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Capacity and tolerance of a new device for ocular drug delivery

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Abstract

A new method to increase the drug-capacity of the OphthaCoil, a flexible and tubular device for delivery of drugs to the tear film of the eye, was explored. Poly(2-hydroxyethyl methacrylate)- and poly(2-hydroxyethyl methacrylate-*co*-1-vinyl-2-pyrrolidone)-microspheres were prepared by suspension polymerization. The resultant particles were swollen in a highly concentrated solution of either the dye fluorescein sodium or the antibiotic chloramphenicol. The loaded particles were placed in the central cavity of the ocular device. In vitro release profiles showed a six-fold increase of the capacity for the dye fluorescein sodium, but not for the antibiotic chloramphenicol. Flexibility measurements revealed that by introducing microspheres in the central cavity of the device, flexibility did not decrease. Finally, a preliminary in vivo evaluation of the device ($n = 5$) was done for a 2 h-period to assess the tolerance of the device in the human eye. Ophthalmologic examinations and photographs of the eye indicated no signs of irritation. Volunteers reported that the presence of the device in the eye could be noticed, but no irritation was reported.

Keywords: Ocular insert; Microspheres; 2-Hydroxyethyl methacrylate; Drug release; In vivo tolerance

1. Introduction

Ocular drugs are normally administered via eye drops. Although this is common practice, there are several problems and drawbacks, such as: (i) limited bioavailability, (ii) rapid elimination, (iii) destabilization of the tear film, (iv) non-compliance by patients, and (v) allergic reactions (Winfield et al., 1990; van Ooteghem, 1993; Lang, 1995; Le Bourlais et al., 1998; Oyster, 1999; Geroski and Edelhauser, 2000; Van Santvliet and Ludwig, 2004; Baudouin, 2005; Gulsen and Chauhan, 2005; Hosoya et al., 2005; Salyani and Birt, 2005; Barbu et al., 2006).

These practical issues have stimulated the search for alternative methods for ocular drug delivery. Much of this work was devoted to ocular inserts, which serve as a platform for the release of one or more active substances. It has become clear, however, that the development of an ocular insert that reliably combines controlled release with absence of any irritation to the patient, poses a formidable technical challenge (Ding, 1998; Herrero-Vanrell and Refojo, 2001; Barbu et al., 2005).

Recently, we have proposed an ocular drug delivery device according to a new concept (Pijls et al., 2004, 2005). The device, called "OphthaCoil" (Fig. 1), consists of a drug loaded adherent hydrogel on a thin metallic wire, which is coiled. The coiled structure accounts for the device's flexibility and integrity. The ends of the coil are sealed with caps using photo-curable cyanoacrylate glue and the device is gas-sterilized before use. The device is placed in the conjunctival fornix. The hydrogel coating starts to swell once the device contacts the tear fluid, and concomitant release of the drug into the tear film occurs. Previously, we have reported that the OphthaCoil is well tolerated in animal models, notably in the eyes of Beagle dogs (Pijls et al., 2005). Moreover, we have shown that it is possible to release antibiotics into the tear film, in such a way that the concentration remains above the minimal inhibitory concentration (MIC-value) for 16 h at least.

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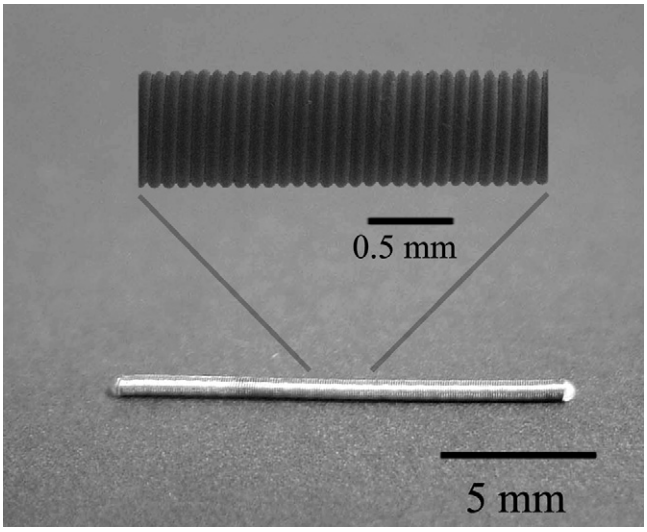
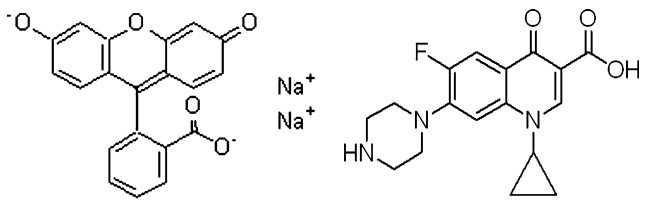


Fig. 1. The ocular drug delivery device.

In this study, we describe further improvement of the OphthaCoil, especially in terms of drug capacity and patient comfort. Two ideas were explored: (i), to use the interior of the coil as an additional drug reservoir; this implies that drug molecules must be introduced into the coil's cavity, and that these molecules must pass the windings of the coil in the release process; (ii) to use polymeric microspheres as drug carriers. Microspheres were used since filling the interior with pure drug (as a powder) is not practical; the use of a concentrated aqueous solution of the drug to fill the lumen is impossible since this would lead to premature swelling of the hydrogel coating; and the use of drug loaded hydrogel coated filaments (as described in our previous work (Pijls et al., 2005)) would translate in an increased stiffness of the device.

The hypothesis is that the use of drug loaded polymeric microspheres offers the possibility to load the interior cavity of the coil, without compromising its flexibility. The microspheres can move independently in the cavity when the coil is bent, and thus maintain the flexibility of the device when compared to when wire filaments are used.

The agents used for loading of the microspheres are the dye fluorescein sodium and the antibiotic ciprofloxacin. The chemical structures of both agents are given in Fig. 2. Ciprofloxacin is a fluoroquinolone antibacterial agent, which is widely used in human and veterinary medicine (Rosemary et al., 2003). Ciprofloxacin is used to treat bacterial infections of the eye, such as conjunctivitis or keratitis (Parks et al., 1993; Ciprofloxacin Ointment/Bacterial Keratitis Study Group, 1993; Ciprofloxacin Bacterial Keratitis Study Group, 1996). It is also prescribed to treat corneal ulcers, when the delicate layer covering the surface of the eye is ulcerated (Health Information Patient UK, 2006). Fluoroquinolones are bactericidal, exert their effect by DNA gyrase inhibition, and have a broad spectrum of activity against both Gram negative and Gram positive bacteria (Ke et al., 2001; Schaefer et al., 2001).



A Fluorescein sodium **B** Ciprofloxacin

Fig. 2. The chemical structure of fluorescein sodium (A) and chloramphenicol (B).

Here, new data on the release of the dye and the antibiotic from three different OphthaCoil prototypes will be reported. The first series has the dye/drug exclusively in the hydrogel coating of the coil; the second series has the dye/drug exclusively in the interior (that is within the hydrogel microspheres); and the third has the dye/drug both in the hydrogel coating and in the interior of the coil. Furthermore, the structural stiffness of prototypes filled on three different ways are measured (a coil with an empty interior, a coil with three hydrogel coated filaments and a coil with the hydrogel microspheres in the interior), and initial evaluations of the OphthaCoil in the human eye were performed.

2. Materials and methods

2.1. Preparation of the ocular device

All chemicals were purchased from Acros Organics (Geel, Belgium), unless stated otherwise. The hydrogel coating Slipskin® was prepared from 1-vinyl-2-pyrrolidone (NVP, Aldrich, Steinheim, Germany) and butyl methacrylate (BMA) as described earlier (Aldenhoff et al., 2004). A NVP:BMA molar ratio of 70:30 was used. The co-polymer was dissolved in 1-methyl-2-pyrrolidinone (NMP) at 10%, w/w under continuous mechanical stirring for 24 h. The procedure to apply the co-polymer onto a thin metallic wire was described earlier (Hanssen et al., 1999; Peerlings et al., 2002; Knetsch et al., 2004). Briefly, a stainless steel wire (thickness of 76 μm) was pulled through the co-polymer solution and guided through a cylindrical oven (maximal temperature 300 °C, height approximately 6 m). This resulted in the evaporation of the solvent NMP and residual-free monomers. In our case, first a 1 μm layer of poly-ethersulfone (PES) metal primer coating was applied followed by a 4–5 μm layer of the co-polymer. This process leads to a dual coating on the metal wire with uniform thickness. In order to coil the wire, a steel core mandrel with a diameter of 432 μm was connected to a speed-controllable electromotor, and the coated wires were wound around the mandrel. Coils of approximately 50 cm length were obtained. Then, the core wire was removed and the coil was cut into pieces of 15 mm in length. The ends of each coil were sealed with a small cap consisting of UV-cured poly(cyanoacrylate), which is a suitable sealing and bonding agent (Ressemann et al., 1996; Kim and Gupta, 2003). These caps serve two purposes: (i) they prevent that any sharp edges (due to cutting of the wire) could damage or irritate the cornea of tissues inside the fornix, and (ii) they close the space inside the coil, i.e. transport of drug from the lumen of the coil to the tear fluid is limited to diffusion along a pathway in between adjacent windings of the coil. Note that the placement of the caps was done stepwise: after placement of the first cap, we first filled the

lumen with (drug loaded) microspheres. Then, the second cap was placed.

To create a drug delivery device, the hydrogel coating of the wire could be incorporated with a drug. The drug was dissolved in the NMP, together with the 70 mol%/30 mol% NVP/BMA co-polymer. Then, the coating procedure was performed as described above to apply the drug loaded coating composition onto the wire.

2.2. Preparation of the microspheres

The monomers 2-hydroxyethyl methacrylate (HEMA) and NVP were used as the principal building blocks for the microspheres. Poly(HEMA) is a well-known material in biomedical applications, because of its non-toxicity, non-irritability and biocompatibility. Examples of its applications are soft contact lenses (Karlgard et al., 2003; Gulsen and Chauhan, 2004; Sato et al., 2005), blood-contacting materials and artificial emboli in endovascular embolization (Jayakrishnan and Thanoo, 1990; Jayakrishnan et al., 1990). Poly(NVP) is used because of its hydrophilicity and swelling properties. It is present in many eye drop formulations (Gobbels and Spitznas, 1992), and is also known to have excellent biocompatibility when implanted in the vitreous body or used as a vitreous substitute (Bruining et al., 1999).

The microspheres were prepared by suspension polymerization of the appropriate monomer in a non-solvent using suitable stabilizing agents to prevent the agglomeration of the monomer droplets during polymerization (Jayakrishnan and Thanoo, 1990; Jayakrishnan et al., 1990). In the case of highly water-soluble monomers like HEMA, the dispersion of the monomer into droplets has to be carried out in concentrated salt solutions in which the solubility of the monomer is very low (Jayakrishnan et al., 1989). One type of microspheres consisted of the monomer HEMA solely, named poly(HEMA)-spheres and the other type consisted of both the monomers HEMA and NVP in a 70/30-weight ratio, named poly(HEMA-*co*-NVP)-spheres.

In the preparation of the poly(HEMA)-spheres, the polymerization of the monomer HEMA was done in an aqueous medium containing 17 wt% sodium chloride and 0.672 wt% magnesium hydroxide as described by Jayakrishnan and Thanoo (1990) and Jayakrishnan et al. (1990). A 250 mL three-neck round-bottomed flask, fitted with a half-moon stirrer was charged with a solution containing 14.45 g sodium chloride and 1.97 g magnesium chloride hexahydrate in 70 mL water. The flask was heated to 70 °C in a thermostatic oil bath and 0.783 g sodium hydroxide was added in 15 mL water with continuous stirring to precipitate the suspension stabilizer magnesium hydroxide. The temperature was raised to 80 °C and the dispersed phase (total weight of 36 g) was introduced drop wise into the flask. The dispersed phase contained the monomer HEMA (96.8 wt%), the cross-linker tetra-ethyleneglycol dimethacrylate (tetra-EGDMA) (3.0 wt%, Fluka Chemie, Steinheim, Germany) and the initiator 2,2′-azobis(2-methylpropionitrile) (AIBN) (0.2 wt%). The suspension was stirred at 150 rpm for 4 h. After cooling down the suspension, the stabilizer magnesium hydroxide was dissolved by adding dilute hydrochloric acid (Merck, Germany). The beads were washed several times with distilled water and dried under reduced pressure at 35 °C.

The preparation of the poly(HEMA-*co*-NVP)-spheres (70 wt%/30 wt%) was similar to the preparation of poly (HEMA)-spheres mentioned above. The only difference was that the dispersed phase contained the monomers HEMA (67.8 wt%) and NVP (29.0 wt%), the cross-linker tetra-EGDMA (3.0 wt%) and the initiator AIBN (0.2 wt%). Also this batch was dried under reduced pressure at 35 °C.

2.3. Characterization and drug loading of the microspheres

The prepared poly(HEMA)- and poly(HEMA-*co*-NVP)-spheres were characterized by size, shape and swelling. The size distribution was measured using standard test sieves (Retsch, Germany). The beads were sieved into different size-fractions using standard test sieves of 200, 300, and 425 μm. The shape and surface of the microspheres were investigated with scanning electron microscopy. The average size ($n \geq 200$) and swelling ($n \geq 30$) of the microspheres was determined with photographs taken with an optical microscope (Nikon Eclipse 800). The photographs were characterized with the program ImageJ (version 1.32j).

To measure the swelling of both types of microspheres, a dry microsphere (size range 200–300 μm) was put under the light microscope and a picture was taken. Then, the microspheres were swollen in an excess of water for 24 h at room temperature. The equilibrium swelling ratio (Q_v) was calculated by the formula:

$$Q_v = \frac{D}{D_0} \quad (1)$$

with D and D_0 the swollen and initial (dry) diameters of the microspheres, respectively (Horák et al., 1997; Oral and Peppas, 2006). To investigate whether the swelling medium will influence the equilibrium swelling ratio Q_v, the microspheres were also measured in simulated lacrimal fluid (SLF) and ethanol.

The agents used for loading of the microspheres are the dye fluorescein sodium and the antibiotic ciprofloxacin. Four different microspheres according to Table 1 were used for the in vitro release experiments. In order to load the microspheres with fluorescein sodium, a concentrated solution of fluorescein sodium of 75 mM was prepared in demineralized water. One gram of poly(HEMA)-spheres was placed in a test sieve (diameter of 25 mm, pore size 100 μm), after which the sieve was placed in the fluorescein-solution. After 8 h, the sieve was removed from the solution and the microspheres were lyophilized. Then,

Table 1
The microspheres used for the in vitro release experiments

Microsphere	Material	Drug
I	Poly(HEMA)	Fluorescein sodium
II	Poly(HEMA-*co*-NVP)	Fluorescein sodium
III	Poly(HEMA)	Ciprofloxacin
IV	Poly(HEMA-*co*-NVP)	Ciprofloxacin

a dispersion of 12 mM ciprofloxacin (Fluka Chemie, Steinheim, Germany) in ethanol was made. This dispersion was less concentrated than the fluorescein sodium solution because of solubility limits of ciprofloxacin in water and ethanol. One gram of poly(HEMA)-spheres was placed in a test sieve, which was subsequently placed in the dispersion for 8 h. The drug loaded microspheres were then lyophilized.

The same procedure was followed to load the poly(HEMA-*co*-NVP)-spheres with fluorescein sodium and ciprofloxacin, respectively.

2.4. Drug release

The drug loaded microspheres were introduced into the lumen of the coil with a miniature funnel. The ends were sealed with the photo-curable cyanoacrylate cap and drug release was measured at room temperature in the following set-up. SLF was perfused through a silicon tube (inner diameter of 1 mm) at a rate of 100 μL/min. A coil was placed at the end of the silicone tube and fractions of approximately 150 μL were collected.

Several microsphere loaded coils were measured to investigate the contribution of the microspheres to the capacity and the release time of the device. Table 2 shows the coils used for the release experiments. First the contribution of drug loaded microspheres in the central cavity of the coil was investigated by comparing six types of coils:

- a coil with a fluorescein sodium loaded coating and an empty cavity (coil A); the hydrogel coating of the fluorescein sodium loaded coils contained 9 wt% of the dye (i.e. 43 μg per coil);
- a blank coil (with no active ingredient in the hydrogel coating) with fluorescein sodium loaded poly(HEMA)-spheres in the inner cavity (coil B); the microspheres released 33 μg fluorescein sodium (vide infra);
- the combination: fluorescein sodium loaded hydrogel coating on the coil and fluorescein sodium loaded poly(HEMA)-spheres in the inner cavity of the coil (coil C); the coils contained 43 + 33 = 76 μg fluorescein sodium;
- a blank coil with fluorescein sodium loaded poly(HEMA-*co*-NVP)-spheres in the cavity (coil D); the microspheres released 70 μg fluorescein sodium (vide infra);
- a blank coil with ciprofloxacin loaded poly(HEMA)-spheres in the inner cavity (coil E); coil E released 1 μg of ciprofloxacin (vide infra);
- a blank coil with ciprofloxacin loaded poly(HEMA-*co*-NVP)-spheres in the inner cavity (coil F); coil F released 6 μg (vide infra).

Table 2
The microsphere loaded coils used for the in vitro release experiments

Coil	Coating coil	Microspheres
A	Fluorescein sodium	None
B	None	I
C	Fluorescein sodium	I
D	None	II
E	None	III
F	None	IV

The constant flow through the tube of 100 μL/min, allows the amount of drug released to be calculated by the determination of the area under the curve (AUC) of the release curve (concentration plotted against time). The concentrations of fluorescein sodium in the fractions collected were measured directly after sampling with a spectrofluorometer (SpectraMax Gemini, containing the SoftMax Pro software) with the absorption and emission wavelengths $\lambda_{abs} = 490$ nm and $\lambda_{em} = 514$ nm, respectively (Mota et al., 1991).

The concentrations of ciprofloxacin in the collected fractions were evaluated using a zone of inhibition assay within a month after the release measurement. The samples were stored at −18 °C until the bio-assay. Plastic petridishes ($d = 14$ cm) were filled level with 60 mL Iso-sensitest™ medium. After coagulation and drying, the indicator bacterium *E. coli* ATCC 25922 was brought onto the plates. Seven holes ($r = 4$ mm) were punched at equal distance in the agar of every petridish. Finally, these holes were filled with 100 μL of the collected fractions or the dilution series. After 24 h of incubation at 37 °C under atmospheric conditions, semi-confluent growth appeared on the total agar surface and the diameters of the inhibition zones were measured. The concentrations of ciprofloxacin in the collected fractions could be inferred from a dilution series (concentrations in the range of 0.02–8.0 μg/mL).

2.5. Flexibility tests

Flexibility tests were done with different coils to determine whether filling the central cavity with microspheres compromises the flexibility of the coils. A three-point bending test was performed on: (i) a coil with an empty cavity, (ii) a coil with three hydrogel coated filaments inside and (iii) a coil with microspheres in the inner cavity.

The flexibility of the coils was measured with a rheometric solids analyser (RSA3), equipped with RSI-Orchestrator V6.5 software. The ends of each coil were placed onto two solid points and a force was applied to the centre of the coil as shown in Fig. 3. The displacement was set at 2.5 mm and the force as a function of the displacement was measured at the centre of the coil. All coils were measured in the dry and wet state (both $n = 4$), to investigate whether wetting of the coils influences the bending forces.

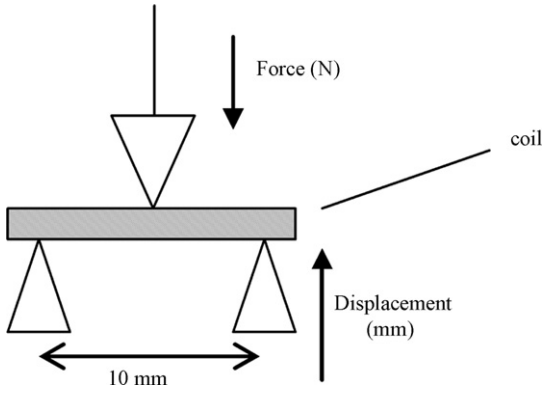


Fig. 3. Schematic set up three-point bending test for the coil (flexibility test).

2.6. Evaluation patient comfort

A preliminary patient comfort study was performed to investigate the insertion and removal procedure of the coil, as well as the tolerance of the coil in the human conjunctival fornix for 2 h. After approval of the Ethical Committee of the Academic Hospital Maastricht, five healthy volunteers (age: 24–57 years) gave informed consent prior to the start of the study. The compliance of the coil was assessed in three ways: (i), qualitative assessment of irritation by an ophthalmologist; (ii), comparison of the diameters of the capillary veins on photographs taken before installation, and after removal of the device; possible irritation would lead to dilatation of the veins and (iii) answers to questionnaires, filled out by the volunteers (subjects).

As a control, the ophthalmologist examined each subject to exclude any eye disease or infection. This qualitative assessment consisted of: best corrected visual acuity, a slit-lamp investigation to exclude any pre-existent anterior surface disease, grading of conjunctival redness (I = no redness, V = extensive redness), tear break-up time, Schirmer tear test, fluorescein pattern of the tear film, corneal punctate staining (diffuse, linear, or none), appearance of the anterior chamber, and photography of the ocular surface. All photographs were taken with the same magnification.

After the control examination, a drug-free coil with hydrogel coated filaments in the coil's interior was inserted in the conjunctival fornix of the right eye, using forceps. The appearance of the eye, the ease of application, and the duration of the insertion procedure were observed. Two hours after installation, a subsequent examination was done. The coil was removed and photographs of the eye were taken.

Finally, 4 h after the removal of the device, each subject had the last examination, including photographs of the eye. The subjects filled out a questionnaire at every time point, answering the questions according to a grading system with numbers scaling from 1 to 5 (1 = not at all, 3 = neutral, 5 = yes, absolutely).

3. Results and discussion

3.1. Preparation of the coils

Preparation of all coils was successful. Coils with the Slipskin® coating were prepared, as well as coils with 9 wt% of fluorescein sodium and 91 wt% Slipskin® in the coating. Inspection of the outer surface of the coated wires and coils (by optical microscopy and scanning electron microscopy) showed perfectly smooth surfaces. Coiling of the coated wires did not lead to any damage of the coating (i.e. there were no visible cracks). Earlier tests showed that the coating of the coil has no residual solvents or monomers after the coating procedure (Hanssen et al., 1999).

3.2. Preparation and characterization of the microspheres

Two different series of microspheres were prepared successfully: cross-linked poly(HEMA)- and cross-linked poly(HEMA-*co*-NVP)-spheres, in which the mass ratio HEMA/NVP was 70/30. The cross-linker was tetra-EGDMA (3% by mass) in all cases. The yields of the poly(HEMA) microspheres was 27.2 g (75%; theoretically expected 36.0 g), and the yield of the poly(HEMA-*co*-NVP) microspheres was 20.0 g (95%; theoretically expected 21.2 g). All microspheres were thoroughly washed with demineralised water, to remove possible leachable substances (oligomers and unreacted monomers).

Table 3
Size distribution of the microsphere batches

Size (μm)	Poly(HEMA) batch	Poly(HEMA-*co*-NVP) batch
<200	19.6 wt%	5.3 wt%
200–300	17.8 wt%	22.4 wt%
300–425	28.4 wt%	25.2 wt%
>425	36.4 wt%	47.1 wt%

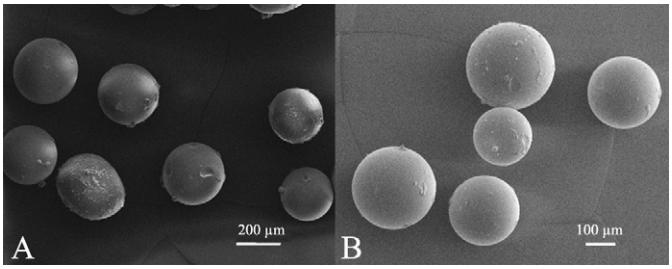


Fig. 4. SEM-photographs of the different microspheres: (A) poly(HEMA) and (B) poly(HEMA-*co*-NVP)).

The average size diameters of the poly(HEMA)- and poly(HEMA-*co*-NVP)-spheres (both $n \geq 200$) were 195 and 300 μm, respectively. Further data on the size distribution are given in Table 3. The desired fraction, diameters between 200 and 300 μm, was selected through sieving. Microspheres in this size range can easily be inserted in the interior of the coil, and there remains sufficient space to allow them to swell. Fig. 4 shows scanning electron microscopy (SEM) photographs of the sieved microspheres, and reveals that both types of microspheres were spherical and had a smooth surface.

Table 4 compiles the data on equilibrium swelling of the microspheres in water, SLF, and ethanol. There was no significant difference in swelling of the poly(HEMA)-spheres and the poly(HEMA-*co*-NVP)-spheres in both water and SLF. However, swelling of both types of microspheres in ethanol resulted in significantly different swelling ratios compared to their swelling in water or SLF ($p < 0.05$).

3.3. Drug release

Fig. 5 displays the release profiles measured for the coils A–C, which were loaded with fluorescein sodium, either exclu-

Table 4
Equilibrium swelling ratio (Q_v) of the microspheres in the swelling media ($n \geq 30$)

	$Q_{v(polyHEMA)}$ (S.D.)	$Q_{v(poly(HEMA\text{-}co\text{-}NVP))}$ (S.D.)
Water	1.166 (0.031)	1.234 (0.015)
SLF	1.176 (0.019)	1.256 (0.033)
Ethanol	1.492 (0.054)	1.409 (0.037)

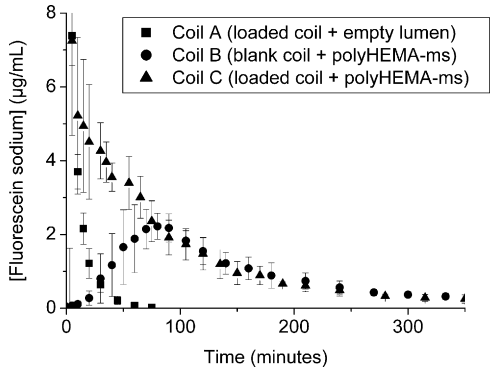


Fig. 5. Contribution of the microspheres in the central cavity of the coil to the release profile.

sively in the hydrogel coating of the coil (coil A), or exclusively in the coil's lumen (coil B), or both in the hydrogel coating and the interior (coil C). Note that the release curves were measured in four-fold. In line with previous observations, coil A releases most of the dye in the first hour; measured concentrations in the effluent SLF drop from 7.4 μg/mL to approx 0.05 μg/mL at $t = 60$ min. Coil B shows virtually no initial release; a maximum concentration of approx. 2.2 μg/mL is reached after 80 min. During the next 4 h, the concentration gradually drops to approximately 0.3 μg/mL. The release curve measured for coil C combines the features of coils A and B: fluorescein release from coil C declines from approx. 7.2 μg/mL initially, to approx. 0.3 μg/mL after 5 h.

From integration of the release curves A and B, it can be calculated that approximately 19 μg of fluorescein sodium was released from coil A and 33 μg from coil B. Integration of the release curve C leads to a total release of approximately 123 μg. Hence, it can be concluded that the device's capacity has increased by approx. 500% by using dye loaded microspheres compared to a coil with an empty interior.

Fig. 6 shows a comparison of the performance of the poly(HEMA)-spheres versus the poly(HEMA-*co*-NVP)-spheres ($n = 4$). The release curves of fluorescein sodium in Fig. 6A show that poly(HEMA-*co*-NVP)-spheres are a much more effective carrier than the poly(HEMA)-spheres. Integration of the curves led to 33 μg of fluorescein sodium released for coil B (poly(HEMA)-spheres) versus 70 μg of fluorescein sodium released for coil D (poly(HEMA-*co*-NVP)-spheres). This difference can be explained, at least in part, by the fact that poly(HEMA) swells much less than poly(HEMA-*co*-NVP) under our experimental conditions. The data reveal that the loading percentages are 2.4% (S.D. 0.41) for the poly(HEMA)-spheres and 5.4% (S.D. 0.65) for the poly(HEMA-*co*-NVP)-spheres. Note that we cannot determine the entrapment efficiency, since the maximum capacity of each of the materials for fluorescein sodium is not known.

The curves in Fig. 6B show the data measured for release of ciprofloxacin under the same conditions. Two conclusions can be drawn directly: (i) much less ciprofloxacin, as compared to fluorescein sodium, is absorbed in both cases (i.e. 1.0 and 6.0 μg for poly(HEMA)- and poly(HEMA-*co*-NVP)-spheres, respectively). The loading percentages are 0.084% (S.D. 0.027) for ciprofloxacin in poly(HEMA)-spheres and 0.367% (S.D. 0.087) for ciprofloxacin in poly(HEMA-*co*-NVP)-spheres; (ii) poly(HEMA-*co*-NVP) is an efficient absorber of ciprofloxacin (compared to poly(HEMA)). The latter observation may be explained by a favourable matrix-host interaction, probably hydrogen bonding between the C=O groups of the NVP-links in the co-polymer, and a proton-donating site of ciprofloxacin.

Closer inspection of the release curves in Fig. 6A and B reveals another difference: release of fluorescein sodium is negligible during the first 20 min. Then, the concentration of the dye rises, passes a maximum and decreases again. This pattern implies that diffusion of the drug across the device's wall is the rate-determining step in the release mechanism. On the other hand, the release curves for ciprofloxacin show an initial maximum and continuous decrease afterwards. This suggests that, for ciprofloxacin, the release from the microspheres is the rate-determining step; the drug easily passes through the windings of the coil.

Obviously, the amount of ciprofloxacin on the microspheres is only marginal. Apparently, the drug loading of microspheres is very dependent on the solubility of the drug and the swelling medium.

3.4. Flexibility test

Flexibility of the coil is an important factor that can affect the comfort of this ocular device and acknowledging that the conjunctival space of the eye has a curvature. The flexibility of several coils was determined with three-point bending tests.

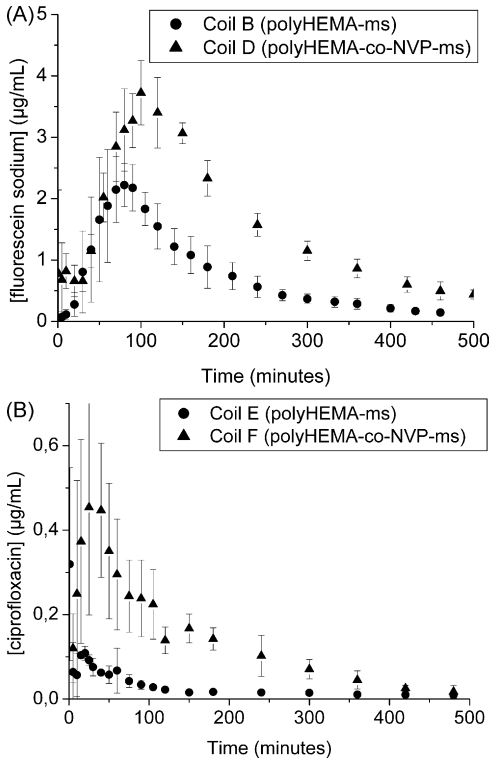


Fig. 6. Release curves of a blank coil filled with all four types of microspheres: (A) fluorescein sodium and (B) ciprofloxacin.

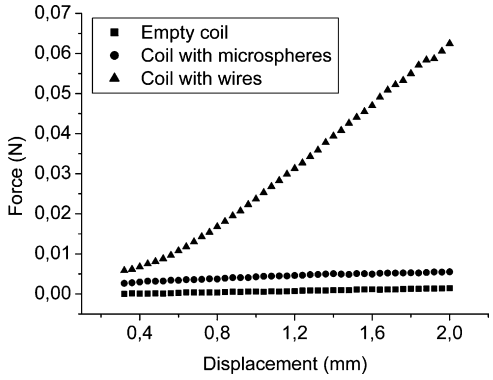


Fig. 7. The flexibility experiment with three different coils.

The coils used had an empty central cavity, hydrogel coated filaments in the inner cavity or microspheres in the lumen. In Fig. 7, the forces are plotted against the displacement of the coil. It can be seen that the coils with an empty cavity and with microspheres in the cavity are more flexible, i.e. less force is necessary for the displacement, than the coils with the hydrogel coated filaments in the inner cavity. The coils with microspheres in the central cavity had a stiffness approximately two times higher than the empty coils (this measured with the displacement set at 2.5 mm out of a length of 10 mm). The coils with the three coated filaments in the central cavity had much higher values for the force/displacement than both other types of coils. Wetting the coils did not have a statistically significant effect on the stiffness.

3.5. Evaluation patient comfort

3.5.1. Ophthalmologic investigations

Slit-lamp examinations showed a normal appearance of the ocular surface in all volunteers after the coils were removed. It was noted that subject #1 had a rather narrow fornix, due to an oriental lid crease. Prior to the experiment, redness was grade II for subjects #1 and #4, and grade I (no redness) for the rest. No changes in redness were observed throughout and after the experiment. All subjects had normal fluorescein patterns of the tear film, as well as a normal appearance of the anterior chamber before installation of the device and after removal. None of the subjects showed corneal punctuate staining before installation of the device, or afterwards.

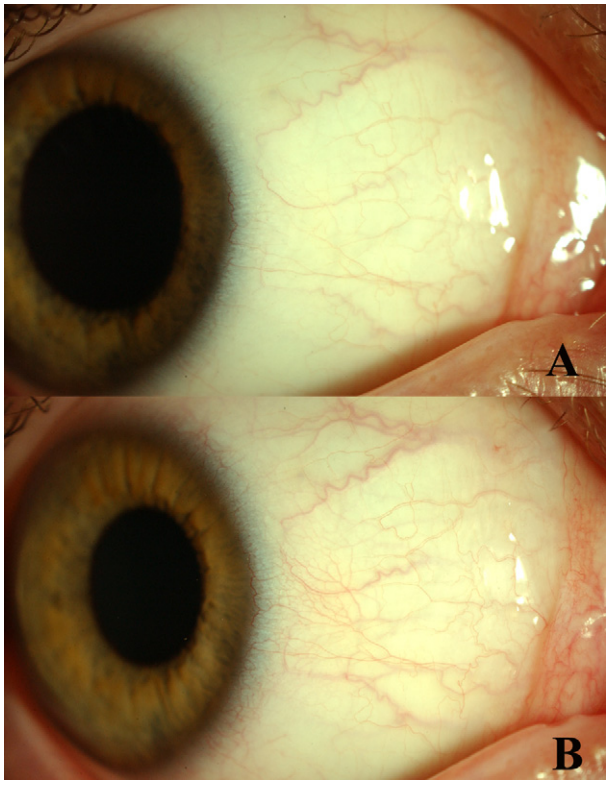


Fig. 8. Photograph of the capillary veins of a volunteer prior to (A) and after (B) the insertion of the coil.

3.5.2. Photographic evaluation

Fig. 8 shows typical photographs that were taken during this study. Fig. 8A shows a photograph of the right eye of subject #5 prior to the experiment; Fig. 8B shows the same eye, immediately after removal of the device that had been in the conjunctival fornix for 2 h. The photographic images of all subjects were

Table 5
Results of the questionnaires filled in by the five subjects. The answers were scored by a grading system (1 = not at all, 5 = yes, absolutely)

Question	Subject#					Average
	1	2	3	4	5	
During wear of the insert						
Was the insertion unpleasant?	4	2	1	1	1	1.8
Do you feel the insert?	4	2	5	4	1	3.2
Do you have a tendency to rub your eye?	1	1	1	3	4	2.0
After removal of the insert						
Was the removal procedure unpleasant?	3	1	1	1	1	1.4
Did you feel the insert in the eye?	4	2	1	4	4	3.0
Was it unpleasant to wear the insert?	4	1	1	2	3	2.2
Did you have a tendency to rub your eye?	1	1	1	2	4	1.8
Did your eye produce more tears?	1	1	1	1	1	1.0
Did your eye itch?	1	1	1	1	1	1.0
Did your eye have a burned feeling?	1	1	1	1	1	1.0
Did your eye turn red?	1	1	1	1	1	1.0

shown to be superimposable. Magnifications of the images were performed to determine whether subtle variations in capillary diameter occurred. In all cases, there was no detectable change of the capillary diameter, which suggests that the eyes were not irritated by the device. The results of the photographic evaluations were consistent for all subjects throughout the experiment.

3.5.3. Questionnaires

Table 5 lists the questions that were asked to the subjects, as well as their answers. The question "Do you feel the insert in the eye?" had the highest score, i.e. 3.2 during wear and 3.0 after removal of the device. This reveals that most of the subjects do notice the presence of the device in the fornix. In most cases, the feeling is characterised as "a little". Subject #5 experienced that he had a slight tendency to rub his right eye during wear of the device. Subject #1 experienced the installation as "a little unpleasant." This can be attributed to the fact that his fornix was relatively narrow due to an oriental lid crease.

To summarize the responses to installation, 2 h-wear and removal of the insert are reminiscent of responses that are seen routinely with new wearers of contact lenses (particularly hard contact lenses) (Morgan et al., 2003).

4. Concluding remarks

The idea to use drug loaded polymeric microspheres to increase the capacity of the OphthaCoil, without affecting the flexibility of the coiled structure, appears to be technical feasible. Using fluorescein sodium as a drug-mimic, it was found that a 500% increase of the released compound could be realized. By introducing dye or drug loaded microspheres in the lumen of the coil not only the capacity can be increased, but also the time window of the dye released can be increased from 1 h to over 5 h. Note that the drug release in vitro is probably faster than in vivo, due to a difference in the flow; i.e. in the in vitro set up the flow was set at 100 μL/min, whereas in vivo the tear turnover rate is only 0.5–2.2 μL/min (Schoenwald, 1997; Ghate and Edelhauser, 2006).

Yet, the total amount of fluorescein sodium released from the OphthaCoil remains low. For example, the total release of fluorescein sodium from coil C (that contained the dye in both the surface coating and in the lumen) was 123 μg. For comparison: a 0.5% eye drop with a volume of 20–30 μL contains 100–50 μg of drug. As mentioned earlier, the bioavailability of an eye drop is less than 5%. This means that only 5–7.5 μg of an eye drop will reach the intraocular tissues. Evidently, the crucial point is the bioavailability of drugs released from the OphthaCoil. Our previous work indicated this to be significantly larger than in the case of eye drops. If the bioavailability of drugs from our insert would be 10%, than the charge of coil C corresponds to two eye drops. However, with increasing bioavailability, the amount of drug absorbed is also increased. For example, a coil with a drug bioavailability of 50% can be compared to 8–12 eye drops. It can be concluded that it is essential to achieve maximum drug loading of the microspheres.

The way of loading the microspheres with a drug by soaking them into a concentrated solution is especially useful for hydrophilic drugs. The uptake of drugs from a concentrated solution is suitable for relatively rapid release. However, also hydrophobic drugs can be incorporated into the microspheres by the synthesis of the microspheres in the presence of the hydrophobic drug, rather than charging them afterwards through swelling. This will result in relatively slower release. This was not done here, because we focus on relatively rapid release (1–12 h).

In our opinion, the results of the preliminary patient study are encouraging, especially with respect to potential short-term applications (i.e. drug delivery to the eye over several hours). Currently, we foresee two such applications:

(i) Treatment of aggressive corneal infections (i.e. corneal ulcers) and severe bacterial conjunctivitis and fungal keratitis. The incidence of these infections is increasing, partly due to (soft) contact lens wear. There is evidence, that contact lens use has surpassed trauma as the most common risk factor for fungal keratitis. The percentage of fungal ulcers caused by non-therapeutic contact lenses doubled from 25% between 1999 and 2001, to approximately 45% in 2005 and 2006 (Iyer et al., 2006). *Fusarium* and *candida* are common causes of fungal keratitis. According to Iyer, most patients can be treated successfully with dual antifungal therapy: natamycin (5%) and amphotericin B (0.15%) are the most commonly used drugs. In some cases, the infection has to be treated by frequent installation of eye drops that contain an antibiotic. To treat corneal ulcers, patients have to apply, day and night, ciprofloxacin eye drops every 15 min for the first 6 h, every 30 min for the next 18 h. On day 2, patients have to administer the drops every 2 h and for the next 3–14 days, every 4 h (Health Information Patient UK, 2006).Evidently this treatment puts a heavy burden on patients and health care workers, as the patients will be hospitalized during the first days and this ultimately has impact on health care cost. We expect that delivery of antibiotics from our device can alleviate this burden. Depending on the potency of the antibiotic agent, we calculated that sustained delivery over a minimum of 5 h could be realized. This implies that the 2-day therapy can be realized with a maximum of 10 device exchanges for the first 48 h of treatment, which compares favorably with >70 drop-installations.

(ii) Controlled delivery of drugs to the eye, prior to cataract surgery. Before cataract operations, it is mandatory that several drugs are administered to the eye. These include an anaesthetic, antibiotic and several mydriatic agents. Normally these agents are administered sequentially through eye drops. A device could be used to deliver multiple drugs either sequentially or simultaneously. The time scale of release required is of the same order of the coil release characteristics, since the health care workers administer antibiotics and mydriatica to patients approximately 2 h prior to surgery.

A clinical pilot study into the second application is currently in preparation. Moreover, further work will concentrate on characterization and optimization of long-term compliance of our

coil. If sustained release over longer periods (e.g., several days or 1 week) can be realized, then the coil may ultimately prove useful in treatment of uveïtis or glaucoma. It might provide a better basis to administer ocular drugs to patients who are allergic to the preservative agents that are normally present in eye-drop solutions as well as providing a treatment that demands less time of health care workers.

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References

Aldenhoff, Y.B., Knetsch, M.L., Hanssen, J.H., Lindhout, T., Wielders, S.J., Koole, L.H., 2004. Coils and tubes releasing heparin. Studies on a new vascular graft prototype. Biomaterials 25, 3125–3133.

Barbu, E., Sarvaiya, I., Green, K.L., Nevell, T.G., Tsibouklis, J., 2005. Vinylpyrrolidone-*co*-(meth)acrylic acid inserts for ocular drug delivery: synthesis and evaluation. J. Biomed. Mater. Res. A 74, 598–606.

Barbu, E., Verestiuc, L., Nevell, T.G., Tsibouklis, J., 2006. Polymeric materials for ophthalmic drug delivery: trends and perspectives. J. Mater. Chem. 16, 3439–3443.

Baudouin, C., 2005. Allergic reaction to topical eyedrops. Curr. Opin. Allergy Clin. Immunol. 5, 459–463.

Bruining, M.J., Edelbroek-Hoogendoorn, P.S., Blaauwgeers, H.G., Mooy, C.M., Hendrikse, F.H., Koole, L.H., 1999. New biodegradable networks of poly(*N*-vinylpyrrolidinone) designed for controlled nonburst degradation in the vitreous body. J. Biomed. Mater. Res. 47, 189–197.

Ciprofloxacin Ointment/Bacterial Keratitis Study Group, 1993. 0.3% Ciprofloxacin ophthalmic ointment in the treatment of bacterial keratitis. Arch. Ophthalmol. 111, 1210–1218.

Ciprofloxacin Bacterial Keratitis Study Group, 1996. Comparison of ciprofloxacin ophthalmic solution 0.3% to fortified tobramycin-cefazolin in treating bacterial corneal ulcers. Ophthalmology 103, 1854–1862 (discussion 1862–3).

Ding, Shulin, 1998. Recent developments in ophthalmic drug delivery. Pharm. Sci. Technol. Today 1, 328–335.

Geroski, D.H., Edelhauser, H.F., 2000. Drug delivery for posterior segment eye disease. Invest. Ophthalmol. Vis. Sci. 41, 961–964.

Ghate, D., Edelhauser, H.F., 2006. Ocular drug delivery. Exp. Opin. Drug Deliv. 3, 275–287.

Gobbels, M., Spitznas, M., 1992. Corneal epithelial permeability of dry eyes before and after treatment with artificial tears. Ophthalmology 99, 873–878.

Gulsen, D., Chauhan, A., 2004. Ophthalmic drug delivery through contact lenses. Invest Ophthalmol. Vis. Sci. 45, 2342–2347.

Gulsen, D., Chauhan, A., 2005. Dispersion of microemulsion drops in HEMA hydrogel: a potential ophthalmic drug delivery vehicle. Int. J. Pharm. 292, 95–117.

Hanssen, H.H., Wetzels, G.M., Benzina, A., van der Veen, F.H., Lindhout, T., Koole, L.H., 1999. 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, 820–828.

Health Information Patient UK, http://www.patient.co.uk/showdoc/30003336/, part of http://www.patient.co.uk/ (accessed 17/10/2006).

Herrero-Vanrell, R., Refojo, M.F., 2001. Biodegradable microspheres for vitreoretinal drug delivery. Adv. Drug Deliv. Rev. 52, 5–16.

Horák, D., Metalova, M., Rypácek, F., 1997. New radiopaque polyHEMA-based hydrogel particles. J. Biomed. Mater. Res. 34, 183–188.

Hosoya, K., Lee, V.H., Kim, K.J., 2005. Roles of the conjunctiva in ocular drug delivery: a review of conjunctival transport mechanisms and their regulation. Eur. J. Pharm. Biopharm. 60, 227–240.

Iyer, S.A., Tuli, S.S., Wagoner, R.C., 2006. Fungal keratitis: emerging trends and treatment outcomes. Eye Contact Lens 32, 267–271.

Jayakrishnan, A., Chithambara Thanoo, B., Rathinam, K., Ravi Mandalam, K., Rao, V.R.K., Lal, A.V., Mohanty, M., 1989. Hydrogel microspheres from cross-linked poly(methyl methacrylate): synthesis and biocompatibility studies. Bull. Mater. Sci. 12, 17–25.

Jayakrishnan, A., Sunny, M.C., Thanoo, B.C., 1990. Polymerization of 2-hydroxyethyl methacrylate as large size spherical beads. Polymer 31, 1339–1342.

Jayakrishnan, A., Thanoo, B.C., 1990. Suspension polymerization of 2-hydroxyethyl methacrylate in the presence of polymeric diluents: a novel route to spherical highly porous beads for biomedical applications. J. Biomed. Mater. Res. 24, 913–927.

Karlgard, C.C., Jones, L.W., Moresoli, C., 2003. Ciprofloxacin interaction with silicon-based and conventional hydrogel contact lenses. Eye Contact Lens 29, 83–89.

Ke, T.L., Cagle, G., Schlech, B., Lorenzetti, O.J., Mattern, J., 2001. Ocular bioavailability of ciprofloxacin in sustained release formulations. J. Ocul. Pharmacol. Ther. 17, 555–563.

Kim, Y.M., Gupta, B.K., 2003. 2-Octyl cyanoacrylate adhesive for conjunctival wound closure in rabbits. J. Pediatr. Ophthalmol. Strabismus 40, 152–155.

Knetsch, M.L., Aldenhoff, Y.B., Schraven, M., Koole, L.H., 2004. Human endothelial cell attachment and proliferation on a novel vascular graft prototype. J. Biomed. Mater. Res. 71A, 615–624.

Lang, J.C., 1995. Ocular drug delivery conventional ocular formulations. Adv. Drug Deliv. Rev. 16, 39–43.

Le Bourlais, C., Acar, L., Zia, H., Sado, P.A., Needham, T., Leverge, R., 1998. Ophthalmic drug delivery systems—recent advances. Prog. Retin. Eye Res. 17, 33–58.

Morgan, P.B., Maldonado-Codina, C., Efron, N., 2003. Comfort response to rigid and soft hyper-transmissible contact lenses used for continuous wear. Eye Contact Lens 29, S127–S130 (discussion S143–4, S192–4).

Mota, M.C., Carvalho, P., Ramalho, J., Leite, E., 1991. Spectrophotometric analysis of sodium fluorescein aqueous solutions. Determination of molar absorption coefficient. Int. Ophthalmol. 15, 321–326.

van Ooteghem, M., 1993. In: Edman, P. (Ed.), Properties Influencing Drug Retention. Biopharmaceutics of Ocular Drug Delivery. CRC Press, Inc., Boca Raton, Florida, pp. 29–33.

Oral, E., Peppas, N.A., 2006. Hydrophilic molecularly imprinted poly(hydroxyethyl-methacrylate) polymers. J. Biomed. Mater. Res. A 78, 205–210.

Oyster, C.W., 1999. The Human Eye: Structure and Function. Sinauer Associates, Inc., Sunderland, Massachusetts.

Parks, D.J., Abrams, D.A., Sarfarazi, F.A., Katz, H.R., 1993. Comparison of topical ciprofloxacin to conventional antibiotic therapy in the treatment of ulcerative keratitis. Am. J. Ophthalmol. 115, 471–477.

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

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

Pijls, R.T., Sonderkamp, T., Daube, G.W., Krebber, R., Hanssen, H.H., Nuijts, R.M., Koole, L.H., 2005. Studies on a new device for drug delivery to the eye. Eur. J. Pharm. Biopharm. 59, 283–288.

Ressemann, T.V., Hackett, S.S., Keith, P.T. (1996). Intravascular catheter with distal tip guide wire lumen. US Patent 5,921,958.

Rosemary, M.J., Suryanarayanan, V., Ganapati Reddy, P., Maclaren, I., Baskaran, S., Pradeep, T., 2003. Ciprofloxacin@SiO_2: Fluorescein nanobubbles. Proc. Indian Acad. Sci. (Chem. Sci.) 115, 703–709.

Salyani, A., Birt, C., 2005. Evaluation of an eye drop guide to aid self-administration by patients experienced with topical use of glaucoma medication. Can. J. Ophthalmol. 40, 170–174.

Sato, T., Uchida, R., Tanigawa, H., Uno, K., Murakami, A., 2005. Application of polymer gels containing side-chain phosphate groups to drug-delivery contact lenses. J. Appl. Polym. Sci. 98, 731–735.

Schaefer, F., Bruttin, O., Zografos, L., Guex-Crosier, Y., 2001. Bacterial keratitis: a prospective clinical and microbiological study. Br. J. Ophthalmol. 85, 842–847.

Schoenwald, R.D., 1997. In: Zimmermann, T.J. (Ed.), Ocular Pharmacokinetics. Textbook of Ocular Pharmacology. Philadelphia, Lippinocott-Raven Publishers, pp. 119–138.

Van Santvliet, L., Ludwig, A., 2004. Determinants of eye drop size. Surv. Ophthalmol. 49, 197–213.

Winfield, A.J., Jessiman, D., Williams, A., Esakowitz, L., 1990. A study of the causes of non-compliance by patients prescribed eyedrops. Br. J. Ophthalmol. 74, 477–480.