Mucoadhesive Properties of Chitosan-Coated Ophthalmic Lipid Emulsion Containing Indomethacin in Tear Fluid

Masazumi Yamaguchi,∗a Kayoko Ueda, a Akiharu Iwasa, a Akira Ohtori, a Hirofumi Takeuchi, b Nobuyuki Ohguro, c and Kakuji Tojo, d

a Research Laboratories, Senju Pharmaceutical Co., Ltd.; 1–5–4 Murotani, Nishi-ku, Kobe, Hyogo 651–2241, Japan; b Department of Pharmaceutical Engineering, Gifu Pharmaceutical University; 5–6–1 Mitahora Higashi, Gifu 502–8585, Japan; c Department of Ophthalmology and Visual Science, Osaka University; 2–2 Yamadaoka,Suita, Osaka 565–0871, Japan; and d Department of Biochemical Science and Engineering, Kyushu Institute of Technology; 680–4 Kawazu Iizuka, Fukuoka 820–8501, Japan. Received November 25, 2008; accepted March 18, 2009

To evaluate the residence of chitosan-coated emulsion (CE) containing indomethacin in tears, the drug retention of CE in tear fluid was compared with non-coated emulsion (NE) after instillation in rabbit eyes. CE had mean concentrations 3.6-fold and 3.8-fold higher than NE at 0.5 h and 0.75 h after instillation, respectively. Mean residence time and half-life of CE were lengthened to 1.5-fold and 1.8-fold those of NE, respectively. Volume of distribution of CE in tear fluid was also 1.6-fold greater than that of NE. These findings indicated that retention of the drug in tears was appreciably prolonged by chitosan-coated emulsion, and that CE had higher distribution on the ocular surface than NE. The drug levels in cornea, conjunctiva, and aqueous humor at 1 h after instillation were clearly higher than those of NE. In a generalized ocular pharmacokinetic model, the ratio of CE to NE for peak concentration values (Cmax) and the area under the concentration/time curve (AUC) nearly corresponded to aqueous humor levels in vivo. Additionally, tensile testing showed that the force of detachment between CE and mucin was significantly larger than that of emulsion containing hydroxypropylmethyl cellulose (HPMCE) with a viscosity similar to CE; the forces of detachment of CE and HPMCE measured using phosphate-buffered saline (PBS) were almost the same since these formulations have similar viscosity. Mucoadhesive strength of CE was confirmed by measurements of force of detachment between formulations and mucin. The residence time of the emulsion in tear fluid was prolonged by chitosan coating because of its mucoadhesive properties.

Key words lipid emulsion; mucoadhesive; tear fluid; chitosan; indomethacin; computer simulation

The physical and biochemical barriers of the eye to topical instillation are tear flow drainage, corneal resistance to diffusion, enzymatic degradation in tears and cornea, and flow of aqueous humor. The ocular bioavailability of instilled drug is therefore extremely low for most ophthalmic solutions, and less than about 5% of the drug may reach the anterior chamber of the eye.1) Usually, poorly soluble drugs are designed as ophthalmic suspensions. However, ocular bioavailability for ophthalmic suspensions is not good, similar to ophthalmic ophthalmic suspensions. Therefore, little is known concerning the pharmacokinetic profiles in tear fluid of instilled chitosan-coated particulate formulations. Additionally, little has been reported on ophthalmic lipid emulsions coated by chitosan to improve ocular bioavailability.

In this study, we examined the effects of coating of ophthalmic lipid emulsion with chitosan on ocular bioavailability. Indomethacin was incorporated as a model drug in the oil droplets of the emulsions. It is an anti-inflammatory agent widely used for the treatment of post-operative inflammation after cataract surgery.10) The marketed eye drops containing it unfortunately exhibit poor bioavailability.20) Thus, the objectives of this study were to evaluate the retention of chitosan-coated emulsion in tear fluid, and then to compare the ocular bioavailability of this formulation with non-coated emulsion. We also simulated drug concentrations in aqueous humor with a pharmacokinetic model using the pharmacokinetic parameters of the drug in tear fluid. The strength of adhesion of the formulation to mucin was therefore assessed by tensile testing, since it was necessary to achieve slow elimination of indomethacin from tear fluid.

© 2009 Pharmaceutical Society of Japan


**Materials**  Chitosan (over 100 kDa) was purchased from Funakoshi Corporation (Tokyo, Japan). Castor oil was purchased from Sioe Pharmaceutical Company (Osaka, Japan). Indomethacin was purchased from Sigma-Aldrich Japan (Tokyo). Polysorbate 80 was purchased from Nikko Chemicals (Tokyo). Hydroxypropylmethyl cellulose (HPMC, 65SH-4000) was purchased from Shin-Etsu Chemical Company (Tokyo). Mucin derived from porcine stomach was purchased from Wako Pure Chemical Industries (Tokyo). Water was purified with a Milli-Q purification system (Millipore, Osaka, Japan) at 8000 rpm for 1 h. The mixture was then cooled to room temperature and adjusted to pH 4.0 with Osaka, Japan). Other reagents were of HPLC grade or the highest grade commercially available. Male Japanese albino rabbits weighing 2.0 to 2.5 kg from Fukusaki Laboratory Animals Inc. (Hyogo, Japan) were used.

**Preparation of Lipid Emulsions, Chitosan-Coated Emulsions, and HPMC Emulsions**  Oil-in-water emulsions composed of indomethacin (0.1% (w/v)) as a lipophilic drug, castor oil (10% (w/v)) as an oil phase, and polysorbate 80 as an emulsifying agent (8.0% (w/v)) were prepared. The emulsions were prepared in two steps; in the first step, 380 ml of water was put into a 1000-ml glass beaker. Polysorbate 80 (40.0 g) and glycerin (22.0 g) were added to the water and mixed, and sodium acetate (0.5 g) was then dissolved in the solution as a buffering agent. Indomethacin (0.5 g) was added to castor oil (50 g) and dissolved at 70 °C, and this mixture was then added to the solution preheated to 70 °C and emulsified by a homogenizer (Robomics, PRIMIX, Osaka, Japan) at 8000 rpm for 1 h. The mixture was then cooled to room temperature and adjusted to pH 4.0 with acetic acid. The coarse emulsion was obtained by adding water to the mixture to 500 ml. In the second step, the coarse emulsion was treated with a high-pressure emulsifier (Microfluidizer M-110EH, Microfluidics Corporation, MA, U.S.A.) at 40 °C with an inlet pressure of 1.5×10^5 kPa. Individual batches were processed through the microfluidizer with 20 passes, and collected into glass beakers. The emulsion was then cooled to room temperature.

Chitosan and HPMC were dissolved in acetate buffer at pH 4.0 to prepare 1.5% and 0.5% solutions, respectively. Chitosan-coated emulsion (CE) was prepared by mixing and stirring the same volumes of emulsion and 1.5% chitosan solution at 10 °C for 1 h. HPMC emulsion (HPMCE) was prepared by the same procedure as for CE to obtain the same viscosity as CE, while non-coated emulsion (NE) was prepared by mixing equal amounts of the acetate buffer and the emulsion. The compositions of these emulsions are presented in Table 1.

**Characterization of These Emulsions**  Mean particle sizes of emulsions were measured by photon correlation spectroscopy (PCS) using a dynamic light scattering particle size analyzer (HPPS, Malvern Instruments, Worcestershire, U.K.) with 100-fold dilution by distilled water at 25 °C.

The surface of the emulsions was evaluated by measuring the zeta potential of the particles with a zeta meter (Zetasizer, Malvern Instruments). The emulsions were diluted 100-fold with water and placed in an electrophoretic cell with 150 mV.

Viscosity was determined by a cone plate viscometer (TV-20, TOKI SANGYO Co., Ltd., Tokyo) at 20 °C. The system was calibrated using standard viscosity fluids (Nippon-Grease Co., Ltd.).

**High-Performance Liquid Chromatography (HPLC)**  The HPLC system (LC-10A, Shimadzu, Kyoto, Japan) was composed of an autosampler (SIL-10ADvp), a pump (LC-10ADvp), a column oven (CTO-10ASvp), a UV detector (SPD-10AVvp), and data processing software (CLASS-VP). An octadecylsilica column (ODS A-302, 150-mm, 4.6-mm i.d., YMC) was used for measurement of indomethacin. The mobile phase was a mixture of methanol and 0.1% phosphoric acid (7 : 3, v/v) at a flow rate of 1.0 ml/min at 40 °C. The wavelength was 254 nm. Standard solution was prepared by dissolving 10 mg of the drug in the mobile phase (100 ml). A portion of each solution was mixed and diluted with the mobile phase.

**In Vivo Instillation Experiments**  Unanesthetized rabbits were maintained in a prone position using restraining boxes. A volume of 50 μl of CE or NE was instilled directly onto the corneal surface of both eyes of each rabbit. Approximately 1 μl of tear fluid was collected by a disposable glass capillary at 0.1, 0.25, 0.5, 0.75, and 1 h after instillation. The tear fluids collected were then added to 200 μl of the mobile phase, and the supernatant was obtained as sample solution after centrifugation. The rabbits were sacrificed by intravenous overdose administration of sodium pentobarbital solution after collection of tear fluid 1 h after instillation. About 200 μl of aqueous humor was collected with a syringe after the eye was washed with saline. The whole eye was enucleated, and then the cornea and conjunctiva were excised and minced in acetonitrile (5 ml) to extract the drug. The supernatant was obtained as sample solution after centrifugation, and 50 μl of the solution was analyzed by HPLC. This animal experiment was approved by our Institutional Committee for the Care and Use of Laboratory Animals.

Pharmacokinetic parameters were determined from mean tear concentration versus time profiles using multi-compartmental model analysis (WinNonlin version 2.1; Pharsight, Mountain View, CA, U.S.A.). The pharmacokinetic parameter areas under curves from time 0.1 to 1 h (AUC), preconical clearance (CL), mean residence time (MRT), volume of distribution in tear fluid (V), elimination rate constant (k) and half-life (t_{1/2}) in tear fluid were obtained.

**Prediction of Aqueous Humor Concentration by Simulation**  Drug concentration in aqueous humor was simulated by a generalized ocular pharmacokinetic model consisting of tear fluid dynamics, bilayer diffusion/partitioning for transcorneal transport, and multicompartment elimination/distribution in the internal tissues of the eye. (21,22) The

---

**Table 1. Compositions of Different Emulsions Investigated**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>CE</th>
<th>NE</th>
<th>HPMCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indomethacin</td>
<td>0.05%</td>
<td>0.05%</td>
<td>0.05%</td>
</tr>
<tr>
<td>Castor oil</td>
<td>5.0%</td>
<td>5.0%</td>
<td>5.0%</td>
</tr>
<tr>
<td>Polysorbate 80</td>
<td>4.0%</td>
<td>4.0%</td>
<td>4.0%</td>
</tr>
<tr>
<td>Glycerol</td>
<td>2.2%</td>
<td>2.2%</td>
<td>2.2%</td>
</tr>
<tr>
<td>Sodium acetate</td>
<td>0.05%</td>
<td>0.05%</td>
<td>0.05%</td>
</tr>
<tr>
<td>Chitosan</td>
<td>0.75%</td>
<td>—</td>
<td>0.25%</td>
</tr>
<tr>
<td>Hydroxypropylmethyl cellulose</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>pH</td>
<td>4.0</td>
<td>4.0</td>
<td>4.0</td>
</tr>
</tbody>
</table>
pharmacokinetic parameters obtained by analysis of drug levels in tears were used to simulate drug concentration in aqueous humor. The following were also used as other physiological and pharmacokinetic parameters; diffusion coefficient (cm²/s): 4.2×10⁻⁹ in corneal epithelium and 3.0×10⁻⁷ in stroma; total corneal thickness (cm): 0.040, epithelium thickness (cm): 0.0035; partition coefficient between epithelium and stroma: 18.6; volume of distribution in aqueous chamber (ml): 0.30; volume of distribution in lens (ml): 0.30; effective corneal surface area (cm²): 2.0; elimination rate constant in aqueous humor (min⁻¹): 0.01; elimination rate constant in lens (min⁻¹): 1×10⁻²; mass transfer coefficient in aqueous humor/corneal endothelium boundary (cm/s): 2×10⁻³; transfer rate constant from aqueous humor to lens (s⁻¹): 3×10⁻⁴; transfer rate constant from lens to aqueous humor (s⁻¹): 1×10⁻⁴; simulation of drug levels in aqueous humor was carried out for 8 h.

Measurement of Detachment Forces Forces of detachment between the emulsions and mucin dispersion were measured by tensile testing with modification of a previously described method; a digital force gauge (Model ZP-5N, IMADA, Aichi, Japan) and a vertical motorized stand (Model MX-500N, IMADA) were used. Filter paper discs (HVLP, Millipore, Tokyo) 20 mm in diameter were attached using double-sided adhesive tape on the cylindrical upper probe and the lower platform of the apparatus. Thirty-five microliters of emulsion (CE, NE, or HPMCE) was applied to the filter paper of the upper probe. Mucin was dispersed in phosphate-buffered saline (PBS, pH 7.4), and a 4% (w/v) mucin dispersion at pH 7.4 was prepared. Sixty-five microliters of the mucin dispersion was applied to the filter paper of the upper platform. After a 5-min interval, the upper probe was lowered, and the filter disc of the probe and the platform were attached for 60 s with a 1 N force of attachment. The probe was then raised at a constant speed of 6 mm/min, and the force of detachment was measured. Blank measurements were also performed using PBS instead of the mucin dispersion. Measurements were performed in triplicate.

Statistical Analysis Statistical analysis was carried out using Student’s t-test, with p-values of 0.05 considered significant. Calculations were performed with SAS statistical package 8.01 (SAS Institute, Cary NC, U.S.A.).

RESULTS AND DISCUSSION

Characterization of Emulsions A castor oil in water emulsion with polysorbate 80 as a nonionic surfactant has good stability, ocular tolerance, and ocular bioavailability. Suggesting that this is promising as an ophthalmic formulation. To further improve the bioavailability of indomethacin, the emulsion was coated with chitosan to prolong the residence time of the emulsion in tear fluid. Chitosan within the concentration range of 0.5 to 1.5% has good tolerance after instillation onto the corneal surface. The residence time of instilled drug for a conventional ophthalmic solution in tear fluid was prolonged by chitosan in this concentration range. Therefore, 0.75% was selected as chitosan content in the emulsion in this study.

Table 2 shows the physicochemical properties of these emulsions. The particle sizes of CE and NE were 117.6 and 94.8 nm, respectively. Coating with chitosan increased particle size. CE and NE had zetas potentials of 27.7 and −6.2 mV, respectively, and the zeta potential reversed from negative to positive charge with chitosan coating. Coating of the emulsion by chitosan was confirmed by increase in particle size and changes in zeta potential. On the other hand, the particle size and zeta potential of HPMCE were almost the same as those of NE, indicating that HPMCE was not coated by HPMC. HPMC was thus not adsorbed onto the surface of the emulsion droplets, and was probably dispersed in the water phase because particle size did not increase. The viscosity of NE was 1.8 mPa·s, whereas those of CE and HPMCE were 7.4 and 7.8 mPa·s, respectively. It was confirmed that the viscosity of HPMC was almost the same as that of CE.

The zeta potential of NE is close to neutral. However, chitosan is adsorbed onto the surface of the oil droplets. Jumaa and Müller developed a chitosan–lipid emulsion using a nonionic surfactant, pluronic F68. They suggested that chitosan molecules localized at the interface of the oil droplets and intercalated with the nonionic surfactant. This may have occurred in our chitosan-coated emulsion as well.

In Vivo Instillation Experiments CE was instilled on the corneal surface of rabbit eyes to evaluate drug concentration in tear fluid. Disposable 1 μl glass capillaries are usually used for sampling of tear fluid in animal pharmacokinetic studies. We collected about 1 μl of tear fluid for sampling. Figure 1 shows the concentration–time profiles of indomethacin in tear fluid after instillation of CE and NE. The drug levels with the two formulations were almost the same until 0.25 h after instillation. The conjunctival sac normally holds 7—9 μl of tears, but can retain up to approximately 20—30 μl without overflow. The normal tear flow rate is 1 μl/min. Therefore, difference in drug level between the two formulations would not appear until return to physiological tear volume. The drug levels in CE were 3.6-fold and 3.8-
fold higher than those in NE at 0.5 and 0.75 h after instillation, respectively ($p<0.05$). One hour after instillation, the drug concentration of CE was 2.2-fold that of NE. This difference may have become less pronounced as time increased. Usually, the viscosity of ophthalmic solution affects residue time on the ocular surface. Optimum viscosity is within the range of 15 to 20 mPa·s. Further increases in viscosity above this range are not advantageous. The values of tear viscosity given in the literature also vary between 1 and 6 mPa·s. The viscosity of CE was 7.4 mPa·s and slightly outside the range of physiological tear viscosity. However, the viscosity of CE is not within the viscosity range clearly affecting drug residue time. Although the viscosity of CE might affect drug residue time, its effect would be small. The advantageous effect of viscosity in most viscous solutions generally appears to persist for as long as about 20 min after instillation. CE maintained higher drug levels than NE from 0.5 to 1.0 h after instillation, and this prolonged period was an effect of the chitosan coating rather than viscosity.

The pharmacokinetic parameters of CE and NE in tear fluid are summarized in Table 3. The $AUC_{0-1h}$ of CE was slightly higher than that of NE. However, the mean resident time ($MRT$) and half-life ($t_{1/2}$) of CE were prolonged to 1.5-fold and 1.8-fold those of NE, respectively. This finding clearly indicates that the residence time of the drug in tears was prolonged with use of chitosan-coated emulsion. The volume of distribution ($V$) of CE was also 1.6-fold that of NE, suggesting that CE had higher distribution on the ocular surface tissue than NE. The half-life of cationic liposomes instilled on the corneal surface was previously reported to be 5 min. The half-life of CE in the present study was about 15 min (0.25 h), and longer than that of the cationic liposomes reported. This finding suggests that the mucoadhesive chitosan contributes strongly to the retention of emulsion in tear fluid.

The drug concentrations of CE and NE in aqueous humor, cornea, and conjunctiva 1 h after instillation are listed in Table 4. The drug concentrations for CE in all tissues were clearly higher than those in NE. The concentration ratios of CE to NE in each tissue were from 1.4-fold to 1.8-fold and in each tissue the concentrations increased with increase in drug levels in tears. The drug concentration in aqueous humor was evaluated using a generalized ocular pharmacokinetic model to predict the profile of aqueous humor level. This model is basically composed of 4 sections: tear flow dynamics, corneal penetration, aqueous humor flow dynamics, and tissue distribution around aqueous humor. The drug instilled in tear fluid is eliminated by tear flow. The pharmacokinetic parameters obtained for the formulations in the in vivo instillation experiment were used as parameters of tear flow dynamics for this simulation. Under these conditions, drug penetration in the cornea usually depends on two physicochemical properties: partition coefficient and diffusion coefficient. The drug partitioned onto the corneal epithelium is then partitioned to the corneal stroma, and also diffuses in epithelium and stroma. The partition coefficient between epithelium and stroma was calculated using a correlation equation with the octanol/buffer partition coefficient of indomethacin. It is also well established that membrane diffusion coefficient is inversely proportional to the exponential multiplier of molecular size or molecular weight of the drug. Diffusion coefficients of pirenoxine, an anti-cataract drug, in epithelium and stroma were determined previously, and the values in corneal epithelium and stroma were $6.6 \times 10^{-7}$ and $3.2 \times 10^{-7}$ (cm$^2$/s), respectively. The diffusion coefficients in epithelium and stroma for this simulation were calculated by multiplying the diffusion coefficients of pirenoxine by reciprocal of the exponential multiplier of the molecular weight ratio of indomethacin/pirenoxine. The partition coefficients and the diffusion coefficients were used as parameters of corneal penetration for this simulation. The drug in aqueous humor is eliminated by aqueous humor flow dynamics and distribution to surrounding tissues such as the lens and iris. The aqueous humor flow rate is approximately 1% of its volume per minute. Assuming perfect mixing in aqueous humor, the absence of enzymatic reaction, and negligible distribution to tissues around the aqueous humor, the elimination rate constant was calculated as 0.01 min$^{-1}$. This was in accord with the elimination rate constants of most drugs. The elimination rate constant in aqueous humor was used as a parameter of aqueous humor flow dynamics for this simulation. Distribution to lens and iris can be simulated based on a three-compartment model if there are appropriate initial conditions. However, appropriate initial conditions of indomethacin are not clear. Parameters of pirenoxine are shown in the literature for analysis using the generalized ocular pharmacokinetic model. We used the parameters of pirenoxine as model parameters of tissue distribution around aqueous humor for this simulation. Physiological values and anatomical values were used for other parameters as well. In this simulation, the physicochemical properties of the formulations affected the parameters in tear flow dynamics.

This simulation was based on some of the assumptions mentioned above for corneal penetration, aqueous humor flow dynamics, and tissue distribution around aqueous humor. Therefore, we used CE/NE ratios as simulated results for concentration and $AUC$ but did not show the simulated...
values. The time at which the $C_{\text{max}}$ is achieved ($T_{\text{max}}$) of CE and NE were estimated to be 1.4 and 1.2 h, respectively. The calculated $C_{\text{max}}$ and AUC of CE were 1.6-fold and 1.7-fold higher than those for NE, and were roughly similar to aqueous humor levels in vivo. These results suggest that prolonging the residence time of the drug in tears can clearly improve the ocular bioavailability of emulsion.

**Measurement of Forces of Detachment** Forces of detachment of the emulsions and mucin dispersion were measured using a tensile testing apparatus for evaluation of mucoadhesive strength, as shown in Fig. 2. The force of detachment of CE and mucin (CE-mucin) was significantly larger than those for the other emulsions ($p<0.05$) investigated. The force of detachment of HPMCE (HPMCE-mucin) was also significantly larger than that for NE ($p<0.05$). In addition, we used HPMC as a viscous agent with poor mucoadhesiveness.\(^{33}\) The forces of detachment of CE and HPMCE for the blank measurement using PBS were almost the same, since these formulations have similar viscosity. For the blank measurement, these forces of detachment were slightly higher than those for NE. Recently, the BIACORE method for measuring interaction between bioadhesive polymers and mucin was reported. Our findings corresponded to the results obtained with the BIACORE method, which showed that chitosan possesses higher adhesivity to mucin than HPMC.\(^{34}\)

Usually, the force needed to move the eyelids during a normal blink is about 0.2 N, and is 0.8 N for a forceful blink.\(^{29}\) It is desirable to evaluate forces of detachment under experimental conditions close to ocular physiological conditions. When over 1 N was applied at contact with emulsion and mucin dispersion, the relative standard deviation of the measured values was less than 10% (data not shown). Therefore, a force of 1 N was selected at contact with emulsion and mucin dispersion in this study. We also used porcine gastric mucin in this study. Gastric mucin is mainly composed of large gel-forming mucins,\(^{35}\) which are responsible for the rheological properties of mucins.\(^{36}\) The rheological behavior of ocular mucin as well as gastric mucin is predominantly determined by the gel-forming mucins. Porcine gastric mucin was therefore used as commercially available gel-forming mucin in this study. In addition, the pH of the mucin dispersion was adjusted to 7.4 to conform to the mean pH value of normal tears.

We approximated ocular physiological conditions in measuring forces of detachment. As expected, CE exhibited mucoadhesiveness by interaction of chitosan and mucin rather than increase in viscosity under experimental conditions close to ocular physiological conditions. These results implied that the residence time of emulsion in tears was prolonged by chitosan coating. Furthermore, drug absorption should be improved by prolonging precorneal retention of the drug. CE should exhibit excellent tolerance on the cornea, since 1.5% chitosan solution was very well tolerated when the solution was instilled onto the rabbit cornea four times a day for a period of 3 d, as reported previously.\(^{33}\) CE is thus a promising formulation for improving the ocular bioavailability of ophthalmic emulsion because of its close adhesion to ocular surface tissues.

**CONCLUSION**

We examined the tear retention of CE containing indomethacin in rabbit eyes. The mucoadhesive properties of CE and NE were measured using tensile testing. This study confirmed that the residence time of indomethacin with CE was prolonged compared with NE in in vivo instillation experiments. Pharmacokinetic parameters indicated higher distribution of CE on the ocular surface than NE. The drug concentrations for CE in cornea, conjunctiva, and aqueous humor were clearly higher than for NE 1 h after instillation. The drug concentration in aqueous humor was simulated by a generalized ocular pharmacokinetic model to predict the profile of aqueous humor levels. The calculated ratios of CE to NE for $C_{\text{max}}$ and AUC nearly corresponded to those for aqueous humor level in vivo. On tensile testing, CE exhibited mucoadhesive properties rather than increase in viscosity under experimental conditions close to ocular physiological conditions. The residence time of emulsion in tear fluid was thus prolonged by coating with chitosan.

**REFERENCES**