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Miconazole-loaded 6-oxychitin–chitosan microcapsules

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Abstract

Oxychitin–chitosan microcapsules were prepared and assayed for the purpose of delivering miconazole. The microcapsules formed upon contacting 4% oxychitin solution with 2% chitosan solution, both containing 1–2% calcium chloride. The encapsulation efficiency for miconazole was 49–67%. The drug-loaded microcapsules (1–2 mm diameter) were spherical, translucent and stable over the observation period of 7 days in the wet state. The microcapsules disintegrated in the presence of sodium dodecyl sulfate at pH 7.1 and the delivery of miconazole was delayed compared to control. The microcapsules were also degraded by lysozyme that, however, was partially inhibited by the oxychitin-rich fragments released by the external layer as well as by the oxychitin entrapped in the core. Oxychitin was also found to be biocompatible towards human keratinocytes that normally grew in the presence of uronans added to the culture medium. It is concluded that the polyelectrolyte complex formed by oxychitin and chitosan is safe and suitable for the preparation of microcapsules, whose performances depend on the selection of the preparation conditions. Oxychitin offers the advantage of depressing the hydrolytic action of lysozyme on the chitosan present in the complex, thus retarding the drug release.

Keywords: Chitosan; Oxychitin; Miconazole; Microcapsules; Drug delivery; Lysozyme

1. Introduction

Among biodegradable polymers, chitosan has increasing importance in the field of drug delivery due to its versatility in pharmaceutical applications. Chitosan, poly[β(1 → 4)-2-amino-2-deoxy-D-glucopyranose], a hydrophilic and biocompatible polymer, displays some dose dependent bioactivity. For example, it inhibits the adhesion of *Candida albicans* to human vaginal epithelial cells thus preventing the development of mycosis, and also has biostimulatory activity in reparative processes of wounded tissues (Jollès & Muzzarelli, 1999; Knapzyk, Macura, & Paulik, 1992; Leher, Segal, & Barr-Nea, 1983). During the last few years this polymer has been studied and used to afford microcapsules and microspheres with the intention of modifying drug release rates, as an excipient for tablets in direct tableting process and as a functional component of wound dressings.

The polyuronan 6-oxychitin is a new chitin derivative obtained by regiospecific oxidation of chitin (Muzzarelli, Muzzarelli, Cosani, & Terbojevich, 1999; Muzzarelli et al., 2000). The oxychitin sodium salt has an average molecular weight of around 9000 Da, degree of oxidation close to 1, complete water solubility over the pH range, 3–12, and the capacity to flocculate proteins; moreover, it is quite compatible with living tissues.

The manufacture of microcapsules by complex coacervation of oxychitin and chitosan required chitosan salts of molecular weight ranging between 150 and 300 kDa, the molar ratio of chitosan to oxychitin between 1:3 and 1:6 (Muzzarelli et al., 1999a; Muzzarelli, Stanic, & Ramos, 1999b), 2% chitosan and at least 1% (preferably 4%) $CaCl_2$ concentration, i.e. the viscosity of the chitosan starting solution above 209 mPa s. The presence of $CaCl_2$ in both chitosan and oxychitin solutions was also necessary for stabilising the complex by cross-linking. The preparation method was straightforward, relatively fast and did not involve the use of organic solvents or high temperature.

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Oxychitin provides an alternative to alginate (Gaserod, Smidsrod, & Skjak-Braek, 1998; Daly & Knorr, 1988; Gaserod, Sannes, & Skjak-Braek, 1999; Takahashi, Takayama, Machida, & Nagai, 1990; Kubota & Kikuchi, 1998), having the advantage of producing microcapsules where both the anionic and the cationic compounds have the same origin, i.e. chitin, and are endowed with biological significance.

Scope of the present work was the preparation of drug-loaded oxychitin–chitosan microcapsules by complex coacervation, their characterization and the evaluation of their in vitro dissolution. Miconazole nitrate, a well-known synthetic broad spectrum antimycotic agent that inhibits the growth of common dermatophytes, is usually administered topically for the treatment of mucosal mycotic infections and oral–vaginal candidiasis.

Because of the intended application to oral and vaginal mucosae, the breakdown and dissolution profile of the microcapules was here evaluated in the presence of lysozyme. This muramidase hydrolyses chitosan by splitting the β(1 → 4) glycosidic bond (Kurita et al., 1996; Muzzarelli, 1992, 1997). For the administration of microcapsules it is important to know how lysozyme degrades the oxychitin–chitosan polyelectrolyte complex, and how biocompatible the latter is towards human cells.

2. Experimental

2.1. Materials

Chitosan glutamate salt, Protasan G210 Mw 150,000 Da, degree of deacetylation 0.86, was purchased from Pronova, Oslo, Norway. 6-Oxychitin sodium salt, Mw 9000 Da, white amorphous powder, soluble in the pH range 3–12, with degree of oxidation ca. 1, was obtained according to Muzzarelli et al. (1999a,b) by regiospecific oxidation. An aqueous suspension of lobster chitin (1 g dry weight + 50 g water) to which the stable nitroxyl radical 2,2,6,6-tetramethyl-1-piperidinyloxy (Tempo®, 12 mg) together with NaBr (0.4 g) was added, was treated with NaOCl (20 ml, 4%). Immediately after introduction of the latter, the pH was adjusted to 10.8 using 0.5 M NaOH and maintained to this value for 30 min; after this time the suspension turned clear. Oxychitin was isolated by dialysis and freeze-dried.

The glucomannans from salep (dry tubers of *Orchis*, Orchidaceae, Merck Index number 8475) and from konjak (traditional food in Japan from the tubers of *Amorthophallus konjak*) (Kishida, Okimasu, & Kamata, 1978), and the pullulan from *Aureobasidium pullulans* were treated in a similar way, and the corresponding uronans, namely oxypullulan and oxyglucomannans, were produced and isolated (Cescutti, Pupulin, Delben, Abbate, Dentini, Sparapano, Rizzo, Crescenzi (2002); Muzzarelli, Mattioli-Belmonte, Miliani, Muzzarelli, Gabbanelli, & Biagini, 2002).

Cibacron Brilliant Red 3B-A (reactive Red 4, C.I. 18105), glycine, *Micrococcus luteus,* miconazole nitrate (1-[2,4-dichloro-β-(2,4-dichlorobenzyl-oxy)phenethyl]imidazole, Merck Index n°. 6266), chicken egg white lysozyme and Tempo®, were purchased from Sigma-Aldrich, Milan, Italy. Sodium lauryl sulfate was from Janssen Chemicals, Beerse, Belgium; sorbitol was from Merck, Milan, Italy; lactose and other compounds were from Farmitalia Carlo Erba, Milan, Italy. All solvents were analytical grade; methanol and acetonitrile were HPLC grade.

The keratinocyte continuous and non-tumourigenic cell line NCTC 2544 was used with buffered isotonic minimum essential medium (MEM) in thermostated chambers at 37 °C under CO_2 control (5%). MEM contained fetal calf serum (10%), penicillin (5000 units), streptomycin (5 mg) in 0.9 M NaCl (1%), L-glutamine (200 mM, 1%), non-essential aminoacids (1%), and amphotericin-B.

2.2. Microcapsule preparation

Chitosan glutamate was dissolved in water together with $CaCl_2$; oxychitin sodium salt and $CaCl_2$ were dissolved in water at different concentrations as shown in Table 1. The oxychitin aqueous solution was mixed with miconazole in methanol solution at 1% w/v final concentration, and stirred to obtain a uniform suspension (due to its low water solubility the drug partially precipitated in the aqueous oxychitin solution). One millilitre of this suspension was carefully dropped, through a syringe with a 18 G needle, into 10 ml of chitosan + $CaCl_2$ solution and gently stirred. Formation of gelatinous microcapsules took place immediately as soon as the drops of oxychitin contacted the chitosan solution. The microcapsules were allowed to settle for 1 h; after this time they were treated with 10 ml of saline and the supernatant was discarded; then the microcapsules were rinsed twice with saline (10 ml) and every time the supernatant was discarded. Finally, they were placed in water and freeze dried at −40 °C and 40 mbar for 48 h. Table 1 reports the composition of the solutions used to make four batches of microcapsules.

Table 1
Concentrations (w/v%) of the solutions used to prepare various batches of microcapsules

Number	Chitosan solution, 10 ml		Oxychitin solution, 1 ml	
	Chitosan glutamate	$CaCl_2$	Oxychitin sodium salt	$CaCl_2$
1	2	4	4	4
2	2	2	4	2
3	2	1	4	1
4	2	2	2	2

2.3. Microcapsule morphology

Dry and wet microcapsules were photographed with a Sony digital Mavica camera. The outer and inner surfaces of dried and wet microcapsules were examined by scanning electron microscopy (Jeol JX 840-A, Jeol Ltd., Tokyo, Japan). Granulometric analysis of microcapsules was performed by sieving, with a sieve series between 1 and 2 mm.

Swelling was determined by sizing the microcapsule diameter before and after soaking in saline (NaCl 0.9% w/v) for fixed times (15, 30, 45, 60, 75, and 90 min).

2.4. Biocompatibility of oxychitin and other uronans

Human keratinocytes (3×10^4 cells) were transferred to fresh growth medium (1 ml per well) containing either oxychitin (0.3%) or one of the other uronans. Cells were counted in triplicate every 24 h, and fresh growth medium was replenished in each well. The Burker chamber and an optical microscope were used. Growth curves showing the cell number increase with time were then plotted.

2.5. Analytical chemistry

Infrared spectra were recorded as previously reported (Muzzarelli et al., 2000). Differential scanning calorimetry (DSC) was performed on both polymers, on the drug, on blank and miconazole loaded microcapsules. For this purpose a calorimeter model 12E (Mettler, Grefenser, Switzerland) equipped with a cryostat D8-G (Haake, Karlsruhe, Germany) was used; scanning was performed in a temperature range between 20 and 400 °C at heating rate of 5 K/min. X-ray diffractometry was performed with a powder diffractometer PW1750 model 1050 (Philips, Eindhoven, Netherlands).

2.6. Reaction index determination

The amount of chitosan that reacted with oxychitin to produce the microcapsules (reaction index) was determined by colorimetry with the anionic reactive dye Cibacron Brilliant Red (Muzzarelli et al., 1999b; Muzzarelli, 1998). The dye concentration was 7.5 mg/l; the calibration curve was drawn for chitosan concentrations in the 7–40 μg/ml range, and the absorbance values were determined with a Beckman DU spectrophotometer at the 575 nm wavelength. The analyses to determine the chitosan reaction index were performed on the chitosan solutions before microcapsule preparation and on final microcapsule supernatants after 1:1 dilution with saline and microcapsule stabilisation. Briefly, 4 ml of water were added to 1 ml of all chitosan solutions, containing $CaCl_2$, and tested before microcapsule preparation, 2 ml of water were added to the supernatants recovered from the first rinse after microcapsules preparation, 25 μl of these solutions were added to 275 μl of 0.1 M glycine hydrochloride buffer solution and then to 3 ml of dye solution, and the solutions analysed. The reaction index was calculated as the difference between the initial and residual amount of chitosan in the supernatant after microcapsule preparation. The values of the reaction index are expressed as percentages of the stoichiometric amount of chitosan that would react with one gram of oxychitin.

2.7. Colorimetric determination of chitosan on miconazole microcapsules in lysozyme solution

Colorimetric determinations of chitosan released from the microcapsules by lysozyme were carried out on all batches of miconazole microcapsules. Fifty microlitre aliquots were withdrawn from the microcapsule suspension, at fixed times up to 24 h; the withdrawn volume was replaced with fresh buffer. The 50 μl aliquot was diluted with 450 μl of buffer, 300 μl of this mixture were added to 3 ml of dye solution and assayed colorimetrically.

2.8. Drug content determination

The miconazole content in the microspheres was determined by HPLC following a method suitably modified (Cavrini, DiPietro, & Gatti, 1989). Microcapsules (5 mg) were dissolved in CH_3OH + 0.1 M HCl 1:1 mixture (10 ml), the samples were sonicated and filtered before analysis. Analyses were carried out by an HPLC model 9010 (Varian, Milan, Italy) equipped with an UV–Vis detector Varian Varichrom UV2550, at 230 nm and a LiChrospher® rp 18 (250 mm × 5 mm × 5 μm) column, the mobile phase was a 85:15 v/v mixture of methanol and triethylammonium acetate, pH 7, the flow rate was 1.2 ml/min.

2.9. Chemical degradability of microcapsules

The microcapsules were suspended for 15 days at 37 °C in the following media: water, saline, 0.1 M HCl, 15 mM SDS pH 7.1, water/methanol mixture 70:30.

2.10. Biodegradability of microcapsules

The microcapsules were soaked in lysozyme solution for 24 h; at fixed times 60 μl of solution were withdrawn and analysed for lysozyme specific activity and chitosan concentration. Lysozyme specific activity was calculated with the following equation:

$$\text{Specific activity : U/mg} = \Delta A_{450}/\text{min}/0.001 \times 0.5 \times [\text{Lysozyme}]$$

Lysozyme activity vs time at different temperatures: lysozyme activity was studied after 2, 20, 44, 68, 190 and 340 h at 4 and 37 °C, starting from 1 μg/ml lysozyme concentration. Lysozyme activity at 22 °C, under the same conditions, was taken as reference.

Lysozyme activity in the presence of oxychitin: lysozyme activity was evaluated after incubation at 37 °C for 1, 2, 3, 5, 20, 24 h in the presence of different concentrations of oxychitin (0.01, 0.05, 0.10, 0.50 and 1.00 mg/ml); four different concentrations of lysozyme were used (0.001, 1, 2.5 and 15 mg/ml).

Lysozyme activity in the presence of chitosan: lysozyme activity was evaluated after incubation at 37 °C for 1, 2, 3 and 5 h in the presence of 0.5 and 1 mg/ml chitosan solutions; lysozyme was used at 1 and 2.5 mg/ml.

Lysozyme activity in the presence of miconazole microcapsules: lysozyme activity was evaluated after incubation at 37 °C with 2 mg of each batch of miconazole microcapsules. The following procedure was used: 2 mg of microcapsules were placed in 1 ml of a 1 mg/ml lysozyme solution; 10 μl of the solution were withdrawn after fixed times, up to 24 h and replaced with the same volume of 1 mg/ml lysozyme solution. Water (990 μl) was added to each 10 μl withdrawn, and 0.5 ml of the resulting solution were used to determine the lysozyme activity.

2.11. In vitro dissolution test

In vitro dissolution tests of miconazole loaded microcapsules were carried out on the four batches prepared and on free lyophilized miconazole (not entrapped in the microcapsules), whose dissolution profile was taken as control. The dissolution media used were either 15 mM SDS or 15 mM SDS containing 0.1% lysozyme. SDS was added to the dissolution medium because of the very low solubility of miconazole in water, to simulate the solubilizing effect exerted by human bile acids. Solubility tests of miconazole in SDS were performed by HPLC to afford the dissolution profile of the drug in a SDS medium. The in vitro dissolution tests were performed in screw cap bottles; 10 mg of microcapsules were placed in 10 ml of 15 mM SDS solution at pH 7.1 and 37 °C and shaken continuously. Aliquots (500 μl) of dissolution medium were removed at fixed times, and replaced with fresh medium. The data obtained were reported as percent of miconazole released vs time.

3. Results and discussion

3.1. Microcapsule characterization

Microcapsules were promptly formed upon contact of the oxychitin solution with the chitosan solution, under the conditions described in Table 1. The granulometric analysis showed that the batches were quite homogeneous, their size being smaller than 1.5 mm (about 25% by weight), and smaller than 2 mm (about 75% by weight).

Miconazole-loaded microcapsules, batches 2, 3 and 4, were well defined, spherical and stable, even after freeze-drying. Batch 1 was less stable; the freeze-drying process caused microcapsule deformation and aggregation.

The morphological evaluation of the microcapsules by SEM (Fig. 1) showed that the outer surface was smooth with no evident porosity; after soaking in saline solution (Fig. 1(b)) the aspect of the outer surface changed and provided evidence of swelling. SEM analysis of the dry or hydrated inner microcapsule membrane (Fig. 1(c) and (d)) revealed the presence of miconazole crystals.

Swelling in saline was quite fast, maximum swelling was observed for miconazole-loaded microcapsules after 30 min soaking and corresponded to an average 20% diameter increase. Further soaking in saline solution did not seem to affect the microcapsule size and shape. The presence of miconazole inside the microcapsules limited their swelling.

The infrared spectra of miconazole and miconazole-loaded microcapsules showed that the drug peaks were superimposed to those of the ionic complex. They showed the composite band at 1630 cm^{-1} resulting from the superimposed oxychitin bands (the carboxylate bands at 1618 and the amide bands at 1650 and 1558 cm^{-1}) with the chitosan bands at 1664 cm^{-1} (CO-hydrogen bonded to NH) and 1591 cm^{-1} (amide II).

Chitosan and oxychitin presented characteristics exothermic peaks at the differential scanning calorimetry, at 270 and 265 °C, respectively, not detected in blank microcapsules, suggesting complex formation. Moreover, both polymers had endothermic peaks below 100 °C, that were maintained in the blank microcapsules (probably due to loss of water). The raw drug presented an endothermic peak at 180 °C whose sharpness was indicative of a pure substance. This peak was partially maintained in the DSC profile for miconazole-loaded microcapsules for batch 1. Not all the miconazole-loaded microcapsules had the same DSC profile: sometimes the miconazole peak was dramatically reduced and other peaks appeared in proximity or superimposed to those belonging to the complex below 100 °C. These results suggested that miconazole was in a crystalline form inside the microcapsules, even though some interaction occurred between drug and polysaccharides (Fig. 2).

While the polyelectrolyte complexes were amorphous and did not present characteristic diffraction peaks, X-ray diffractograms of miconazole-loaded microcapsules showed depressed drug crystallinity.

3.2. Drug content and chitosan reaction index

Batches 1, 2 and 3 resulted in good encapsulation efficiency, the values obtained were well reproducible as shown by the low standard deviations. Batch 4, prepared using oxychitin at 2% w/v concentration, resulted in higher encapsulation efficiency but also high variability in drug

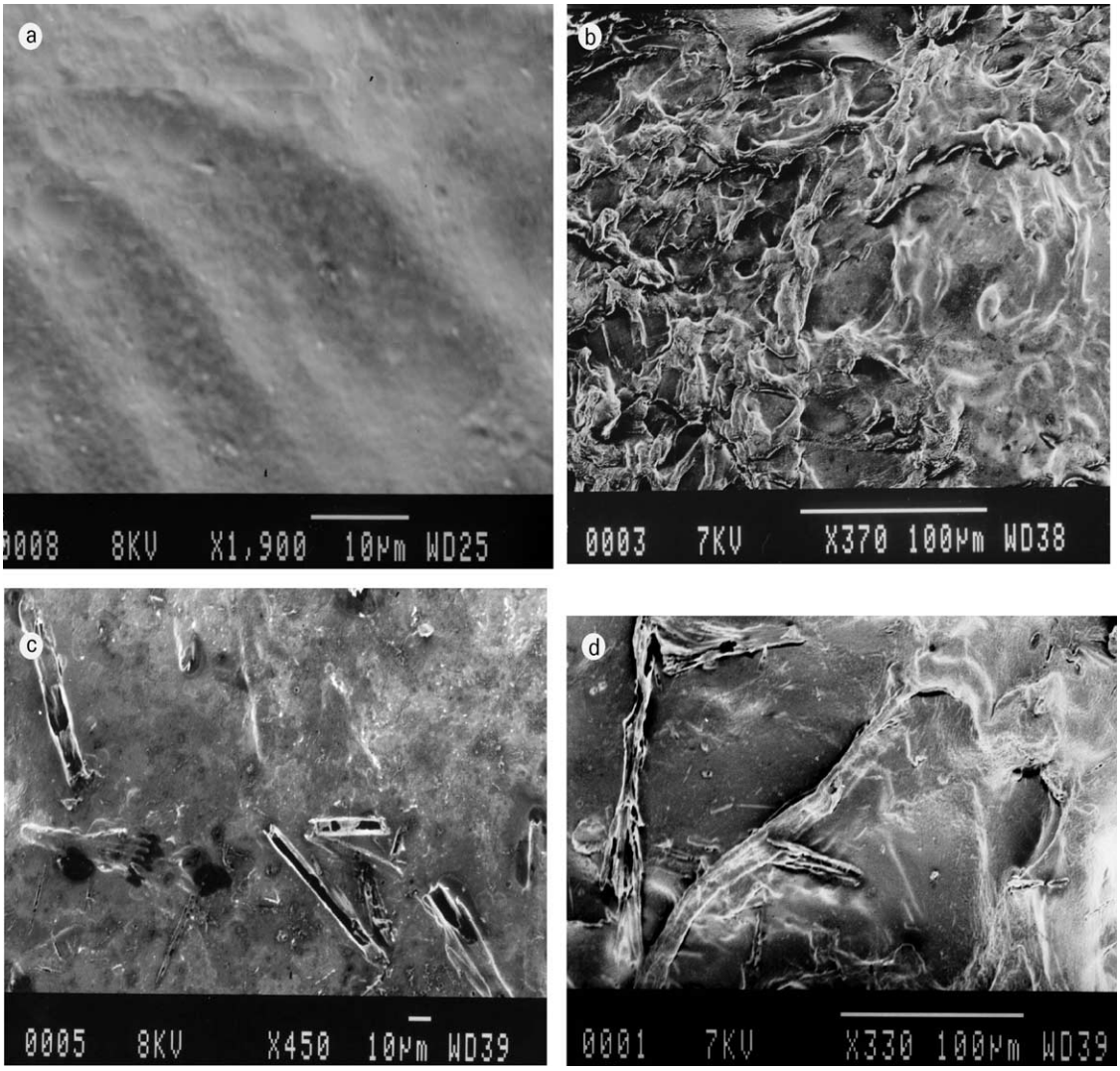

Fig. 1. Micrographs of the microcapsule membranes: (a) outer membrane, dry; (b) outer membrane, wet; (c) inner membrane, dry; (d) inner membrane, wet.

content, underlined by the large standard deviation values (Table 2).

Batches 2 and 3, prepared with 4% oxychitin and 1 and 2% $CaCl_2$, respectively, had the highest chitosan reaction indexes indicative of the highest microcapsule stability; moreover, the small standard deviations indicated good reproducibility. The data for batches 1 and 4 proved that membrane formation was hampered and these preparations were less reproducible.

3.3. Effects of polysaccharides on lysozyme

The results of the lysozyme assay showed first of all that the enzyme was stable for the period of time tested (11 days) at 4 and 37 °C, and that its activity was not depressed by the presence of chitosan, as expected in view of the fact that chitosan is a substrate for lysozyme (Muzzarelli, 1992).

A linear decrease of lysozyme activity was brought about by oxychitin in the concentration range 0.01–1.00 mg/ml, from 33 to 17 U/mg, upon 1 and 2 h contact time at 37 °C.

For 5 h contact the 0.05–0.10 mg/ml oxychitin concentration lowered the lysozyme activity by ca. 25%. These

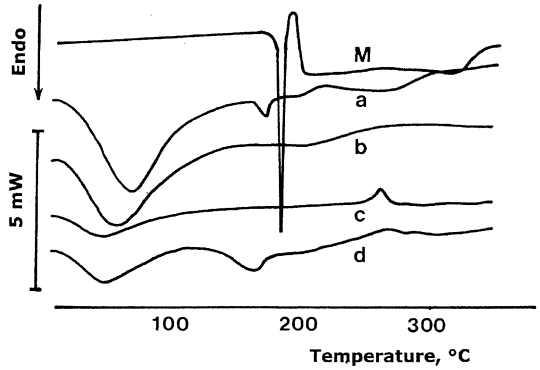

Fig. 2. Differential scanning calorimetry curves for (M) plain miconazole, (a) miconazole-loaded microcapsules, (b) blank microcapsules, (c) oxichitin, (d) chitosan.

Table 2
Drug encapsulation efficiency and chitosan reaction index

Number	Drug encapsulation efficiency, % ± std dev.	Reaction index, % ± std dev.
1	41.18 ± 5.2	33.50 ± 10
2	67.47 ± 1.2	41.00 ± 0.8
3	48.78 ± 1.4	41.22 ± 3.0
4	84.36 ± 11.5	35.78 ± 18

The reaction index corresponds to the percent of the stoichiometric amount of chitosan that would react with 1 g of oxychitin to form the microcapsules.

results showed the high affinity of oxychitin for proteins, and the capacity of oxychitin to exert a partial inhibition of lysozyme.

3.4. Biodegradation of miconazole loaded microcapsules

Microcapsules swelled in saline, 0.1 M HCl, SDS solution and methanol/water mixture, but remained undamaged translucent spheres, even after 170 h. The microcapsules immersed in lysozyme solution initially swelled, but after 1 h and a half progressive detachment of fragments took place from the outer membrane. Those fragments disintegrated later on. Therefore lysozyme was active not only on chitosan, but also on its polyelectrolyte complex.

All microcapsules were completely degraded after 4 h incubation; correspondingly, the lysozyme activity was lowered to 2% of the initial activity. Fig. 3 shows the parabolic activity decrease with time for the four microcapsule batches, the differences among batches were not significant. The decrease of enzymatic activity was attributed to the release of oxychitin-rich fragment from the electrolyte complex.

The biodegradation was followed by measuring the amount of chitosan released from the microcapsules after enzymatic attack. Batch 1 showed the highest chitosan release rate. The compositions of batches 2 and 3 seemed to

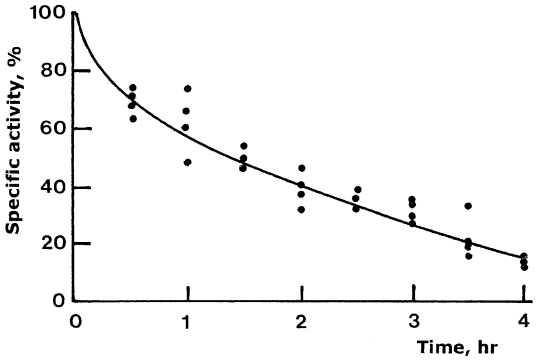

Fig. 3. Lysozyme specific activity as a function of time of contact with oxychitin–chitosan microcapsules. The curves for the four batches of microcapsules are not significantly different.

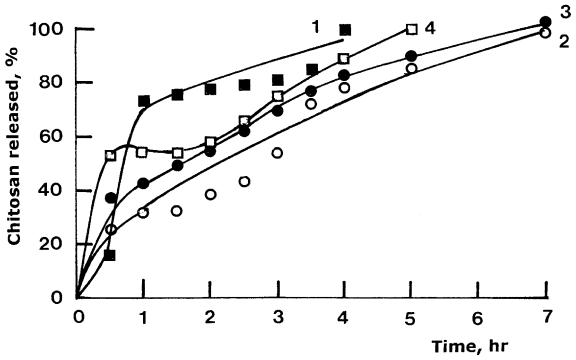

Fig. 4. Chitosan release as a function of time of contact with oxychitin–chitosan microcapsules. Numbers refer to the four batches of chitosan.

be optimal for their stability: these batches were less susceptible to the enzyme due to the higher bond strength between chitosan and oxychitin. Fig. 4 shows in fact that for batches 1 and 4 the chitosan release was was over 50% in 1 h and complete in 4 h, while for batches 2 and 3 it was ca. 40% in 1 h and required at least 7 h to go to completion.

3.5. In vitro dissolution

In vitro dissolution profiles of the miconazole loaded microcapsules and the drug alone in 15 mM SDS pH 7.1 are reported in Fig. 5 where a significant difference among the dissolution rates of the four batches of microcapsules is evident. The highest dissolution rates were obtained for batches 1 and 4, while batches 2 and 3 had slower dissolution profiles: with batch 3, 90% of the drug release was achieved in 7 h, and was completed in 20 h. The presence of lysozyme in the dissolution medium increased significantly the drug dissolution rate as 100% of drug was released from all batches after about 2 h.

3.6. Biocompatibility of oxychitin

The growth of keratinocytes in minimum essential medium modified to incorporate oxychitin, oxypullulan or

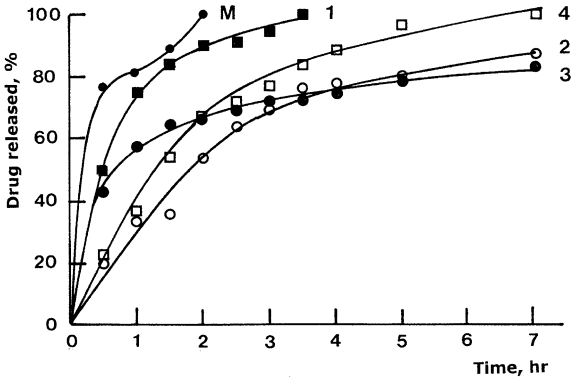

Fig. 5. Release of miconazole from oxychitin–chitosan microcapsules as a function of time of contact with lysozyme. Numbers refer to the four batches of chitosan. M is for plain miconazole.

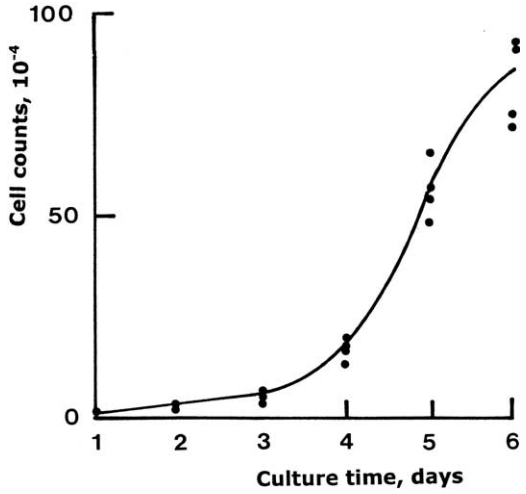

Fig. 6. Growth curve for keratinocytes in the presence of oxychitin. Standard deviations omitted for clarity; the oxychitin curve is not significantly different from those of the other uronans.

oxyglucomannans from salep and konjak was described in terms of cell number increase with time during the cell logarithmic phase. The data in Fig. 6 for the four polysaccharides were not significantly different from the control, and therefore they indicated full biocompatibility with human keratinocytes.

This information is of value in assessing the biocompatibility of the polyelectrolyte complex; in fact, the biocompatibility of chitosans has already been assessed (Berscht, Nies, Liebendorfer, & Kreuter, 1995; Guerra, Cerrai, Tricoli, Maltinti, & Sbarbati, 1998), but for oxychitin direct biocompatibility data are here presented for the first time. Some indirect evidence of biocompatibility of both polysaccharides with human cells is available (Muzzarelli et al., 2001; Jollès & Muzzarelli, 1999; Mattioli-Belmonte et al., 1999).

4. Conclusions

The most suitable conditions for the preparation of miconazole-loaded oxychitin–chitosan microcapsules are: 4% oxychitin + 1 to 2% $CaCl_2$ + miconazole 1% dropped in excess 2% chitosan + 1 to 2% $CaCl_2$. The encapsulation efficiency, typically 49–67%, and the reproducibility of the preparations were satisfactory.

The microcapsules are characterised by an external layer of polyelectrolyte complex and a core of oxychitin with miconazole crystals. Their swelling in various media amounted to 20–45% and made the microcapsules translucent: they were, however, stable for at least one week in the wet form. A modest interaction between miconazole and the polysaccharides in the microcapsule was detected by X-ray diffraction and DSC.

Dissolution of the microcapsules took place under the action of chemicals and enzymes: while sodium dodecyl sulphate dissolved completely the loaded microcapsules in 20 h, lysozyme degraded them in shorter times, by splitting anhydroglycosidic bonds in the partially deacetylated chitosan. The progress of the enzymatic hydrolysis was, however, depressed by the oxychitin present in the polyelectrolyte complex and in the core. The release of miconazole may be modulated by proper preparation conditions and may be retarded mainly by the inhibitory action of oxychitin upon lysozyme. Both chitosan and oxychitin are biocompatible with human cells and therefore their polyelectrolyte complexes can be used safely.

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References

Berscht, P. C., Nies, B., Liebendorfer, A., & Kreuter, J. (1995). In vitro evaluation of biocompatibility of different wound dressing materials. *Journal of Material Science, Materials in Medicine*, *6*, 201–205.

Cavrini, V., DiPietra, A. M., & Gatti, R. (1989). Analysis of miconazole and econazole in pharmaceutical formulation by derivative UV-spectroscopy and liquid chromatography. *Journal of Pharmaceutical and Biomedical Analysis*, *7*, 1535–1543.

Cescutti, P., Papulin, R., Delben, F., Abbate, M., Dentini, M., Sparapano, L., Rizzo, R., & Crescenzi, V. (2002). New exopolysaccharides produced by Aureobasidium pullulans grown on glucosamine. *Carbohydrate Research*, *337*, 1203–1209.

Daly, M. M., & Knorr, D. (1988). Chitosan–alginate complex coacervate capsules: Effects of calcium chloride, plasticizers and polyelectrolytes on mechanical stability. *Biotechnology Progress*, *4*, 76–81.

Gaserod, O., Sannes, A., & Skjak-Braek, G. (1999). Microcapsules of alginate–chitosan. II. A study of capsule stability and permeability. *Biomaterials*, *20*, 773–783.

Gaserod, O., Smidsrod, O., & Skjak-Braek, G. (1998). Microcapsules of alginate-chitosan. I. A quantitative study of the interaction between alginate and chitosan. *Biomaterials*, *19*, 1815–1825.

Guerra, G. D., Cerrai, P., Tricoli, M., Maltinti, S., & Sbarbati, R. (1998). In vitro cytotoxicity testing of chitosan-containing polyelectrolyte complexes. *Journal of Material Science, Materials in Medicine*, *9*, 73–76.

Jollès, P., & Muzzarelli, R. A. A. (Eds.), (1999). *Chitin and Chitinases*. Basel, Switzerland: Birkhauser Verlag.

Kishida, N., Okimasu, S., & Kamata, T. (1978). Molecular weight and intrinsic viscosity of konjak glucomannan. *Agricultural Biological Chemistry (Tokyo)*, *42*, 1645.

Knapzyk, K. J., Macura, A. B., & Paulik, B. (1992). Simple tests demonstrating the antimycotic effect of chitosan. *International Journal of Pharmacy*, *80*, 33–38.

Kubota, N., & Kikuchi, Y. (1998). Macromolecular complex of chitosan. In S. Dumitriu (Ed.), *Polysaccharides: Structural Diversity and Functional Versatility* (pp. 595–625). New York: Marcel Dekker.

Kurita, K., Akao, H., Kaji, Y., Kojima, T., Hirakawa, M., Kato, M., Ishii, S., & Mori, T. (1996). Relationship between lysozyme susceptibility and

the structure of chitin and its derivatives. In R. A. A. Muzzarelli (Ed.), (*Vol. 2*) (pp. 483–490). *Chitin Enzymology*, Italy: Atec.

Leher, N., Segal, E., & Barr-Nea, L. (1983). In vitro and in vivo adherence of *Candida albicans* to mucosal surface. *Annals of Microbiology, 134B*, 293–306.

Mattioli-Belmonte, M., Nicoli-Aldini, N., DeBenedittis, A., Sgarbi, G., Amati, S., Fini, M., Biagini, G., & Muzzarelli, R. A. A. (1999). Morphological study of bone regeneration in the presence of 6-oxychitin. *Carbohydrate Polymers*, *40*, 23–27.

Muzzarelli, R. A. A. (1992). Depolymerization of methyl pyrrolidinone chitosan by lysozyme. *Carbohydrate Polymers*, *19*, 29–34.

Muzzarelli, R. A. A. (1997). Depolymerization of chitins and chitosans with hemicellulase, lysozyme, papain and lipase. In R. A. A. Muzzarelli, & M. G. Peter (Eds.), *Chitin Handbook* (pp. 153–163). Italy: Atec.

Muzzarelli, R. A. A. (1998). Colorimetric determination of chitosan. *Analytical Biochemistry*, *260*, 255–257.

Muzzarelli, R. A. A., Biagini, G., DeBenedittis, A., Mengucci, P., Majni, G., & Tosi, G. (2001). Chitosan-coated prosthetic materials. *Carbohydrate Polymers*, *45*, 35–41.

Muzzarelli, R. A. A., Mattioli-Belmonte, M., Miliani, M., Muzzarelli, C., Gabbanelli, F., & Biagini, G. (2002). In vivo and in vitro biodegradation of oxychitin–chitosan and oxypullulan-chitosan complexes. *Carbohydrate Polymers*, *48*, 15–21.

Muzzarelli, R. A. A., Miliani, M., Cartolari, M., Genta, I., Perugini, P., Modena, T., Pavanetto, F., & Conti, B. (2000). Oxychitin–chitosan microcapsules for pharmaceutical use. *STP Pharma Sciences*, *10*, 51–56.

Muzzarelli, R. A. A., Muzzarelli, C., Cosani, A., & Terbojevich, M. (1999a). 6-Oxychitins, novel hyaluronan-like polysaccharides obtained by regioselective oxidation of chitins. *Carbohydrate Polymers*, *39*, 361–367.

Muzzarelli, R. A. A., Stanic, V., & Ramos, V. (1999b). Enzymatic depolymerization of chitins and chitosans. In C. Bucke (Ed.), *Methods in Biotechnology: Carbohydrate Biotechnology Protocols*. Totowa: Humana Press.

Takahashi, T., Takayama, K., Machida, Y., & Nagai, T. (1990). Characteristics of polyion complexes of chitosan with sodium alginate and sodium polyacrylate. *International Journal of Pharmacy*, *61*, 35–41.