilize toxic compounds like trichloroethane or heavy metals inside their rings, or they can form complexes with stable substances like trichlorfon (an organophosphorus insecticide), or sewage sludge, enhancing their decomposition.[15,16] Other interesting applications arise from their ability to form inclusion complexes with fragrance/flavor materials like menthol to obtain, for example, polymeric fibers with odor properties.[17] This study applies a technique consisting in grafting a β-CD crosslinked polymer on the surface of cellulosic substrates with different morphologies, to confer them bactericidal properties through the encapsulation of active agent molecules.

To date, studies establishing the most appropriate pathway of grafting cyclodextrins onto textiles[18–20] and nonwovens[21–23] were performed. Moreover, studies for grafting cyclodextrins onto cellulosic fabrics loaded by miconazole nitrate[24] or silver ions[25] were also carried out, to prepare new fabrics with antimicrobial properties. In the medical domain, cyclodextrins were used to obtain polyvinylidene fluoride regenerative membranes for periodontal applications,[26] to produce bone implants with drug release properties,[27,28] polyvinyl alcohol hydrogels for sustained release of ocular therapeutics,[29] or to fabricate functionalized polyester vascular prosthesis with a postoperation release of antibiotics to prevent infections.[30] Nowadays, these studies are extended for commercial cellulosic materials, to provide them new or added bactericidal properties, thus yielding a final product with the highest added value possible; this is one of the reasons why medical application is targeted. The authors of this article compare for the first time different kinds of paper substrate, and an optimum of grafting is proposed. An additional challenge consists on maintaining the release for within a prolonged period of time.

EXPERIMENTAL

Materials

The three different cellulosic substrates used in this work were a noncoated paper (70 g/m^2), a 100% cotton-based medical bandage (70 g/m^2), and a medical crepe paper (60 g/m^2, from Ahlstrom, France) used for sterilization purposes. Citric acid, sodium dihydrogen hypophosphite ($NaH_2PO_4{\cdot}H_2O$), β-CDs, and chlorhexidine digluconate were commercial chemical grade products, supplied from Aldrich Chemicals (Saint Quentin Fallavier, France). They were used as received.

Cyclodextrin Grafting Process

The grafting of paper by β-CD was based on the pad-dry-cure textile finishing method, previously reported.[31] All the samples (4 cm × 4 cm) were oven dried at 105°C for 20 min before soaking and immediately weighted. After, each single paper sheet was soaked into 100 mL aqueous solution containing 10 g of β-CD, 3 g of catalyst (sodium dihydrogen hypophosphite), and 10 g of crosslinking agent (citric acid). Then, the samples were dried at 105°C during 10 min. The grafting reaction occurs via a polyesterification reaction which is described in the section entitled "Cyclodextrin Grafting"; the structures of the cyclodextrin/cellulose/citric acid adducts were described in detail in previous publications.[23] The grafting reaction occurred in a ventilated stove at different temperatures (between 130°C and 160°C) and times (between 5 and 50 min). After the reaction, the ensuing specimens were abundantly washed with hot (80°C) distilled water to remove the nonlinked CDs, oven dried (at 104°C for 30 min) and weighted. The weight gain (WG) (%) representing the yield of the grafting reaction was calculated using the expression:

$$\mathrm{WG}\ (\%) = (M_f - M_i)/M_i \times 100 \tag{1}$$

where M_i and M_f are the sample weights, before and after the grafting treatment, respectively. The data reported in this work are the average values of three experiments.

Drug Adsorption

Modified and unmodified sheets were dried at 105°C for 20 min before being weighted. Then, the samples were treated with antibacterial agent by dipping them at room temperature into 20% (w/v) chlorhexidine digluconate (digCHX) solution, for 1 h. Afterward, the sheets were dried with blotting paper to remove the excess of solution and oven dried at 40°C in a ventilated oven during 24 h. The resulting sheets were finally considered totally dried (without any residual humidity) and immediately weighted.

The weight gain ($\Delta M_{\mathrm{digCHX}}$) represents the amount of antiseptic agent loaded onto sample sheets, as calculated from the following equation:

$$\Delta M_{\mathrm{digCHX}} = M_{f_2} - M_f \tag{2}$$

where M_f is sample weight after grafting treatment and M_{f_2} is the sample weight after loading with the antiseptic agent. The amount of CHX adsorbed has been reported in grams per gram of the substrate, as follows:

$$g_{\mathrm{digCHX}}/g_{\mathrm{impregnatedpaper}} = \Delta M_{\mathrm{digCHX}}/M_{f_2} \tag{3}$$

UV Spectroscopy and Release Analyses

An UV spectrophotometer (UNIcam UV500 Thermospectronic) was used to characterize the prolonged liberation behavior by measuring concentration of the digCHX. Each experiment was conducted in triplicate.

Samples were dipped in distilled water into vials filled with 30 mL, waiting for release under constant stirring. At pre-established intervals, the water was completely renewed and the drug content in the withdrawn fluid was determined by UV spectrophotometry. The first measure was taken at few hours after the beginning of the release kinetic, while the following ones were taken at regular intervals of 24 h. Measures taken every 24 h give the number of cycles with renewed distilled water the functionalized support can carry on releasing the active agent, that is, the prolonged liberation behavior.

Scanning Electron Microscopy Imaging

An analysis of the surface and cross-section of the different substrates was performed using an environmental scanning electron microscope (ESEM) (Quanta 200, FEI Company, Hillsboro, Oregon) under moderate vacuum at an operating voltage of 7 kV. Dried cellulose-based fibre-mats were gold-coated by sputtering for 15 s.

Fourier Transform Infrared Spectroscopy

A Perkin Elmer PARAGON 1000 FTIR spectrometer equipped with spectrum software was used to perform the Fourier transform infrared spectroscopy (FTIR) analyses. The spectra were obtained by analyzing directly the surface by using ATR system (GoldenGate, JASCO). The FTIR spectra were collected with a resolution of 4 cm^{-1} in the range of 4000–400 cm^{-1}. Thus, 16 scans were taken within this interval.

Cobb Absorption, Bendtsen Roughness, and Bendtsen Permeability

Properties that would have an influence on the functionalization and liberation behavior of substrate were tested, namely: Cobb

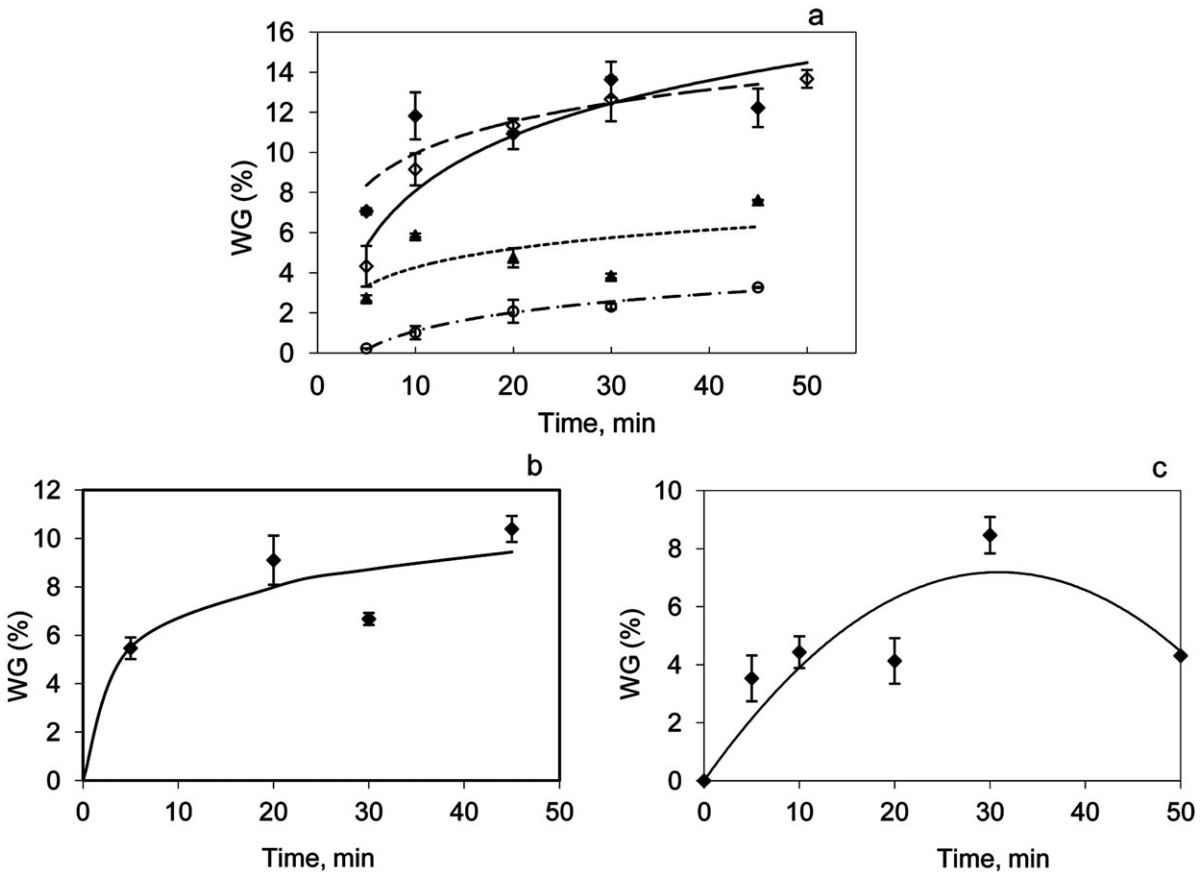

Figure 1. Time–temperature kinetics for noncoated paper (a). Key: 130°C (dash-point curve, ○); 140°C (point-curve, ▲); 150°C (continuous curve, ◊); 160°C (dashed curve, ♦). (b) and (c) show the weight increase kinetics for medical bandage and crepe paper, respectively, at fixed conditions of 150°C for 30 min. Grafting yield is expressed as the WG (%) of the sheets upon reaction.

absorption, Bendtsen roughness, and Bendtsen permeability. All these parameters may play an important role on the way the β-CD solution and the digCHX are captured by the sample surface and internal diffusion on the sheet structure. All these tests were carried out according to commonly used international standards (Cobb absorption: EN 20535-1994; Bendtsen roughness: NF Q03-049-1972; Bendtsen permeability: NF Q03-076-1986).

RESULTS AND DISCUSSION

Cyclodextrin Grafting

The WG of the sheets results from the grafting of the cellulose fibers by a crosslinked polymer formed between β-CD and the citric acid. This occurs via a polyesterification reaction, as previously reported for material treated with other CDs derivatives. Under the influence of heat and catalyst, the polycarboxylic acid (citric acid) is dehydrated. A first esterification reaction occurs between the formed anhydride and a hydroxyl function of the cyclodextrin. In a second step, an anhydride is formed between the other two remaining acid functions; a second esterification reaction can then take place either with the cellulose which is going to be functionalized (—OH functions of cellulose, for example) or with an hydroxyl of another cyclodextrin to form a crosslinked polymer.[19,30,31] The reaction between citric acid and cyclodextrins results in a three-dimensional polymer network whose structure is based on cyclodextrin moieties linked to each other via esterified citric acid residues carrying free carboxylic groups. Depending on the pH, those free carboxylic groups will appear under carboxylate form and experience an ionic interaction with the amino groups present in digCHX molecules. Therefore, the loading of the digCHX onto the functionalized cellulosic substrates can be expected to occur either entrapped into the β-CD cavity by hydrophobic complexation, or adsorbed onto the polymer structure via acid–base interactions.[32]

Figure 1(a) reports the WG values of the treated noncoated paper for up to 45 min at temperatures between 130°C and 160°C. Regression lines were used to indicate the observed trend. The highest WG was observed after 30 min curing at 150°C and 160°C, as shown in Figure 1(a). In fact, a maximum WG of 13 ± 1% was achieved within 30 min at 160°C and for 45 min at 150°C. At lower temperatures, the WG increased more smoothly and reached around 3.3% and 7.6%, after 45 min of curing, for 130°C and 140°C, respectively. Thus, working at 150°C for 30 min was fixed as the optimal condition of curing. The WG under these conditions for the noncoated paper was around 13%.

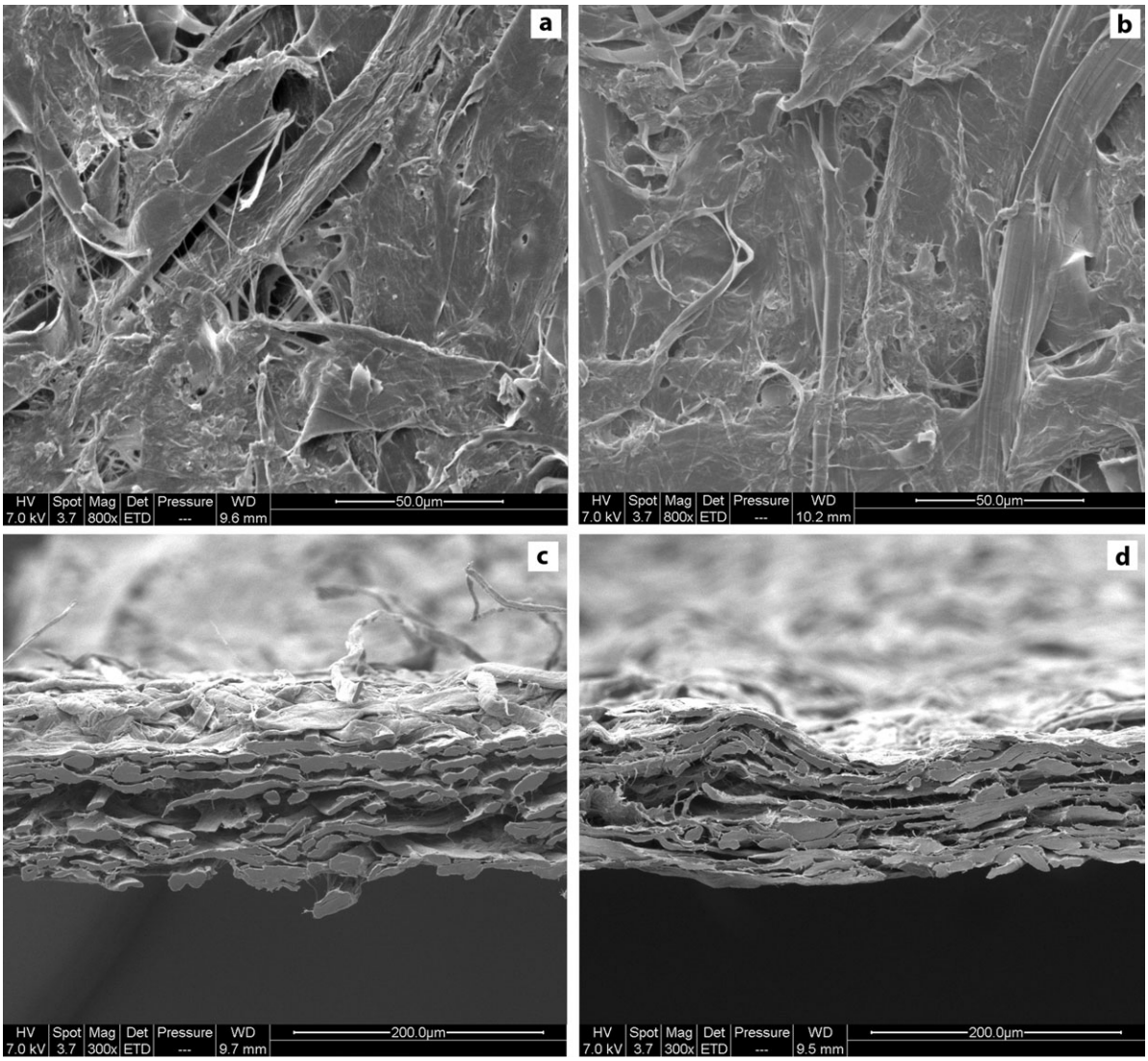

Figure 2. SEM images of the noncoated paper surface (a) initial, and (b) grafted with WG = 13 ± 1%; images (c) and (d) correspond to the crepe paper cross-section; (c) initial, and (d) grafted with WG = 8 ± 1%.

The same temperature was used in the case of the medical bandage and crepe paper. For those substrates, only the curing time was studied, as reported in Figure 1(b,c). The best curing time for both materials was also set at 30 min. In the case of the medical bandage, the curve reached a plateau after 30 min; for crepe paper, functionalization tends to decrease after such a delay, possibly due to loss of fibers in fabric after washing, caused by the degradation and embrittlement of cellulose with temperature. The WG was about 9% and 8%, for the medical bandage and crepe paper, respectively.

The surface morphology of the reference and that of the CD-grafted samples were analyzed by SEM, as displayed in Figure 2. Very few differences were observed after grafting, and material with similar features was achieved. Nevertheless, the compactness of the treated samples seems to be higher. Moreover, porous structure is maintained in both cases, and there is no formation of continuous film at the surface of the grafted materials. This may be due (at least partially) to the grafting but most probably to the role of citric acid and/or drying process at high temperature and under released constraints. The latter two phenomena are well known in papermaking and arise from the partial collapsing (physical for drying and chemical for crosslinking with citric acid) of the fiber cell walls.[33]

Table I shows the mean results of paper physical properties obtained with the Bendtsen and Cobb tests for 10 specimens of each substrate. Bendtsen permeability and roughness were not possible to measure on the medical bandage, because it has very open structure. As mentioned before, the method used to graft the β-CD onto the cellulosic sheets was a bulk-type approach, that is, their soaking into an aqueous citric acid and β-CD solution. This is the reason why the parameters which most probably correlate properly the effect of such a modification are the WG and the Cobb absorption. Indeed, the standardized Cobb absorption test (in g/m^2) gives the amount of water which is

Table 1. Bendtsen and Cobb Values for the Three Substrates

	Bendtsen Permeability [cm^3/cm^2 Pa s]		Bendtsen Roughness [ml/min]		Cobb absorption [g/m^2]	
Support	Mean	StD	Mean	StD	Mean	StD
Non coated	0.39	0.03	304	12	56	1
Crepe paper	3.15	0.05	2534	45	15	1
Medical bandage	-	-	-	-	36	3

Mean and standard deviation were calculated from samples of 10 specimens.

retained by unit area after a certain period of time. As the citric acid and β-CD solution behave similarly to water, higher is the impregnation level, bigger is the absorbed amount of citric acid and β-CD. It is therefore easy to understand that the amount of grafted β-CD onto the fibers is higher. Figure 3 shows the relationship between the amounts of citric acid and β-CD on the different substrates after curing (represented by WG) and the Cobb absorption. As expected, the noncoated paper was better impregnated by the aqueous solution, thus leading to the highest grafting level, whereas samples based on crepe papers which has the lower Cobb values consequently underwent much less to the grafting operation and gave the lower WG.

FTIR spectra of the reference samples and those corresponding to the grafted sheets were compared. The esterification reaction that occurs with the —OH functions of cellulose macromolecules (or those of cyclodextrins) produces chemical functions (ester groups) not present in the cellulose. The detection of these groups in the modified samples evidence that the grafting reaction had took place.[34,35] The retained material could be either adsorbed and crosslinked at the surface of the samples or chemically bonded to it. Of course both mechanisms could occur simultaneously. The elimination of non-crosslinked and/or nongrafted molecule is assessed considering the washing procedure. Figure 4 shows a comparison of the FTIR curves for initial crepe paper before and after modification. The spectrum of the surface of the grafted sheets shows a peak of C=O stretching vibration observed at 1726 cm^{-1}. This signal was not detected in the spectrum associated with the initial substrate.

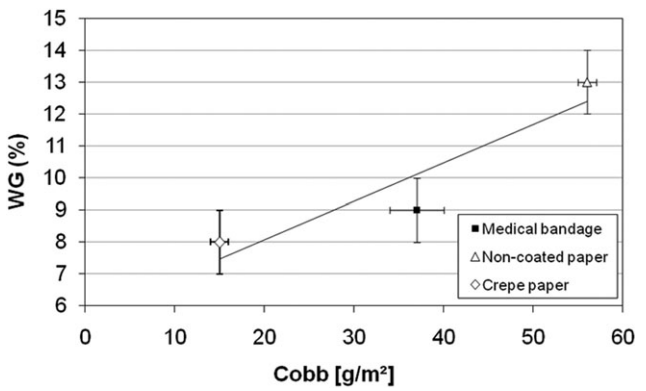

Figure 3. Relationship between the Cobb absorption, expressed in g/m^2, and the amount of β-CD grafted on the substrate expressed as WG increase (%).

Similar results were obtained for the noncoated paper and for the medical bandage.

Release Studies of Noncoated Paper

UV spectrophotometry was used to characterize the release of the digCHX retained by the β-CD. The use of the digCHX was suitable in terms of molecular size to enter the β-CD cavities, since it was reported in previous works.[26] The controlled release effect of digCHX from CD-finished cellulosic substrates is explained by the inclusion of this molecule into the internal cavity of CD,[36] and also due to ionic interactions of digCHX with the free carboxylic groups on the crosslinked polymer, as explained in previous sections. More precisely, ionic exchange probably exists between the gluconate counter ions of the positively charged digCHX and the carboxylate groups present on the grafted substrates. The digCHX molecule has two absorbance peaks between 200 and 300 nm. The peak corresponding to a wavelength of 254 nm (λ_{max}) was used to determine the digCHX concentration using the Beer Lambert law.[37–40] Despite knowing the optimal grafting time and temperature for each substrate, other grafting conditions were tested in terms of release. The idea was to confirm the hypothesis which considers that the maximum number of grafted cyclodextrins corresponds to the highest release level of active agent. The first set of experiments was carried out on each substrate separately. It is important to notice that, the released amounts of digCHX in the following results may appear to be weak, but they are interesting from the point of view of bacterial inhibition, as it will be discussed in next paragraphs.

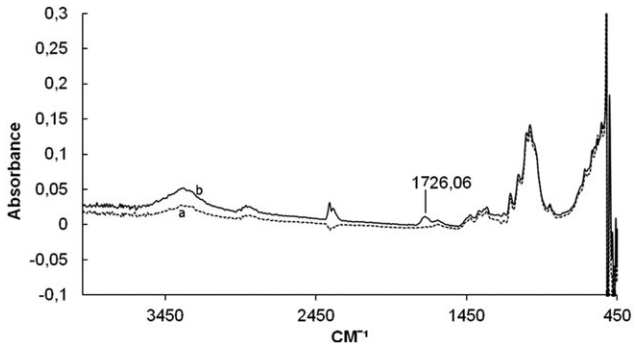

Figure 4. ATR-FTIR spectra of (a) crepe paper and (b) crepe paper treated with β-CD after washing.

Table 2. Characteristics of the Samples Tested in Terms of Liberation Behavior, and the Amount of the Loaded digCHX

	Number of Samples	Loaded digCHX in g	g digCHX / g of Loaded Paper
Non coated Paper			
5 min. 150°C	3	0.042 ± 0.002	0.271 ± 0.011
30 min. 150°C	3	0.028 ± 0.002	0.19 ± 0.01
Non grafted and oven dried at 150°C for 30 min	3	0.033 ± 0.002	0.24 ± 0.01
Non grafted and not oven dried	3	0.035 ± 0.003	0.24 ± 0.02
Crepe Paper			
5 min. 150°C	3	0.045 ± 0.001	0.219 ± 0.003
Non grafted and oven dried at 150°C for 30 min	3	0.032 ± 0.005	0.181 ± 0.010
Medical Bandage			
5 min. 150°C	3	0.045 ± 0.007	0.178 ± 0.025
Non grafted and oven dried at 150°C for 30 min	3	0.035 ± 0.001	0.145 ± 0.006

For noncoated paper, two grafting levels (5 and 30 min at 150°C) were tested and compared with the references. Table II summarizes the characteristics of samples and the amount of the loaded digCHX into the substrate before setting up the kinetics of the release. Concerning noncoated paper, all samples tend to retain similar amounts of digCHX, except those which were grafted at the optimum time and temperature, as described previously. This fact can be explained from the point of view of the sheet structure. After grafting, the structure of sheets becomes more closed (Figure 2), and the cavities are filled by β-CDs. Moreover, the citric acid causes the intrafibers and interfibers crosslinking, yielding substrates with lower capacities of absorbing active agent (i.e., lower porosity and affinity to water-based baths). It is important to notice that this fact is not worrisome as the amount of digCHX which is filled into the surface hollows will not necessarily charge the cavities of β-CDs and will be quickly released. What we want to characterize is the lasting release ability of the grafted β-CDs.

Figure 5 displays the kinetic of digCHX release for the nongrafted and grafted samples [Figure 5(a,b)] and shows that the major part of digCHX is released already after 12 h. A plateau is classically observed for each sample, and a slight but lasting release is observed for the grafted materials. This gives an indication about the prolonged release of active material for the grafted paper. Figure 5(a,b) provides evidence that for nongrafted samples the released amount of digCHX corresponds to that initially loaded (100%). Figure 5(c,d) shows how the initially loaded amount is not achieved after 19 days, indicating that a significant amount of digCHX still remains inside the CDs cavities.

Figure 6 shows the prolonged digCHX release after successive washing for noncoated paper samples that were grafted at different degrees. The release is represented as a percentage of the initially digCHX loaded amount. It is obvious to expect that the maximum amount that can be released for one sample is theoretically 100%. However, the initially loaded amount of digCHX (which corresponds to 100%) is calculated by a mass difference method as explained in the Experimental section. Only the initially loaded digCHX amount is estimated by mass difference; the reason is because there are samples that never release the entire active agent after the period of the kinetic study, so there is no other way to know the loaded digCHX at the beginning. It is important to notice that the difference between the UV measurements and the mass difference method used to calculate the amounts of digCHX may lead to a slight discrepancy, as already shown in Figure 5(a) where the released amount is slightly above 100%. However, Figure 5(b) confirms that such difference could be neglected.

The release values after 12 h evidence that the main part of the loaded digCHX is released in the first hours. Nongrafted samples released practically the total amount of the loaded active agent, while grafted specimens tend to release only about 50% after a first cycle of measurement (curing conditions 30 min-150°C), and 60% (curing conditions 5 min-150°C). Several conclusions can be drawn from Figure 6 comparing the tested samples. The grafted samples using optimal conditions (30 min-150°C) were found to stop the release of the active digCHX molecules after 5 days. The release kinetic is considered accomplished when the concentration of digCHX is so weak that its dosage became impossible to detect from the absorbance peak. Nongrafted samples (dried and nondried) have found to release the total amount of digCHX molecules within the first 12 days, even if it is worth to mention that the major part of the active agent is released within the first measurement. Samples grafted for 5 min at 150°C were found to carry on the releasing after the highest number of washings. The effect of β-CD grafting is clearly observed confirming that such a grafting procedure strongly prolonged the liberation behavior of the active molecules.

However, samples grafted using the optimum curing time and temperature (the highest WG) did not provide the highest release performance. The reason might be the strong

Applied Polymer SCIENCE

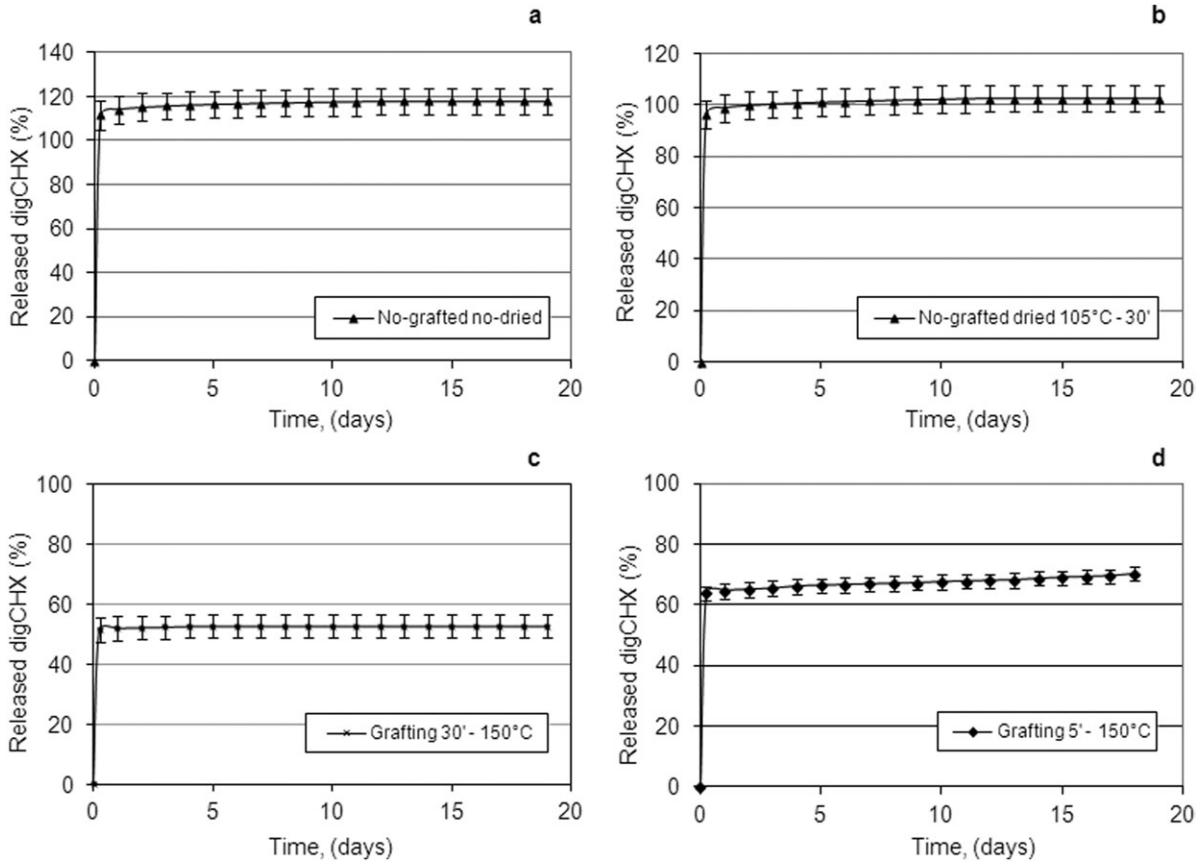

Figure 5. Accumulated release kinetics for noncoated paper samples compared to their theoretical loaded amounts (expressed as % of the initially loaded digCHX). (a and b) show the nongrafted samples while (c and d) show the grafted ones.

crosslinking density with citric acid. At the optimal conditions, a high amount of β-CDs are grafted on the substrate, but the strong cross-linking of the β-CDs layer, the citric acid, and the fibers with themselves produce a closed mesh, which makes cyclodextrin's cavities less accessible to the active molecules. From this point of view a lower grafting level lead to a higher prolonged release behavior, suggesting the necessity of finding a compromise.

The released amounts in the range of 0.2–0.5% correspond to digCHX concentrations around 4–10 mg/L. They may appear to be weak, but they are interesting from the point of view of bacterial inhibition.[41] At relatively low concentrations, the action of chlorhexidine is bacteriostatic, and at higher concentrations, it becomes rapidly bactericidal, with the actual levels varying somewhat from species to another. Chlorhexidine minimum inhibitory concentration (MIC) varies from 0.25 to 128 mg/L for a wide range of gram-positive and gram-negative bacteria.[42] Chlorhexidine's MIC against microorganisms found in endodontic infections was found to be in a range from 2.67 to 80.00 μg/mL.[43] Chlorhexidine's MICs were ranged from 0.625 to 50.00 μg/mL, in the case of pathogenesis related with skin diseases.[44]

The digCHX loaded in the nongrafted samples is completely released, while the sum of the released amounts for the grafted samples never reaches the 100% after 20 days of release kinetic. This means that there's a certain amount (probably negligible) of digCHX which remains strongly entrapped into the CDs cavities. The experience showed that when a longer period of time between two UV measurements is used (longer than 24 h), the amounts of released digCHX increase. It is therefore possible that 24 h is not enough to allow the entrapped digCHX to be liberated from the CDs, after a few days of starting the experiment. In spite of the impossibility to perform UV measurements (due to the small amounts released), a significant amount

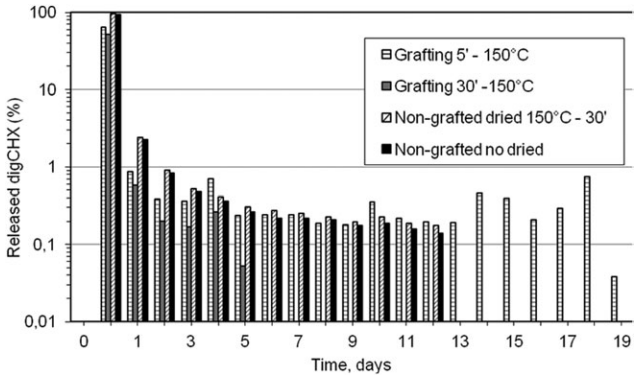

Figure 6. Prolonged digCHX release for noncoated paper samples as (%) of the initially loaded digCHX in the sheets.

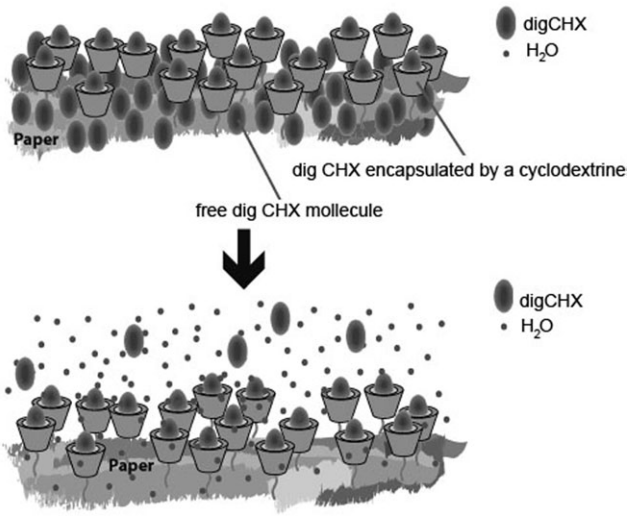

Figure 7. Schematic representation of massive release.

still remains onto the grafted sheets. The criterion used was to consider that experiments were finished when daily measurements were not possible to be performed. Such a rule was applied for all the investigated substrates.

Comparison Between the Cellulosic Substrates

Taking into account the previous results, grafted samples for 5 min at 150°C were tested for the liberation behavior of the other two cellulosic substrates. Table II summarizes the properties of the samples and the amount of the loaded digCHX on each substrate, before starting the investigation of the release kinetics. Similar observations on the digCHX release kinetics were obtained for crepe paper and medical bandage. The results show that in all cases the main part of the loaded digCHX is released in the first few hours.

For crepe paper, grafted samples retained around 30% of the digCHX after 12 h while, for the same delay, the nongrafted samples retained less than 4%. The release of digCHX was found to stop after 4 days for the nongrafted samples, whereas for the grafted samples (5 min-150°C) 9 days were necessary to reach the decay of the digCHX liberation. As previously

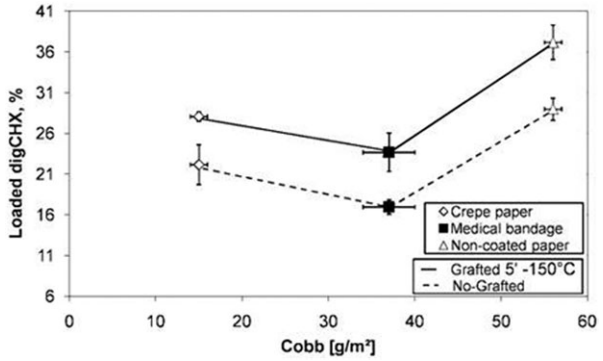

Figure 8. WG due to the loaded digCHX vs. Cobb absorption (in g/m^2) aiming at comparing the capacity of uptaking digCHX of the grafted and the nongrafted samples.

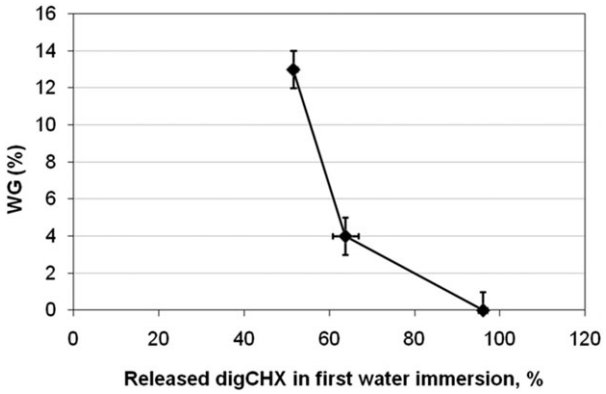

Figure 9. Relationship between the WG (%) and the released digCHX for the noncoated paper samples after the first water immersion of the liberation kinetic. The released digCHX is expressed as % of the initially loaded.

reported, the sum of the released amounts for the grafted samples does never reach the 100% after 9 days.

For the medical bandage, the retention of the digCHX, after the first measurement (12 h), was around 30% and 10% for the grafted and nongrafted samples, respectively. After 2 days, nongrafted samples did not release the digCHX anymore, whereas for grafted samples (5 min-150°C), this delay was extended to 4 days. Concerning the theoretical loaded amount of digCHX, it is completely released after 3 days for nongrafted samples, while for the grafted samples, it continued releasing, even at least, after 4 days.

It is therefore possible to conclude that grafted samples are capable to retain the active molecules and to release them for much longer periods. The nongrafted samples also retained digCHX molecules but their release was so fast that after few days they stopped to liberate such active molecules.

In a general way, all functionalized (grafted) substrates are unable to achieve the theoretical loaded digCHX levels within the period of release measurements, indicating that the active ingredient remains strongly retained by cyclodextrins and its release is much more slowed. Initially loaded digCHX consists of linked and nonlinked molecules on the grafted sheet's surface. When the study of liberation kinetic is started, there is a first fast step in which a massive release is produced (Figure 7). During this period, the water immersion produces an easy release of all the free digCHX molecules which are not strongly linked to CDs. Then, a very weak but steady step of releasing starts for the grafted samples. Nongrafted samples have not digCHX molecule anymore and consequently, the release is stopped. The ability of CDs to retain the active agent is clearly the reason for the prolonged release of the grafted samples.

Relationship Between Loaded digCHX and Cobb Absorbance

Cobb absorbance has an influence on the ability of samples to be loaded by digCHX, as shown in Figure 8. Taking into account the paper substrates, we can observe the higher is the Cobb absorption the greater is the loaded digCHX into the sheets. As previously explained, the load of digCHX is

Applied Polymer SCIENCE

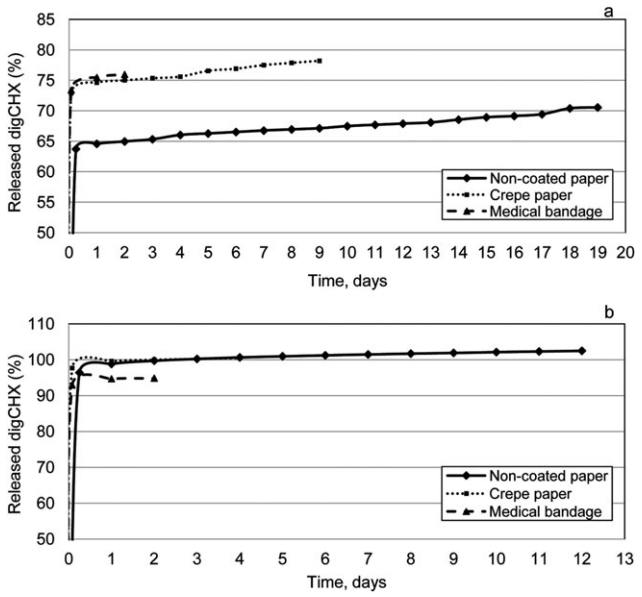

Figure 10. Release kinetics of (a) grafted and (b) nongrafted samples. The grafting conditions are 5 min at 150°C. The released digCHX is expressed as % of the initially loaded.

performed by soaking the sheets into an aqueous environment containing the active agent. The Cobb absorption has proven to be a good indicator to determine that the grafting and the loading of the grafted cellulose-based increase with increasing the Cobb values. Figure 8 also shows that grafted samples increase the ability of the sheets to be loaded with digCHX, for all the substrates.

Relationship Between Grafting Level and digCHX Retention

The ability to retain active agent when sheets are soaked into an aqueous environment under stirring is strongly related to the amount of grafted cyclodextrin within the sheets, as illustrated in Figure 9. In fact, this figure shows the relationship between the grafting level and the released digCHX, after the first cycle of water immersion for the noncoated paper samples. Higher is the grafting level, higher is the substrate ability to retain active molecules in the aqueous environment.

Comparison of Substrates in Terms of Liberation Behavior

The three tested substrates present different release behavior. Thus, the released amount of digCHX of the grafted samples (grafted 5 min-150°C), as a function of time, is shown in Figure 10(a). The noncoated substrate was found to retain the highest amounts of digCHX (release only about 65% in the first measurement). Moreover, it releases active agent for the longest time (19 days). Retention levels for crepe paper and the medical bandage are similar, but the release time lasts 9 days for crepe paper, and only 3 days for medical bandage. Grafted samples never reached the theoretical loaded amounts of digCHX when the kinetics were stopped.

Considering the nongrafted samples (dried at 105°C for 30 min) [Figure 10(b)], the noncoated paper is also the best substrate in terms of the release, since it lasted 12 days. The crepe paper and the medical bandage have released digCHX for periods of 4 and 2 days, respectively. In the nongrafted samples, the active agent released amounts were close to 100% and the release kinetics stopped earlier.

CONCLUSIONS

This study opens a new way of providing bactericidal properties to cellulose-based materials and more specifically to finished commercial paper substrates with different morphologies. Different kinds of paper substrates are compared for the first time, and an optimum of cellulose grafting is proposed. All the studied substrates can be successfully functionalized using the CDs technique, obtaining weight gain between 7% and 15%, depending on the initial substrate properties. It has been found that in all substrates the functionalized samples increased the substrate ability to maintain the release for longer time periods, reaching periods up to 20 days. The optimum grafting conditions, (based on the experimental conditions giving the highest WG) does not give the best material in terms of digCHX release performance. Thus, the best performing substrates in terms of digCHX delivery were those in which the WG was situated between 3% and 7%, that is, 5 min at 150°C. Cobb absorption test was found to be a good indicator to select the substrates to be grafted, as well as their ability to be loaded with active agent.

The obtained results allow building the foundation of a new area dealing with "biologically active cellulose paper substrates." Since the functionalization treatment is applied superficially, the paper manufacturing process is not affected, and the technique becomes very versatile to be potentially applied to a wide range of paper grades. However, this work is a first approach on the use of this functionalization technique applied to this kind of materials, and antimicrobial analysis will be carried out to confirm and characterize the antimicrobial effect.

ACKNOWLEDGMENTS

The authors are especially grateful to the FUNCICEL project (CTQ2009-12904) and BIOFIBRECELL project (CTQ2010-20238-CO3-01) within the framework of the Spanish's MICINN.

REFERENCES

1. Daoud, W. A.; Xin, J. H.; Zhang, Y. *Surf. Sci.* **2005**, *599*, 69.
2. Hu, A.; Tan, Y.; You, T.; Tang, Q. *Faming Zhuanli Shenqing Gongkai Shuomingshu* **2006**, 2005-10032452; 2005–10032452, 9.
3. Romi, R.; Lo Nostro, P.; Bocci, E.; Ridi, F.; Baglioni, P. *Biotechnol. Prog.* **2005**, *21*, 1724.
4. Bergamasco, R. C.; Zanin, G. M.; Moraes, F. F. *J. Inclusion Phenom. Macrocyclic. Chem.* **2007**, *57*, 75.
5. Lo Nostro, P.; Fratoni, L.; Baglioni, P. *J. Inclusion Phenom. Macrocyclic Chem.* **2003**, *44*, 423.
6. Dodziuk, H. Cyclodextrins and Their Complexes; Wiley-VCH: Germany, **2008**, p 489.
7. Szejtli, J.; Osa, T. Comprehensive Supramolecular Chemistry, Cyclodextrins; Pergamon Press: Oxford, **1996**; Vol. 3, p 693.

8. Duchene, D. New Trends in Cyclodextrins and Derivatives; Editions de Santé, Paris, **1991**, p 635.
9. Szejtli, J. Cyclodextrin Technology; Kluwer Academic Publishers: Dordecht, Holland, **1988**.
10. Szejtli, J. Cyclodextrins and Their Inclusion Complexes. 1st ed.; Akademiai Kidao, Budapest, **1982**, p 296.
11. Bender, M. L.; Komiyama, M. Cyclodextrin Chemistry, Springer Verlag, New York **1978**. p 96.
12. Krasowska, H.; Bojarski, *J. Farm. Pol.* **1991**, *47*, 239.
13. Grigoriu, A.; Luca, C.; Grigoriu, A. *Cell. Chem. Technol.* **2008**, *42*, 103.
14. Uekama, K. *Denpun Kagaku* **1983**, *30*, 247.
15. Gruiz, K.; Fenyvesi, E.; Kriston, E.; Molnar, M.; Horvath, B. *J. Inclusion Phenom. Mol. Recognit. Chem.* **1996**, *25*, 233.
16. Yoshii, H.; Furuta, T.; Shimizu, J.; Kugimoto, Y.; Nakayasu, S.; Arai, T.; Linko, P. *Biol. Zh. Arm.* **2001**, *53*, 226.
17. Uyar, T.; Hacaloglu, J.; Besenbacher, F. *React. Funct. Polym.* **2009**, *69*, 145.
18. Martel, B.; Blanchemain, N.; Boschin, F.; Haulon, S.; Delcourt-Debruyne, E.; Morcellet, M.; Hildebrand, H. F. *PCT Int. Appl.* **2006**, 2005-FR2829; 2004–12086, 41.
19. Martel, B.; Weltrowski, M.; Ruffin, D.; Morcellet, M. *J. Appl. Polym. Sci.* **2002**, *83*, 1449.
20. Voncina, B.; Vivod, V.; Chen, W. *J. Appl. Polym. Sci.* **2009**, *113*, 3891.
21. Le Thuaut, P.; Martel, B.; Crini, G.; Maschke, U.; Coqueret, X.; Morcellet, M. *J. Appl. Polym. Sci.* **2000**, *77*, 2118.
22. Martel, B.; Le Thuaut, P.; Crini, G.; Morcellet, M.; Naggi, A.; Maschke, U.; Bertini, S.; Vecchi, C.; Coqueret, X.; Torri, G. *J. Appl. Polym. Sci.* **2000**, *78*, 2166.
23. Martel, B.; Le Thuaut, P.; Bertini, S.; Crini, G.; Bacquet, M.; Torri, G.; Morcellet, M. *J. Appl. Polym. Sci.* **2002**, *85*, 1771.
24. Wang, J.; Cai, Z. *Carbohydr Polym.* **2008**, *72*, 695–700.
25. Bajpai, M.; Gupta, P.; Bajpai, S. K. *Fibers Polym.* **2010**, *11*, 8.
26. Tabary, N.; Lepretre, S.; Boschin, F.; Blanchemain, N.; Neut, C.; Delcourt-Debruyne, E.; Martel, B.; Morcellet, M.; Hildebrand, H. F. *Biomol. Eng.* **2007**, *24*, 472.
27. Hoang Thi, T. H.; Chai, F.; Lepretre, S.; Blanchemain, N.; Martel, B.; Siepmann, F.; Hildebrand, H. F.; Siepmann, J.; Flament, M. P. *Int. J. Pharm.*. **2010**, *400*, 74.
28. Tang, W.; Zhao, J.; Sha, B.; Liu, H. *J. Appl. Polym. Sci.* **2012**.
29. Xu, J.; Li, X.; Sun, F.; Cao, P. *J Biomater Sci, Polym. Ed.* **2010**, *21*, 1023.
30. Blanchemain, N.; Karrout, Y.; Tabary, N.; Neut, C.; Bria, M.; Siepmann, J.; Hildebrand, H. F.; Martel, B. *Acta Biomater.* **2011**, *7*, 304.
31. Martel, B.; Morcellet, M.; Ruffin, D.; Ducoroy, L.; Weltrowski, M. *J. Inclusion Phenom. Macrocyclic Chem.* **2003**, *44*, 443.
32. Blanchemain, N.; Karrout, Y.; Tabary, N.; Bria, M.; Neut, C.; Hildebrand, H. F.; Siepmann, J.; Martel, B. *Carbohydr. Polym.* **2012**, *90*, 1695.
33. Caulfield, D. F. *Tappi J.* **1994**, *77*, 205.
34. Yang, C. Q. Book of Abstracts, 217th ACS National Meeting, Anaheim, CA, March 21–25, **1999**; CELL-026.
35. Yang, C. Q.; Xu, Y.; Wang, D. *Ind. Eng. Chem. Res.* **1996**, *35*, 4037.
36. Boschin, F.; Blanchemain, N.; Bria, M.; Delcourt-Debrayne, E.; Morcellet, M.; Hildebrand, H. F.; Martel, B. *J. Biomed. Mater. Res. Part A.* **2006**, *79*, 78.
37. Aguiar, M. M. G. B.d.; Pereira de Albuquerque, R.; Marinho, D. S.; Braga, B. R. S.; Dornelas, C. B.; Oliveira, A.; Pereira de Sousa, V.; Torres, S. R.; Alviano, D. S.; Alviano, C. S.; Cabral, L. M.; Holandino, C. *Drug Dev. Ind. Pharm.* **2010**, *36*, 594.
38. Wu, W.; Xi, Z.; Xue, H.; Zhang, K.; Li, J.; Chen, Y. *Yiyao Daobao* **2005**, *24*, 624.
39. Yao, X.; Wang, L.; Xu, Y.; Yang, C.; Luo, B. *Zhongchengyao* **2009**, *31*, 789.
40. Zhan, G.; Pan, D. *Zhongguo Yaofang* **2006**, *17*, 1175.
41. Woodcock, P. M. In Industrial Biocides. Critical Reports on Applied Chemistry; Payne, K. R., Ed.; John Wiley & Sons: Chichester, New York, **1988**, p 19.
42. Denton, G. W. In Disinfection, Sterilization, and Preservation; Block, S. S. Ed.; Lea & Febiger: Philadelphia, **1991**, p 274.
43. do Amorim Crystiane, V. G.; Aun, C. E.; Mayer Marcia, P. A. *Braz. Oral Res.* **2004**, *18*, 242.
44. Odore, R.; Colombatti, V. V.; Re, G. *Vet. Res. Commun.* **2000**, *24*, 229.