Available online at www.sciencedirect.com
SCIENCE DIRECT®
European Journal of Pharmaceutics and Biopharmaceutics 57 (2004) 465–472
European Journal of Pharmaceutics and Biopharmaceutics
www.elsevier.com/locate/ejpb

Research paper

Carrageenan–gelatin mucoadhesive systems for ion-exchange based ophthalmic delivery: in vitro and preliminary in vivo studies

Maria Cristina Bonferoni[a,*], Patrizia Chetoni[b], Paolo Giunchedi[c], Silvia Rossi[a], Franca Ferrari[a], Susi Burgalassi[b], Carla Caramella[a]

[a]Department of Pharmaceutical Chemistry, Pavia, Italy
[b]Department of Bioorganic Chemistry and Biopharmaceutics, Pisa, Italy
[c]Department of Pharmaceutical Sciences, Sassari, Italy

Received 17 July 2003; accepted in revised form 15 December 2003

Abstract

Lambda-carrageenan is an anionic polymer able to ionically interact with alkaline drugs. This results in a complex that releases the drug slowly, due to displacement by the counterions of the release medium. The aim of this work was to assess the possible ophthalmic employment of such a complex. As a model drug, an alkaline anti-glaucoma drug, timolol maleate, was chosen. Systems in which lambda-carrageenan interacted both with the drug and a mucoadhesive polymer such as gelatin were studied. The combination of carrageenan and gelatin in different ratios proved to be useful in modulating the drug release profiles, the rheological properties of the hydrated formulations and their mucoadhesive properties. Both films and microspheres were prepared and tested in vitro. A microsphere formulation was also tested in vivo in albino rabbits. The drug concentration and bioavailability in the aqueous humour were significantly high in comparison with commercial formulations.

Keywords: Ophthalmic delivery; Mucoadhesion; Lambda-carrageenan; Gelatin; Ionic interaction

1. Introduction

The bioavailability of ophthalmic formulations is strongly affected by precorneal losses due to lachrymal flow and palpebral blinking. These factors cause quick elimination of conventional formulations. Improvement of the ocular bioavailability has been obtained by using hydrogels or solutions that change to a gel after instillation, such as those based on Gelrite® [1,2]. Ocular inserts can control the drug release for a long period of time; the low compliance of the patient towards this kind of formulation can be to some extent improved if soluble polymers are used, so that the insert undergoes slow erosion and finally does not need to be removed. More recently, the administration of microparticulates has also been proposed [3,4].

For all these formulations a further improvement of bioavailability can be achieved with the employment of mucoadhesive polymers that prolong the precorneal residence time by interacting with the ocular mucin layer [5]. However, it has been pointed out that the use of a mucoadhesive polymer can give no increase in bioavailability if the drug is not efficiently retained in the formulation. Therefore, the success of the mucoadhesive approach depends on the capability of the formulation to retain the drug associated with the vehicle. A confirmation of this principle has been obtained with formulations incorporating the drug in mucoadhesive hydrogels as a poorly soluble or diffusible complex [6].

Controlled release systems that exploit ionic interaction between soluble polymers and oppositely charged drugs have been recently proposed for the oral route [7,8]. Few examples of ophthalmic application of this approach can be found in the literature [9,10].

The aim of the present work was to evaluate the ophthalmic use of an ionic complex between lambda-carrageenan and an alkaline drug. Lambda-carrageenan is an anionic polysaccharide derived from algae, with a higher sulphate substitution level than the other natural types, iota

* Corresponding author. Address: Department of Pharmaceutical Chemistry, V.le Taramelli 12, 27100 Pavia, Italy. Tel.: +39-0382-507357.
E-mail address: cbonferoni@unipv.it (M.C. Bonferoni).

0939-6411/$ - see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejpb.2003.12.002

and kappa: a higher capacity of interaction with the basic drugs can therefore be expected. The anti-glaucoma drug, timolol maleate, was used as a cationic model drug. Carrageenans have been proposed as viscolysers in eye-drop formulations [11], but have not yet been evaluated for ophthalmic controlled release formulations. As the muco-adhesive properties of carrageenan are quite controversial [11], ternary systems in which carrageenan interacts both with the drug and a mucoadhesive polymer such as gelatin should improve this aspect. Gelatin is a polyelectrolyte with a net charge depending on the pH and the type of gelatin. Gelatin A is obtained from collagen by acidic hydrolysis, and has an isoelectric point between 7 and 9. Gelatin B is obtained by alkaline hydrolysis and has an isoelectric point between 4.7 and 5.3 [12]. Lambda-carrageenan and gelatin together are therefore expected to interact to give a polymer network whose performance in the control of drug release is likely to depend on several factors: pH, ionic character of the drug, and presence of salts in the release medium. Moreover mucoadhesion, rheological properties and polymer–drug interaction can be controlled by varying the ratio between the two polymers.

Both films, to be used as inserts, and microspheres were prepared based on the ternary system under investigation. Preliminary in vivo tests were performed on the microsphere samples as they were easier to administer and they were characterized by better compliance.

2. Materials and methods

2.1. Materials

Gelatin A 75–100 Bloom from porcine skin and gelatin B 75 Bloom from bovine skin and bones were obtained from Sigma Chemical Co., St. Louis, MO, USA. The isoelectric point is 7–9 for type A and 4.7–5.3 for type B [12]. Lambda-carrageenan Viscarin GP 109 NF (FMC corporation) was donated by Prodotti Gianni, Milan, Italy. Timolol maleate was donated by Sifavitor S.p.a, Milan, Italy.

Mucin type II crude from porcine stomach was purchased from Sigma Chemical Co., St Louis, MO, USA.

2.2. Preparation of the films

The films were made by casting solutions of polymer or polymer mixtures. Mixtures of the two polymers were obtained by adding their solutions prepared in distilled water. The polymer concentration was, in all cases, 1.5% (w/v). Glycerol was added as a plasticiser at the 0.35% w/v concentration (23% with respect to the polymer weight). After the addition of the drug, the solutions were deaerated under vacuum and poured on PTFE plates (1 ml of solution/cm^2). The plates were dried overnight at 40 °C. The films were stored at a constant level of humidity (50% RH) in the presence of a saturated solution of $Mg(NO_3)_2$. In all the formulations the final concentration of timolol maleate was 0.75 mg/cm^2. The polymer compositions in mg/cm^2 are given in Table 1.

Table 1
Polymer composition of the films (mg of polymer/cm^2)

Films	Carrageenan	Gelatin A	Gelatin B
50/50	7.50	7.50	
50/50 B	7.50		7.50
25/75	3.75	11.25	
Carrageenan	15		
Gelatin		15	

2.3. Preparation and characterization of the microspheres

The microspheres were prepared by hydrating 2 g of gelatin A and 2 g of carrageenan in 260 ml of distilled water. Timolol maleate 200 mg was dissolved in 8 ml distilled water and added to the preparation. The final carrageenan/gelatin/timolol ratio in the microspheres was 1/1/0.1 which corresponded to the composition of the 50/50 films.

A co-current flow type spray-dryer (model Mini Spray HO, W. Pabish, Milan, Italy) equipped with a 0.7 mm nozzle was used. Inlet temperature was 100–105 °C, outlet temperature was 70–75 °C. Spray flow rate was 5 ml/min; spray pressure was 3 atm.

Size and shape of the obtained microspheres were checked by means of SEM microphotographs (Zeiss DSM 962, Zeiss, Germany). The sample was placed on double-sided tape which had previously been secured to aluminium stubs and then analysed at 20 kV acceleration voltage after gold spattering, in an argon atmosphere.

2.4. Characterization of the films

2.4.1. Measurement of water uptake

The water uptake was measured by means of a modified Enslin apparatus [13] connected to a personal computer for continuous recording of the amount of water absorbed.

Artificial lachrymal fluid was used as buffer. Artificial lachrymal fluid was prepared by dissolving $NaHCO_3$ 2.2 g/l; NaCl 6.26 g/l; KCl 1.79 g/l; $CaCl_2{\cdot}2H_2O$ 0.0735 g/l; $MgCl_2{\cdot}6H_2O$ 0.0964 g/l in distilled water [14]. The pH was adjusted to 7.5 with 0.1 M HCl. An accurately weighed piece of film (about 15–20 mg) was put on a filter paper covering the sample holder. Three replicates were performed for each sample.

2.4.2. Rheological measurements

Rheological measurements were performed on the hydrated films with a rotational rheometer (Bohlin CS Rheometer, Bohlin, Cirencester, UK). A plate–plate combination with the diameter of 20 mm was used. Discs were cut from the films by means of a round blade, weighed,

hydrated by addition of 11 ml/g of lachrymal fluid. The measurement was performed at 37 °C after a 60 s lag time. Oscillatory tests were performed in the linearity range, where the viscoelastic parameters are independent of the applied stress. The viscoelastic parameters, storage modulus (G') and loss modulus (G''), were measured at frequencies ranging from 0.1 up to 5 Hz.

2.5. Drug release

2.5.1. Release test

The drug release from both the films and the microspheres was measured by means of a Franz cell with a 20 mm diameter orifice. The temperature was kept at 37 °C by means of a water jacket. The test was performed in lachrymal fluid and in distilled water. A preboiled 12000–14000 Da cut-off dialysis membrane (Visking tubes, Emanuele Mires, Milan, Italy) was cut and placed to cover the orifice. The sample, consisting of either a piece of film or a corresponding amount of microspheres hydrated for 10 min with 11 ml/g of fluid, was laid on the top of the membrane. Timolol concentration in the receptor chamber was detected spectrophotometrically at 294 nm wavelength (DU®-50 spectrophotometer, Bechman Instruments, Inc, Fullerton, California, USA).

2.5.2. Simultaneous release and 'washability' test

A modified Franz cell was used in this case [15]. The lid was closed except for two side arms which allowed a buffer to stream tangentially over the sample. A hole was present in the upper part to allow the exit of air; it was closed by a screw at the beginning of the measurement. Lachrymal fluid, thermostated at 37 °C, was fluxed over the sample at 0.3 ml/min by means of a HPLC pump (Ginkotek model 300, Munch, Germany). After flowing over the sample, the buffer was collected in a beaker and maintained under stirring. At the same time, samples were both collected from the receptor chamber and the beaker and the drug concentration was spectrophotometrically determined.

2.6. Measurement of mucoadhesion

Mucoadhesion measurements were made with a detachment apparatus [16], consisting of a movable carriage and a load cell to measure the force of detachment connected to a personal computer. A piece of the film to be tested was hydrated with 11 ml/g of lachrymal fluid for 10 min, and 50 mg was put on a filter paper attached to the sample holder; a second piece of paper was fixed to the movable carriage and hydrated with 50 μl of either lachrymal fluid (blank) or an 8% mucin dispersion (test). The two papers were put in contact and a 3000 mN preload was applied for 3 min, then the sample was moved apart at constant speed (4 mm/min). The maximum force of detachment was recorded for both the blank and the test with mucin. Six replicates were performed for each sample. The mean values were calculated and the statistical significance of the difference between the tests and the blanks was checked with a two independent sample t-test. Moreover, the relative difference $\Delta F/F$ values were calculated as follows:

$$\Delta F/F = (F_s - F_b)/F_b$$

where

F_s, maximum detachment force in the presence of mucin
F_b, maximum detachment force of the blank (without mucin).

2.7. In vivo testing

In vivo testing was performed on the microsphere sample, in comparison with two commercial formulations: Droptimol® (Farmigea S.p.A. Pisa, Italy) a conventional eye drop, and Timoptic® XE (Merck and Co. Inc. West Point, PA, USA), an in situ gel forming solution based on Gelrite®.

Male, New Zealand albino rabbits, ranging in weight from 2.5 to 3.0 kg (Pampaloni Rabbitry, Fauglia, Italy) were used and treated as prescribed in the publication *Guide for the Care and the Use of Laboratory Animals* (NIH Publication No. 92–93, revised 1985). The in vivo experimental protocol was approved by the Ethical-Scientific Committee of the University of Pisa and the experiments were carried out under veterinary supervision.

The animals were housed singly in standard cages, in a light-controlled room (10 h dark/14 h light cycle) at 19 ± 1 °C and 50 ± 5% RH, with no restriction of food or water. During the experiments the rabbits were placed in restraining boxes: they were allowed to move their heads freely, and their eye movements were not restricted.

An amount corresponding to 50 μl of the preparations Droptimol®, Timoptic® and a microsphere dispersion (50/50 microspheres) was administered in the lower conjunctival sac of one eye of each rabbit. In the study, a total of 26 rabbits was used: two groups of eight animals and one group of 10 animals, respectively, for commercial preparations, Droptimol® and Timoptic® XE, and 50/50 microspheres dispersion. The administered timolol dose was, in all cases, 0.25 mg.

The 50/50 microsphere formulation was dispersed immediately before in vivo administration, adding an appropriate amount of isotonic phosphate buffer (pH 7.4) to a sample of the microspheres having a 50/50 carrageenan/gelatin A ratio.

The animals' eyes were examined with a slit-lamp to evidence possible signs of irritation every 10 min up to 1 h after administration of the microsphere dispersion [17].

At appropriate time intervals the rabbits were anaesthetized by i.m. administration of 30 mg/kg ketamine (Inoketam 1000 solution, Virbac S.r.l., France) and 5.0 mg/kg xilazine (Rompum 2.0% solution, Bayer AG, Leverkusen, Germany). A sample of 60–80 μl of aqueous

humour was removed from the anterior chamber using a 1.0 ml syringe (29G, Micro-Fine, Beckton Dickinson, Dublin, IRL). The aqueous humour samples were immediately frozen and stored at −18 °C. For analysis, the samples were mixed with an equal volume of methanol containing 6% v/v perchloric acid and after centrifugation using a Microfuge 11 (Beckman Instruments, Palo Alto, CA, USA) for 3 min at 13 000 rev/min, 20 μl of the supernatants were submitted to HPLC analysis [18].

2.7.1. Analytical methods

The analyses were performed with HPLC using a Shimadzu apparatus consisting of an LC-6AV pump, an SPD-10A UV detector, a C-R4A integration system and a 20 μl sample loop (Rheodyne, Cotati, CA, USA). The column was a reversed phase C18 (Bondclone 300 × 3.9 mm, Phenomenex, Torrance, CA, USA) with 10 μm packing material and the mobile phase (flow rate, 1.6 ml/min) was CH_3CN:CH_3COONa 0.01 M (25:75 v/v ratio). Timolol was detected at wavelength 294 nm. The coefficient of variation was less than 2.1% in the 0.1–20 μg/ml range. The limit of quantitation (LOQ) was 0.6 nmol/ml and the retention time of timolol was 6.4 min.

The amount of drug in the samples was determined by comparison with appropriate standard curves, obtained by adding timolol to blank aqueous humour samples.

2.7.2. Statistical data analysis

The statistical significance of data was assessed by one-way analysis of variance followed by multiple comparisons using the Fisher PLSD (Protected Least Significant Difference) test (Statview Software, Abacus Concepts, Inc., Berkeley, CA, USA) [19]. The AUC values (area under the drug concentration versus time curves in aqueous humour) were calculated from the beginning (t_0) to the end of the observation time (t_{last}), using the linear trapezoidal rule (Kaleidagraph, Synergy Software, PA, USA).

3. Results

3.1. Characterization of the films

3.1.1. Water uptake

The uptake profile of lachrymal fluid showed no relevant differences between the films of lambda-carrageenan, gelatin A, their mixture 50/50 and 25/75 and the 50/50 mixture of carrageenan and gelatin B. The profiles were all characterized by an initial phase (5–10 min) in which an increase in liquid uptake with time is observed, followed by steady state corresponding to about 11 ml/g of buffer absorbed (ranging between 9.2 ± 1.6 and 13.0 ± 2.1 mg/g). The test allowed an estimate of the amount of fluid to be used to hydrate the samples prior to further characterizations.

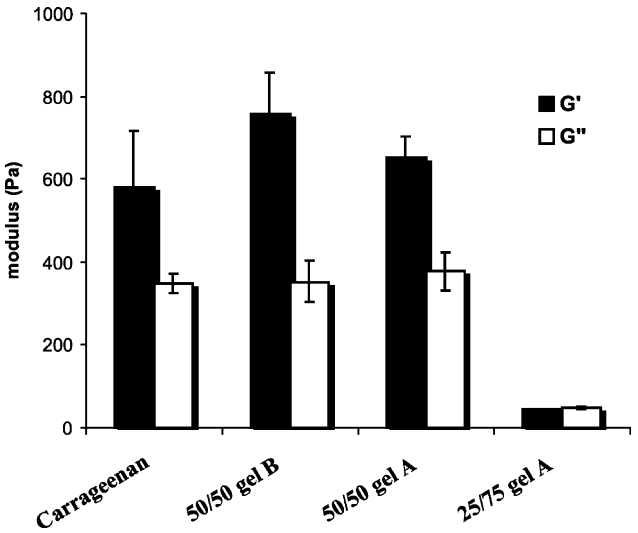

Fig. 1. Rheological characterization of the hydrated films: elastic (G') and viscous (G'') modulus at 1 Hz frequency.

3.1.2. Rheological characterization of the samples

Fig. 1 shows the values of the storage modulus G' and of the loss modulus G'' measured at 1 Hz frequency, for films of carrageenan, 50/50 mixture with gelatin B and 50/50 and 25/75 mixtures with gelatin A. The viscoelastic behaviour is sensitive to network structure. G', elastic modulus, is a measure of the elastic behaviour and is an expression of the presence of strong links that can be deformed but not broken under the stress applied, so that the structure can be elastically recovered. The viscous behaviour (G'') is a measure of the slipping of planes and chains entangled without rigid links. Gelatin alone (both A and B) had very low mechanical properties at 37 °C so that no linear behaviour could be observed even at very low stress values and therefore viscoelastic measurement could not be performed. In the case of both 50/50 mixtures, involving gelatin A and B, the values of viscoelastic parameters are very similar to those of carrageenan, indicating that up to this ratio the gelatins do not affect the mechanical properties of the films. Only slight and non-significant differences can be observed between 50/50 mixture with gelatin A and 50/50 mixture with gelatin B. Higher amounts of gelatin, as in 25/75, results in a clear decrease of both G' and G''.

3.2. Drug release

3.2.1. Release test

The release profiles in the Franz diffusion cell in distilled water and in lachrymal fluid for the films based on carrageenan, gelatin A and their 50/50 and 25/75 mixtures are shown in Fig. 2a and b, respectively.

By comparing the results in distilled water and in lachrymal fluid, the influence of the salts of the medium on drug release could be established. Looking at the profiles in distilled water, it can be observed that the release from pure

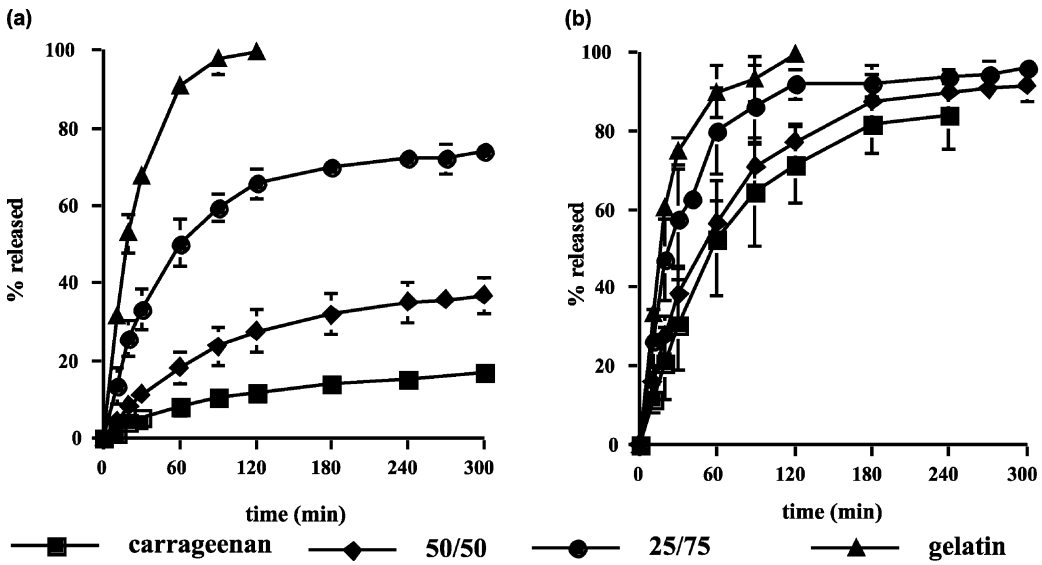

Fig. 2. Release profiles from the films based on carrageenan, gelatin A, and their mixtures in distilled water (a) and in lachrymal fluid (b). Mean ± SD ($n = 6$).

gelatin A film is completed after 2 h. On the other hand, when the amount of carrageenan is increased, an increasing amount of drug is retained in the formulation. This is clearly due to drug–polymer interactions; no salts that can compete with the drug are, in fact, present in the release medium.

The results in lachrymal fluid show that the release profile is superimposable on that observed in distilled water only from gelatin film. In both the media, the release with only gelatin is the fastest, probably because there are no interactions retarding the drug release and because the consistency of the hydrated film is very low.

The effect of the salts present in the medium on the drug displacement is remarkable for all the films containing carrageenan, for which an increase in the release rate with respect to distilled water was observed. This effect is moreover related to the amount of carrageenan in the film: the pure carrageenan films gave the slowest release, followed by 50/50 while 25/75 films are closer to gelatin. This behaviour can be partly explained by the different consistencies of the hydrated films, evidenced by the rheological test that can affect drug diffusion through the hydrated gel.

In Fig. 3 the comparison between films based on 50/50 mixture of carrageenan and gelatins A and B is shown. It can be observed that the profiles are superimposable indicating no influence of the type of gelatin on the drug release. As both rheological characterization and release test indicated lack of differences between films based on gelatin A and B, the work was continued with gelatin A only.

The SEM microphotographs of the microspheres, given in Fig. 4, showed that they were spherical and with dimensions lower than 10 μm.

As can be seen in Fig. 5, no notable difference can be observed between the release from the microspheres and the release from the 50/50 films having the same composition.

3.2.2. Simultaneous release and 'washability' test

The test is intended to measure, simultaneously, the sensitivity to erosion of the formulations ('washability') and its effect on drug release.

Fig. 6 shows, as a function of time, both the percentage of timolol washed away by the tangential stream over the sample (Fig. 6a) and the percentage that is released at the same time to the receptor chamber (Fig. 6b). Although the flow rate of 0.3 ml/min is arbitrarily chosen and cannot be considered to mimic the in vivo behaviour, the test should allow the determination of a rank order of the different sensitivities of the tested formulations to physiological stresses (lachrymal secretions and blinking) responsible for precorneal losses of the formulation. The results are in accordance with the rheological properties of the films: the carrageenan formulation is washed away at the lowest rate and therefore the final amount released to the receptor chamber is the highest. The increasing amount of gelatin

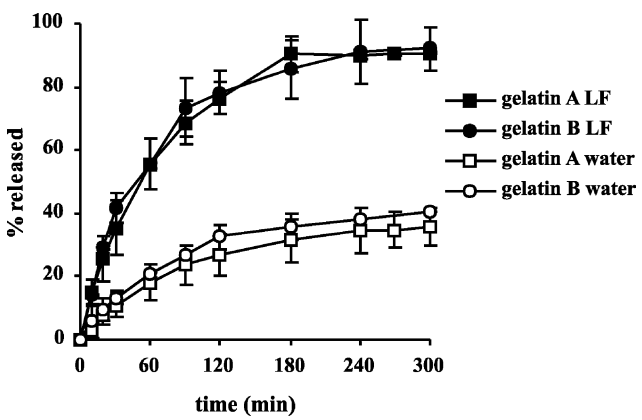

Fig. 3. Comparison of the release profiles obtained from 50/50 films with gelatin A and B. Mean ± SD ($n = 6$), in distilled water and in lachrymal fluid (LF).

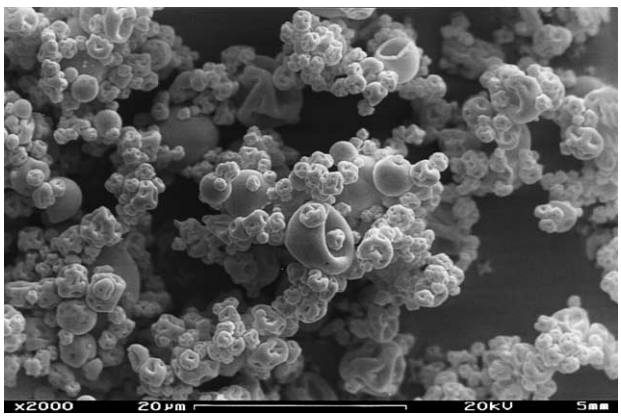

Fig. 4. SEM microphotograph of the 50/50 microspheres.

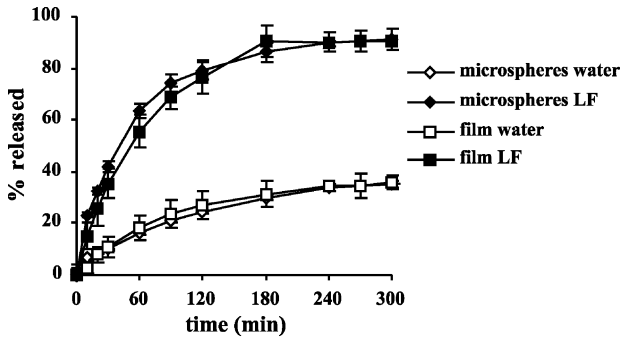

Fig. 5. Comparison of the drug release profiles obtained from 50/50 gelatin A film and from microspheres having the same composition, in distilled water and in lachrymal fluid (LF). Mean ± SD ($n = 6$).

corresponds to faster erosion, particularly evident for the 25/75 and gelatin A formulations; lower cumulative amounts are therefore released in these cases to the receptor chamber.

3.3. Mucoadhesion results

In Fig. 7, the detachment force between the films and the mucin dispersion is compared to the detachment force between the films and the buffer (blanks).

A negative effect was observed for the pure lambda-carrageenan film. Statistically significant and positive differences between the tests performed with mucin and the blanks were found for the 50/50, the 25/75 and gelatin A films. It can be moreover observed from the $\Delta F/F$ values given in the film that the relative differences between mucin and blank increase with the increase of the gelatin amount. This indicates that gelatin is responsible for the mucoadhesive properties of the formulation.

3.4. Preliminary in vivo tests

Preliminary in vivo tests showed that the combination of carrageenan and gelatin had an excellent ocular tolerance. No eyes examined with the slit-lamp during the pharmacokinetic experiments showed symptoms of ocular irritation.

The results of the in vivo tests are summarized in Table 2 where the relevant pharmacokinetic parameters: peak time (T_{max}), maximum concentration (C_{max}), and area under the concentration versus time curves (AUC) are listed for each formulation.

The microsphere formulation produced a drug concentration of 8.17 μg/ml in aqueous humour 60 min after instillation, with an increase of 2–4 fold, when compared with the commercial preparations (2.11 and 3.54 μg/ml for Droptimol® and Timoptic XE® with statistically significant differences). As shown in Fig. 8, the drug concentration in aqueous humour after administration of microsphere suspension was significantly higher (Fisher PLSD $P < 0.05$) at all times, except for 10 min after instillation of the microsuspension with respect to the reference solutions.

The improved ocular bioavailability of the microsphere formulation was evidenced by AUC values: a moderate AUC increase (about 2-fold) with respect to Timoptic XE® and a remarkable increase (exceeding 5.0 times) with respect to Droptimol® were observed.

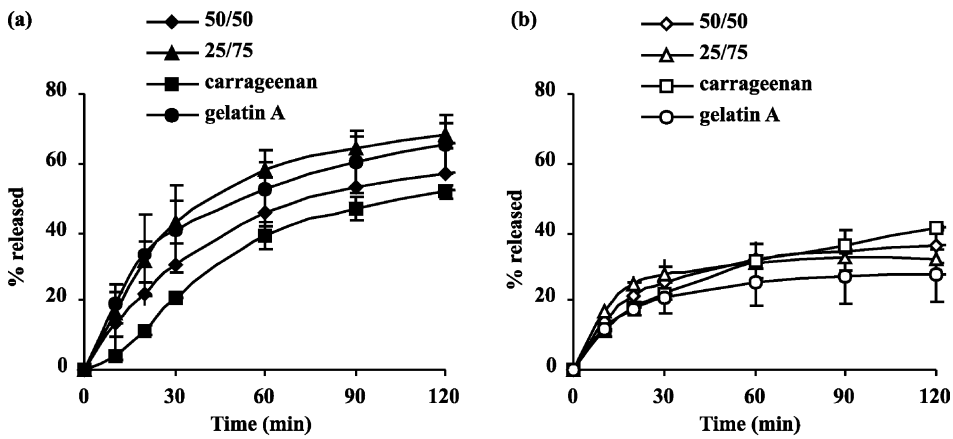

Fig. 6. Results of the simultaneous release and 'washability' test on the films. (a) Amount of drug washed away by the tangential stream (close symbols); (b) amount of drug released to the receptor chamber (open symbols). Mean ± SD ($n = 6$).

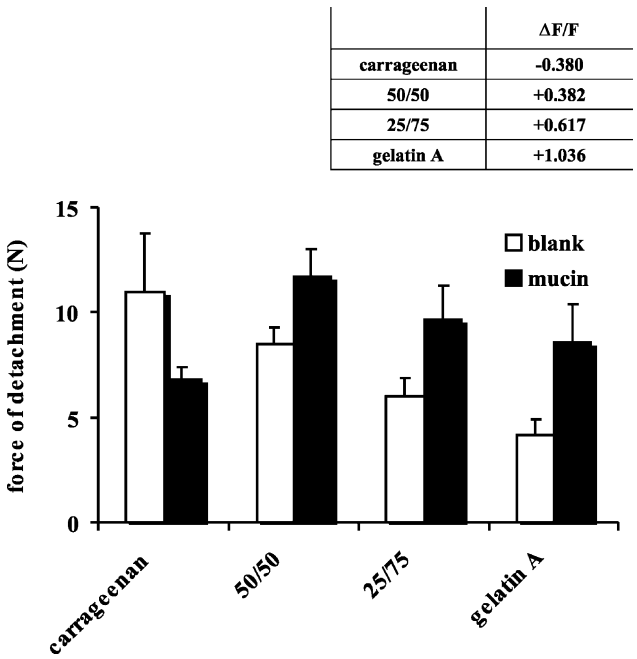

Fig. 7. Mucoadhesion behaviour of the films based on carrageenan, gelatin A, and their mixtures, expressed as force of detachment (mean ± SD; $n = 6$). In the insert, the relative differences $\Delta F/F$ between the values obtained with and without mucin (blanks) are given.

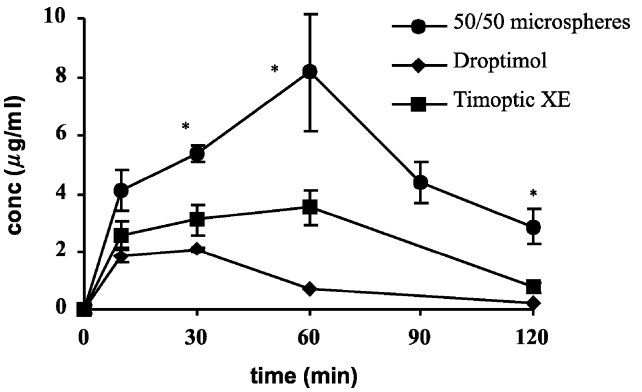

Fig. 8. Timolol concentration profiles in the aqueous humour of rabbits after administration of 50/50 microsphere formulation and the two reference formulations (mean ± SE).

4. Discussion

The results obtained in this study show that carrageenan–gelatin mixtures are suitable for preparing ophthalmic formulations with improved drug residence time in the precorneal area and, in turn, transcorneal absorption.

In particular, it has been confirmed that the addition of gelatin to carrageenan is responsible for mucoadhesive properties that increase proportionally to the amount of gelatin.

The capability of carrageenan to interact with alkaline drugs can also be exploited to control drug release in ophthalmic preparations; the release rate and amount in this case depend on the lachrymal fluid turnover. This can be adequately evaluated only in more systematic in vivo studies. The in vitro results show, however, that by changing the carrageenan–gelatin ratio in the formulation, it is possible to change the amount of drug whose release is affected by the salts contained in the medium. This could be an important factor for the modulation of drug release rate.

Furthermore, the carrageenan/gelatin ratio affects the rheological properties of the hydrated films, due to the fact that at 37 °C, gelatin consistency is very low. This effect, however, can be observed only for relatively high amounts of gelatin. Upto a ratio of 50/50 the rheological parameters of the hydrated films do not differ from those of the pure carrageenan, probably because of the positive effect of polymer–polymer interactions. The consistency of the formulation can be relevant for its performance, as is shown by the effect on the sensitivities to erosion evidenced in the 'washability' test. This is likely to correspond in vivo to different sensitivities toward lachrymal turnover and mechanical stress due to blinking. As carrageenan–gelatin interactions are likely to be mainly of an ionic nature, a difference could be expected between the interaction of carrageenan with gelatin A and B. However, no difference was observed, either in rheological properties or in the release profiles that were superimposable. To better exploit the differences between the two gelatin types it would probably be necessary to use gelatins of higher molecular weights, capable of stronger interactions with carrageenan, or, possibly, samples with more narrow isoelectric point interval.

Carrageenan–gelatin mixtures can also be used to obtain microspheres with adequate morphological and size characteristics. Microsphere and film formulations having the same composition showed similar in vitro behaviour. In both cases a gel was obtained after hydration: this is probably the reason why differences due to surface area or morphology could not be put in evidence.

Preliminary in vivo studies performed on the 50/50 microsphere sample showed that this formulation was promising for ocular administration. The drug concentration in the aqueous humour was higher than that of commercial formulations, suggesting an improved ocular bioavailability.

Table 2
Pharmacokinetic parameters in aqueous humour after administration of the formulations under study

Formulations	C_{max} (μg/ml ± SE)	T_{max} (min)	AUC (min μg/ml ± SE)	AUC rel
Droptimol®	2.11 ± 0.28	30	117.2 ± 18.9	1
Timoptic XE®	3.54 ± 0.61	60	286.7 ± 51.2	2.5
Microspheres	8.17 ± 2.05	60	651.1 ± 114.3	5.6

Acknowledgements

The work was partially supported by FAR (Fondo di Ateneo per la Ricerca) funds.

References

[1] M.B. Sintzel, S.F. Bernatchez, C. Tabatabay, R. Gurny, Biomaterials in ophthalmic drug delivery, Eur. J. Pharm. Biopharm. 42 (1996) 358–374.
[2] A. Rozier, C. Mazuel, J. Grove, B. Plazonnet, Gelrite®: a novel, ion-activated, in situ gelling polymer for ophthalmic vehicles. Effect on bioavailability of timolol, Int. J. Pharm. 57 (1989) 163–168.
[3] A.M. Durrani, N.M. Davies, M. Thomas, I.W. Kellaway, Pilocarpine bioavailability from mucoadhesive liposomal ophthalmic drug delivery system, Int. J. Pharm. 88 (1992) 409–415.
[4] P. Giunchedi, U. Conte, P. Chetoni, M.F. Saettone, Pectin microspheres as ophthalmic carriers for piroxicam: evaluation in-vitro and in-vivo in albino rabbits, Eur. J. Pharm. Sci. 9 (1999) 1–7.
[5] M.F. Saettone, S. Burgalassi, P. Chetoni, Ocular bioadhesive drug delivery systems, in: E. Mathiowitz, D.E. Chickering, C.M. Lehr (Eds.), Bioadhesive Drug Delivery Systems, Fundamentals, Novel Approaches and Development, Marcel Dekker, New York, 1999, pp. 601–640.
[6] S. Burgalassi, P. Chetoni, M.F. Saettone, Hydrogels for ocular delivery of pilocarpine: preliminary evaluation in rabbits of the influence of viscosity and of drug solubility, Eur. J. Pharm. Biopharm. 42 (1996) 385–392.
[7] N. Konar, C. Kim, Drug release from drug-polyanion complex tablets: poly(acrylamido-2-methyl-1-propanesulfonate sodium-co-methyl methacrylate), J. Control. Rel. 57 (1999) 141–150.
[8] C. Caramella, M.C. Bonferoni, Complex between carrageenan and a water soluble drug having a specific granulometry and relative controlled release pharmaceutical compositions, US Patent, 6,355,272 (2002).
[9] M.F. Saettone, D. Monti, M.T. Torracca, P. Chetoni, Mucoadhesive ophthalmic vehicles: evaluation of polymeric low-viscosity formulations, J. Ocul. Pharmacol. 10 (1994) 83–92.
[10] B.S. Lele, A.S. Hoffman, Insoluble complexes of polyacrylic acid with a cationic drug for use as a mucoadhesive, ophthalmic drug delivery system, J. Biomater. Sci. Polym. Ed. 11 (2000) 1319–1331.
[11] E. Verschueren, L. Van Santvliet, A. Ludwig, Evaluation of various carrageenans as ophthalmic viscolysers, STP Pharma. Sci. 6 (1996) 203–210.
[12] A. Wade, P.J. Weller (Eds.), Handbook of Pharmaceutical Excipients, Second ed., American Pharmaceutical Association, London, 1994, pp. 199–200.
[13] F. Ferrari, M. Bertoni, S. Rossi, M.C. Bonferoni, C. Caramella, M. Waring, M.E. Aulton, Comparative rheomechanical and adhesive properties of two hydrocolloid dressings: dependence on the degree of hydration, Drug Dev. Ind. Pharm. 22 (1996) 1223–1230.
[14] M. Albasini, A. Ludwig, Evaluation of polysaccharides intended for ophthalmic use in ocular dosage forms, Il Farmaco 50 (1995) 633–642.
[15] M.C. Bonferoni, S. Rossi, F. Ferrari, C. Caramella, A modified Franz diffusion cell for simultaneous assessment of drug release and washability of mucoadhesive gels, Pharm. Dev. Technol. 4 (1999) 45–53.
[16] F. Ferrari, S. Rossi, M.C. Bonferoni, M. Bertoni, C. Caramella, Sensitivity to rate of stress application of different mucoadhesive polymers, Int. J. Pharm. Adv. 1 (1995) 27–37.
[17] F. Bottari, B. Giannaccini, B. Cristofori, M.F. Saettone, N. Tellini, Semisolid ophthalmic vehicles I. A study of eye irritation in albino rabbits of a series of gel type aqueous bases, Il Farmaco Ed. Pratica 10 (1978) 434–446.
[18] S. Burgalassi, P. Chetoni, L. Panichi, E. Boldrini, M.F. Saettone, Xyloglucan as a novel vehicle for timolol: pharmacokinetics and pressure lowering activity in rabbits, J. Ocul. Pharm. Ther. 16 (2000) 497–509.
[19] J.H. Zar, Biostatistical Analysis, Second ed., Prentice Hall, Englewood Cliffs, NJ, 1984, Chapters 11 and 12, pp. 162–203.