Research paper

Studies on a new device for drug delivery to the eye

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Abstract

Delivery of drugs to the anterior side of the eye is routinely done with eye drops, but this method results in low bioavailability and low patient compliance. Herein, we describe a new device for the delivery of drugs to the eye. The device, called the OphthaCoil, consists of a drug-loaded adherent hydrogel coating on a thin metallic wire, which is coiled. The drug release rates of the dye fluorescein and the antibiotic chloramphenicol have already been evaluated in vitro. In this report the drug release rate of the anti-infective pradofloxacin was evaluated in vitro and in vivo. The data show that the OphthaCoil is capable of sustained drug delivery to the tear film in dogs. Drug levels in the tear fluid of the dogs were well above the MIC-values of relevant bacteria after 16 h, but it should be noted that pradofloxacin has an exceptionally high antimicrobial activity. The study indicates that the OphthaCoil holds promise as a platform for sustained release of drugs to the eye. The device was well tolerated, but the devices were lost when left overnight. Most probably, this is due to the third eyelid pushing the device out of the conjunctival sac during sleep. It should be noted that this complication has no immediate implication for extended wear of the OphthaCoil in humans, as humans do not have third eyelids.
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Keywords: Drug delivery; Hydrogel; Pradofloxacin; In vivo; Ophthalmology

1. Introduction

Hydrogels are three-dimensional, hydrophilic, polymeric networks capable of taking up large amounts of water or biological fluids [1]. The networks are composed of homopolymers or co-polymers and are insoluble due to the presence of chemical or physical cross-links. The physical properties of hydrogels make them attractive for a variety of biomedical applications [2–6]. Hydrogels are especially suitable as platforms for controlled local delivery of drugs. In the simplest form, a drug is impregnated in a dry hydrogel matrix. When implanted, the matrix will swell (uptake of water from the environment), with concomitant diffusion-controlled release of the drug. More sophisticated strategies may be based on stimuli-responsive hydrogels. For example, pH-sensitive hydrogels have been devised for insulin delivery [2]. The role of hydrogels as drug delivery vehicles has been highlighted in several recent reviews [1–3].

In this article, we specifically focus on the use of hydrogels for administration of drugs to the anterior side of the eye. Delivery of drugs to the tear film is routinely done with eye drops, which are well-accepted and for most patients easy to use. However, most of the solution in each eye drop is blinked out or drained into the nasal cavity. This leads to poor bioavailability of the drug [7]. Frequent administrations are required to maintain therapeutic levels, resulting in low patient compliance [8]. Development of an alternative to eye drops via sustained delivery of a drug to

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the tear film is a major challenge. Hence, numerous studies have been devoted to developing devices for sustained drug delivery to the tear film; all of these are based on hydrogel technology [7–14]. This work resulted in several commercial products (e.g. Mydriasert® [9] and Ocusert® [10]). Several other concepts are still under investigation, e.g. a mucoadhesive ocular insert based on thiolated poly(acrylic acid) [8] and a Gelfoam® based ocular device [7].

In general, commercialisation of ocular inserts for drug delivery has proven troublesome, probably in part due to psychological factors. Doctors and patients generally feel more comfortable with traditional eye drops. Use of hydrogels for controlled drug delivery to the tear film is often associated with the complication that the insert becomes weak during the process of swelling and drug release. This may hamper the removal of the insert when drug release is completed. This complication has been described for the mucoadhesive ocular insert based on thiolated poly(acrylic acid) [8]. It should be noted, that biodegradable matrices for drug delivery to the tear film have also been described. In theory, these have the advantage that no removal is necessary, due to enzymatic or chemical degradation. However, biodegradable inserts may also have disadvantages [8]; erosion or disintegration into smaller pieces may result in occasional blurring of vision. The disintegrated insert may also move around the ocular surface causing irritation and might be easily lost.

Herein, we describe a new device for controlled delivery of drugs to the eye, which is also based on hydrogel technology. The major difference between the new device and its predecessors is that a thin hydrogel coating is used; this coating is a drug-loaded adherent hydrogel on a thin stainless steel wire, which forms a short coil. The new device, which is called OphthaCoil, is shown in Fig. 1.
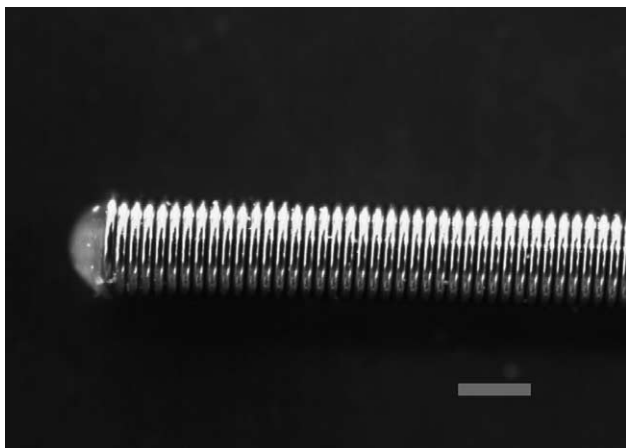

Fig. 1. Photograph showing the OphthaCoil (bar = 0.5 mm). The adjacent windings of the coiled structure, as well as one of the polymeric caps, closing the lumen of the device, are clearly visible. The outer diameter of the coil is 600 μm; the diameter of the coated coiled wire is approximately 90 μm.

The OphthaCoil has several characteristic features:

- The device consists of a flexible coil, which has a drug-loaded biocompatible coating. The coil is inserted into the lower conjunctival sac, i.e. behind the lower eyelid.
- The ends of the coil are closed with a spherical cap to avoid sharp edges.
- The device has a lumen, which is used as a second drug reservoir. Drug molecules can escape from the lumen towards the tear fluid via diffusion in between adjacent windings of the coil; in principle, this allows long-term drug release.
- After swelling of the coating and complete release of the drug, the device retains its integrity.

Release of drugs from thin coatings on wires and coils has been previously studied by our group [15–19]. In all cases, we used co-polymers of *N*-vinylpyrrolidone (NVP) and *n*-butylmethacrylate (BMA) as the coating. Mechanistic studies were done with the help of impregnated dyes and the antibiotic chloramphenicol [15–19]. Based on the in vitro release data, it was anticipated that a short coil as shown in Fig. 1 can contain and release substantial amounts of a drug, i.e. sustained release using the OphthaCoil should, in principle, be possible. It is a *conditio sine qua non*, however, that the OphthaCoil is well tolerated in the eye, i.e. the device should not cause any irritation or inconvenience to the patient.

Presently, we report the results of our first tests on the tolerance of the OphthaCoil in an in vivo model. OphthaCoils, charged with pradofloxacin, a new fluoroquinolone developed by Bayer HealthCare exclusively for veterinary medicine, were used. First, the release of pradofloxacin in vitro was studied. Then, OphthaCoils charged with pradofloxacin were placed in the eyes of six Beagle dogs. The animals were examined, samples of the tear fluid were taken, and the concentration of the antibiotic was monitored as a function of time.

2. Materials and methods

2.1. The OphthaCoils

The hydrogel used is the co-polymer of the hydrophilic monomer NVP and the hydrophobic monomer BMA, in the molar ratio 70:30. The co-polymer was prepared in a free-radical bulk polymerisation reaction, using 2,2′-azobis(2-methylpropionitrile) (AIBN) as the initiator. The procedure resulted in the desired co-polymer as a glassy, opaque rod. The rod was cut into pieces, which were dissolved in *N*-methylpyrrolidone (NMP) by mechanical stirring overnight.

A thin stainless steel wire with a diameter of 76 μm, was first coated with a thin layer of a binding polymer (polyethersulfone, thickness 1 μm), and then coated twice with a polymer solution consisting of 220 mL NMP, 15 g of the co-polymer NVP:BMA 70:30, and 10 g of pradofloxacin

(Bayer HealthCare AG, Leverkusen, Germany). An extrusion-like coating procedure, as described before [17,18], was used. The thickness of the coating layer was measured online with a laser system and afterwards it was verified with scanning electron microscopy.

Half of the wire was stored (wire A); this wire had a coating thickness of 3.75 μm. The other half was processed twice more (four passages), resulting in a drug-containing hydrogel coating with a thickness of 7.25 μm (Wire B). Wire A was coiled around a core wire of 432 μm thickness, and the resulting coil was cut into pieces of 16 mm. Wire B was cut into pieces of 15 mm and three of these wires were put into the coil. The coils were carefully closed with a photoreactive glue, using a technique which is well-known in the catheter industry. The caps effectively prevented sharp edges, see Fig. 1. The dimensions of the coils were 16 mm long and ± 0.6 mm in diameter.

Based on several known parameters, it is possible to calculate the total charge of pradofloxacin on each OphthaCoil. These parameters are: length of wire A (35 cm), thickness of the pradofloxacin-containing coating of wire A (3.75 μm), length of wire B (15 mm), thickness of the pradofloxacin-containing coating of wire B (7.25 μm) and the concentration of pradofloxacin in the hydrogel coating (40%). This resulted in an amount of 135.6 μg pradofloxacin on the coil (wire A) and 3×11.7 μg $= 35.1$ μg on wires B. The calculated total charge of pradofloxacin was then 170.7 μg per OphthaCoil. It is worth noting that, wire B has a contribution of 20% to the capacity of the device.

2.2. In vitro release experiments

Pradofloxacin-charged OphthaCoils were immersed in 1 mL sterile PBS (phosphate buffered saline) that was refreshed at the following time-points: 5, 15, 30 min, 1, 3, 6, 12, 24 and 48 h. The pradofloxacin concentrations in these solutions were determined with a zone-of-inhibition assay [20]. Plastic Petri dishes were filled with Iso-sensitest™ medium which was used as culture medium. E. coli 14 was used as indicator bacterium, because E. coli infections are common in the eyes of dogs. After coagulation and drying of the culture medium, the indicator bacterium was brought onto the medium and holes were punched with a radius of 4 mm at equal distance in the agar bottom. These holes were filled with 0.1 mL of the solutions with the released pradofloxacin. Other holes were filled with 0.1 mL of a dilution series, so a calibration curve could be made to determine the concentration of pradofloxacin in the measurements. After 24 h of incubation at 37 °C the diameters of the inhibition zones were measured and the concentrations were calculated.

2.3. Tolerance in vivo

The tolerance and safety study was done with three female adult Beagle dogs, and one OphthaCoil per animal.

In these experiments, the hydrogel coating did not contain any active substance. The dogs were about 2 years old and they weighed between 10.9 and 12.4 kg. The animals were housed individually in cages with tiled floor and identification was assured through an individual, unchangeable ear tattooing provided by the breeder. The relative humidity varied between 50–60% and the temperature varied between 15–21 °C. Lighting was controlled to ensure approximately 12 h of artificial light from 6.00 a.m. to 6.00 p.m. Once a day the dogs were fed a commercially available dry dog food and water was provided for ad libitum consumption.

To avoid unwanted defense reactions during application of the coil, one drop of the local anaesthetic oxbarucain (Oxbarukain®uno, Chauvin Ankerpharm GmbH, Rudolstadt, Germany) was administered to the eye. The anaesthetic was administered five minutes before application of the coil. Oxbarucain is known to be active for about 30 min. The coils were placed in the lower conjunctival sac of each dog's left eye. The right eyes served as controls. The dogs were continuously observed for 8 h. Then, the presence and the position of the coils were checked. The next day, after 24 h, the coils were checked again and were removed when still present in the eye.

Before the application, and after the removal of the coil, an ophthalmological examination took place, where both eyes were examined for alterations of conjunctiva, third eyelid, cornea and sclera. The following parameters were examined: the ocular discharge, the third eyelid, the cloudiness and surface of the cornea, the redness, secretion and swelling of the conjunctivae, the sclera and the behaviour of the dog. The results were compiled in a four-stage grading system ($-$, normal; $+$, slight change; $++$, moderate change; $+++$, severe change).

2.4. In vivo release experiment

The kinetic study was done with a group of six female Beagle dogs and one pradofloxacin-charged OphthaCoil per animal. All dogs were between 1½ and 2½ years old. Their weight ranged between 10.9 and 13.9 kg. The animals were housed individually in cages, as in the previous experiment. Before application of the coil a tear fluid sample was taken with a Schirmer test strip (Liposic, dr Mann Pharma, Berlin, Germany) and again one drop of oxbarucain was administered five minutes before application of the coil. Tear fluid samples were taken at the following time-points: after 1, 2, 4, 6 and 8 h. The dogs were continuously observed and after 8 h the coil was removed and stored in an Eppendorf vial. The next day the same coils were inserted and tear fluid samples were taken again after 1, 2, 4, 6 and 8 h. Then the coil was left in the eye to check the presence and position of the coil overnight.

The day before the application of the coil and on the last day, after the removal of the coil, an ophthalmological examination took place, where both eyes of the animals were examined, as mentioned in Section 2.3. The concentrations

of pradofloxacin in the tear fluid samples were determined by HPLC and tandem mass spectroscopy [21].

3. Results and discussions

3.1. Build-up of the OphthaCoils

The OphthaCoil consists of a coil (wire A) with three shorter pieces of a wire with a thicker coating thickness (wire B) inside. This is schematically shown in Fig. 2.

3.2. Release of pradofloxacin in vitro

Fig. 3 shows the results of the release experiments in vitro. The concentration of the released pradofloxacin is plotted as a function of time. The initial release is fast, which is in agreement with our previous observations on the release of, e.g. rhodamine and heparin from comparable coils [15–17]. At the end of the experiment, i.e. after 24–48 h of immersion, a released pradofloxacin concentration of approximately 0.6 μg mL^{-1} was found in both cases. This concentration is still far above the MIC-values, which are 0.015–0.03 μg mL^{-1} for *E. coli* ATCC 8739 and 0.03–0.06 μg mL^{-1} for *Staphylococcus aureus* ATCC 6538 [22]. This implies that the OphthaCoil shows sustained release of the anti-infective in vitro, at least for 48 h.

The total amount of pradofloxacin released after 48 h was calculated to be 32 and 35 μg. This implies that approximately 20% of the anti-infective, that was present in and on the device at the start of the experiment, is actually released. Most likely, release of small quantities of the anti-infective goes on after 48 h, although we also anticipate that a substantial fraction of active species remains entrapped in the hydrogel coating, even after swelling to complete equilibrium [17].
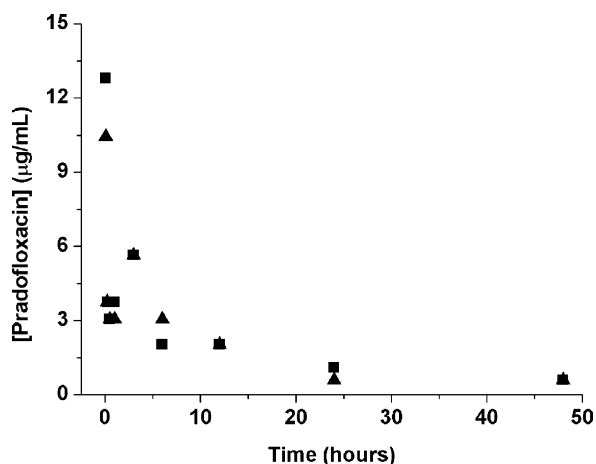
Fig. 3. The release of pradofloxacin in vitro ($n = 2$). While the initial release is fast, concentrations after 48 h are significantly higher as compared to the MIC-value of pradofloxacin.

3.3. Tolerance in vivo

These experiments were performed with three Beagle dogs and three OphthaCoils, which did not contain an active substance. All animals were healthy during the general examination the day before the application. Immediately before the application one dog had slightly reddened conjunctivae and one dog showed slight seromucous discharge from the eye. Introduction of the OphthaCoils (one coil in the left eye of each animal) proceeded without difficulties. During and immediately after the application all animals were calm and did not show signs of discomfort due to the coils.

During examination of the animals after 8 h, which involved lifting of the lower eyelid, one insert partially came out, showing that all manipulations must be executed very carefully. Based on the observations, it was decided to leave the OphthaCoils in place overnight. The next morning, however, we found that all three animals had lost the insert. One of the dogs still had slight discharge from the eye and two dogs had slightly reddened conjunctivae of both eyes.

While we do not have a conclusive explanation for the overnight loss of the device, we presume that the coil is pushed out during sleep, when the eyeball is retracted into the orbit and the third eyelid moves into place [23]. A photograph of a dog with its third eyelid is shown in Fig. 4.

In an attempt to verify this explanation, an additional experiment was executed with a dog that had its third eyelids removed because of lachrymal gland hyperplasia of the third eyelid. The animal received an OphthaCoil in the right eye and the next morning the coil was still present in the eye. This experiment supports the hypothesis that the movement of the third eyelid over the eye is the reason for the loss of the coil overnight.
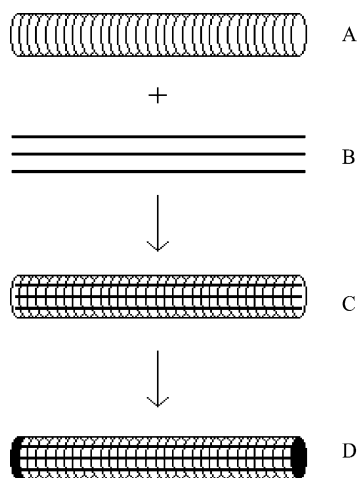
Fig. 2. Schematic view of the OphthaCoil manufacturing: (A) Coil of wire A; (B) Three straight pieces of wire B; (C) Wires B placed inside the lumen of the coil; (D) Closing of the ends by a polymeric cap.
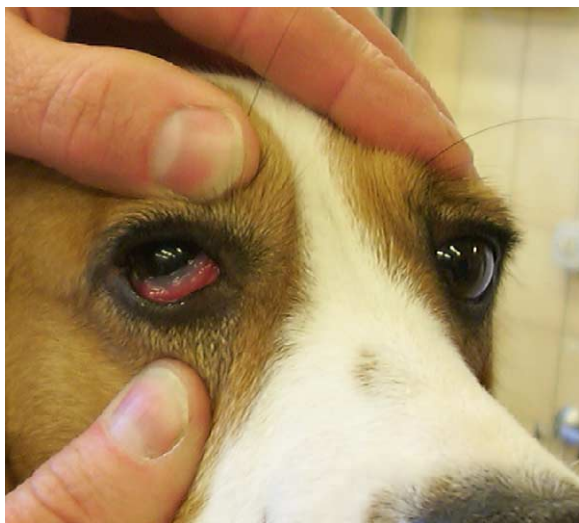
Fig. 4. Detailed photograph showing the third eyelid. Presumably, the third eyelid pushes the device out of the animal's eye during sleep.

3.4. Release of pradofloxacin in vivo

These experiments were performed with six Beagle dogs and six OphthaCoils charged with pradofloxacin. The concentration of the anti-infective in the tear fluid was monitored as a function of time. The tear fluid samples were taken with so-called Schirmer test strips. These paper-like porous strips (5×35 mm) absorb tear fluid when the end of the strip is bent and placed into the lower conjunctival sac. The procedure is harmless to the animals and, in our experience, the animals were not irritated or disturbed during repeated sampling.

Our experience with the unloaded OphthaCoils (3.3 vide supra) led us to insert the devices for 2×8 h, under continuous observation of the animals. The OphthaCoils were inserted on the morning of day 1, taken out after 8 h, and stored. The next day, the OphthaCoils were re-inserted and left in place for another 8 h. Samples were taken at regular time points on both days. No major difficulties were encountered, except for dog #6, which was slightly smaller than the other five animals. Dog #6 lost the OphthaCoil after 4 h on the first day. It was decided to re-insert a new OphthaCoil on the second day. However, the new coil was also lost just before the sampling after 8 h. It was clear that this animal had smaller eyes with tighter eyelids, resulting in a non-optimal fit of the device. No problems were encountered with the other animals. They were quiet during the observations, although sometimes a little frightened. We experienced that the sampling procedure had to be executed with great care, as placing of the Schirmer test strip required lifting of the lower eyelid. In some cases, this led to partial escape of the OphthaCoil from the conjunctival sac. This problem occurred with two animals and could be avoided after some practice.

In view of the apparent tolerance, we decided to leave the OphthaCoil in place at the end of the second day.
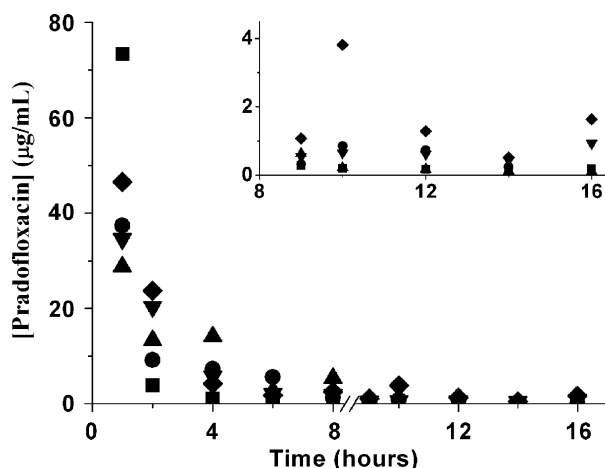

Fig. 5. Release of pradofloxacin in vivo. As in the in vitro experiments, most of the drug is released in the beginning of the experiment. Concentrations after 16 h, albeit low (see inset), are still far above the MIC-value of pradofloxacin. Note that the interruption of the experiment after 8 h is indicated on the *x*-axis and the different symbols (■, ♦, ●, ▼, ▲) refer to the five different animals that participated in this experiment.

On the morning of the third day, however, none of the OphthaCoils were still in place. We ascribe this complication to the third eyelid which covers and protects the eyes during sleep. It should be noted that this complication has no immediate implication for extended wear of the OphthaCoil in humans, as humans do not have third eyelids.

Fig. 5 shows the data which were generated in these experiments. The pradofloxacin concentrations, as measured by re-dissolution of the drug from the Schirmer test strips and analysis by HPLC-MS/MS, are plotted as a function of time. Data from only five dogs are given, because dog #6 was excluded due to its anatomic characteristics, as mentioned above.

Fast initial release is found, with concentrations in the range 30–70 µg mL^{-1} after 1 h. The concentration drops by two orders of magnitude within the next 7 h. Nevertheless, it is clear that the release continues in the period 8–16 h (see insert in Fig. 5). While the concentrations of pradofloxacin on the second day were low, the drug was clearly detectable and its levels were still far above the MIC-values, which are 0.015–0.03 µg mL^{-1} for *E. coli* ATCC 8739 and 0.03–0.06 µg mL^{-1} for *Staphylococcus aureus* ATCC 6538 [22]. But it should be noted that pradofloxacin has an exceptionally high antimicrobial activity.

4. Concluding remarks

Based on the current data, we believe that the OphthaCoil holds promise as a platform for sustained release of drugs to the eye. A particular advantage of the device may be that it has a stable, flexible, rod-shaped geometry. Already in 1977, Katz and Blackman suggested that rod-shaped ocular inserts, having a relatively high aspect ratio (that is, the ratio

of length:width), are better tolerated in the conjunctival sac as compared to tablet-shaped inserts, which have a relatively low aspect ratio [10,24]. It must be noted that the thin, coiled metallic wire plays an important role in providing sufficient stability to the device. Without the metallic wire the hydrogel would lack sufficient strength and durability, i.e. the device could then fall apart in situ. Furthermore, the OphthaCoil is readily visible in the conjunctival sac, and its removal using a forceps was relatively easy.

However, much more developmental work is necessary. Three major questions should be specifically addressed:

- What is the optimal geometry and flexibility for the coil to ensure that use of the device leads to maximum safety and minimum discomfort?
- How can the lumen of the coil be used to maximise the capacity of the device?
- What are the most important parameters controlling the drug release kinetics? These parameters should be optimised to achieve the desired in vivo release profile for each particular drug.

Further research and development work aimed at answering these questions is currently in progress in our laboratories.

References

[1] N.A. Peppas, P. Bures, W. Leobandung, H. Ichikawa, Hydrogels in pharmaceutical formulations, Eur. J. Pharm. Biopharm. (Review) 50 (2000) 27–46.

[2] P. Gupta, K. Vermani, S. Garg, Hydrogels: from controlled release to pH-responsive drug delivery, Drug Discov. Today (Review) 7 (10) (2002) 569–579.

[3] A.S. Hoffman, Hydrogels for biomedical applications, Adv. Drug. Del. Rev. 54 (2002) 3–12.

[4] B.D. Ratner, A.S. Hoffman, F.J. Schoen, J.E. Lemons, Biomaterials Science: An Introduction to Materials in Medicine, Academic Press, San Diego, 1996.

[5] J. Li, X. Ni, K.W. Leong, Injectable drug-delivery systems based on supromolecular hydrogels formed by poly(ethylene oxides) and alpha-cyclodextrin, J. Biomed. Mater. Res. 65A (2) (2003) 196–202.

[6] T. Coviello, M. Grassi, R. Lapasin, A. Marino, F. Alhaique, Scleroglucan/borax: characterization of a novel hydrogel system suitable for drug delivery, Biomaterials 24 (16) (2003) 2789–2798.

[7] Y.C. Lee, J. Millard, G.J. Negvesky, S.I. Butrus, S.H. Yalkowsky, Formulation and in vivo evaluation of ocular insert containing phenylephrine and tropicamide, Int. J. Pharm. 182 (1999) 121–126.

[8] M. Hornof, W. Weyenberg, A. Ludwig, A. Bernkop-Schnurch, Mucoadhesive ocular insert based on thiolated poly(acrylic acid): development and in vivo evaluation in humans, J. Control. Release 89 (2003) 419–428.

[9] J.M. Aiache, S. El Meski, G. Serpin, E. Beyssac, R. Conti, Local tolerance and in vivo release study of a new ocular drug delivery system, Drug Deliv. Syst. Sci. 1 (3) (2001) 85–87.

[10] M.F. Saettone, L. Salminen, Ocular inserts for topical delivery, Adv. Drug Deliv. Rev. 16 (1995) 95–106.

[11] J. Ceulemans, A. Vermeire, E. Adriaens, J.P. Remon, A. Ludwig, Evaluation of a mucoadhesive tablet for ocular use, J. Control. Release 77 (2001) 333–344.

[12] H. Sasaki, T. Nagano, K. Sakanaka, S. Kawakami, K. Nishida, J. Nakamura, N. Ichikawa, J. Iwashita, T. Nakamura, M. Nakashima, One-side-coated insert as a unique ophthalmic drug delivery system, J. Control. Release 92 (2003) 241–247.

[13] P. Chetoni, G. Di Colo, M. Grandi, M. Morelli, M.F. Saettone, S. Darougar, Silicone rubber/hydrogel composite ophthalmic inserts: preparation and preliminary in vitro/in vivo evaluation, Eur. J. Pharm. Biopharm. 46 (1998) 125–132.

[14] A. Karatas, T. Baykara, Studies on indomethacin inserts prepared by water-soluble polymers. II. II. The relation between dissolution rate and swelling behaviour, Farmaco 56 (2001) 197–202.

[15] C.C. Peerlings, H.H.L. Hanssen, R.T. Bevers, E.J. Boelen, B.J. Stelt, E.J. Korthagen, L.H. Koole, Heparin release from slippery-when-wet guide wires for intravascular use, J. Biomed. Mater. Res. 63 (2002) 692–698.

[16] H.H.L. Hanssen, L.H. Koole, Heparin-releasing intravascular guide-wires, Med. Device Technol. 13 (2002) 20–22.

[17] H.H.L. Hanssen, G.M.R. Wetzels, A. Benzina, F.H. Van der Veen, T. Lindhout, L.H. Koole, Metallic wires with an adherent lubricious and blood-compatible polymeric coating and their use in the manufacture of novel slippery-when-wet guidewires: possible applications related to controlled local drug delivery, J. Biomed. Mater. Res. 48 (1999) 820–828.

[18] R.T. Pijls, H.H.L. Hanssen, R.M.M.A. Nuijts, L.H. Koole, Flexible coils with a drug releasing hydrophilic coating: a new platform for controlled delivery of drugs to the eye?, Biomed. Mater. Eng. 14 (4) (2004) 383–393.

[19] L.P. Cruysberg, R.M. Nuijts, D.H. Geroski, L.H. Koole, F. Hendrikse, H.F. Edelhauser, In vitro human scleral permeability of fluorescein, dexamethasone-fluorescein, methotrexate-fluorescein and rhodamine 6G and the use of a coated coil as a new drug delivery system, J. Ocul. Pharmacol. Ther. 18 (2002) 559–569.

[20] W. Levinson, E. Jawetz, Medical Microbiology and Immunology: Examination and Board Review, seventh ed., Lange Medical Books, New York, 1998.

[21] R. Krebber, Analytical method for the determination of pradofloxacin in serum and urine by turbulent flow chromatography/tandem mass spectrometry, J. Vet. Pharmacol. Therap. 26 (1) (2003) 102–103.

[22] H.G. Wetzstein, Comparative mutant prevention concentrations of pradofloxacin and other veterinary fluoroquinolones, Abstracts of the 103rd Interscience Conference on Antimicrobial Agents and Chemotherapy, American Society for Microbiology, San Diego, Z-010 2003.

[23] C.L. Martin, Augenkrankheiten bei Hund und Katze, M&H Schaper, 1995, pp. 31–54.

[24] I.M. Katz, W.M. Blackman, A soluble sustained-release ophthalmic delivery unit, Am. J. Ophthalmol. 83 (1977) 794–800.