Journal of Biomedical Optics 7(1), 27–33 (January 2002)

Minimally invasive spectroscopic system for intraocular drug detection

Joe Miller
University of Strathclyde
Department of Electronic and Electrical Engineering
Glasgow, Scotland, United Kingdom

Clive G. Wilson
University of Strathclyde
Department of Pharmaceutical Sciences
Glasgow, Scotland, United Kingdom

Deepak Uttamchandani
University of Strathclyde
Department of Electronic and Electrical Engineering
Glasgow, Scotland, United Kingdom

Abstract. A novel, minimally invasive measurement technique has been developed for the detection of drugs in the anterior chamber of the eye. Presently there is no satisfactory, real-time detection method available to the ophthalmic community. Accurate determination of drug concentrations in the eye would be of great value and assistance to researchers and manufacturers of ophthalmic drugs and ocular implants, to enable a better understanding of intraocular pharmacokinetics. At present researchers use techniques of direct sampling of the aqueous humor from laboratory animal eyes into which the drug has been introduced topically or systemically. Rabbit eyes are frequently used in this context. Sampling via paracentesis is invasive, and does not yield a continuous measurement. Our approach to addressing this measurement requirement is, in effect, to turn the eye into a cuvette and use optical absorbance spectroscopy measurements to detect drug concentrations. A novel contact lens has been designed using commercial, off-the-shelf, optical design software. The lenses have been optimized to direct light across the anterior chamber of a rabbit's eye. Practical demonstration and characterization of light propagation across the eye have been undertaken and are reported. Preliminary results of the identification of drug compounds introduced into model eyes are also reported.
[DOI: 10.1117/1.1427045]

Keywords: aqueous humor; spectroscopy; contact lens design; minimally invasive; intraocular drug detection.

Paper JBO-102103 received Mar. 2, 2001; revised manuscript received Sep. 4, 2001; accepted for publication Sep. 18, 2001.

1 Introduction

In this article we report the viability of a novel, minimally invasive technique for the measurement of drug concentrations within the aqueous humor of the eye. The technique involves the use of a simple, custom made lens which, when placed in contact with the surface of the cornea, allows a beam of light to be directed laterally through the cornea and across the anterior chamber. This effectively turns the anterior chamber of the eye into a cuvette, enabling absorption spectroscopy measurements of the aqueous humor to be made *in situ*.

The anterior chamber is the front chamber of the eye bounded by the cornea and the iris. This chamber is filled with aqueous humor, and this is a constantly recirculating fluid that delivers nutrients and removes waste from this part of the eye. The aqueous humor is formed from plasma by epithelial cells of the ciliary body; freshly formed aqueous is secreted into the posterior chamber and this is the smaller chamber bounded by the iris and the lens. The aqueous then flows out through the pupil into the anterior chamber where it is drained via the trabecular meshwork.[1] Drugs used to treat various eye disorders can be delivered topically or systemically to the aqueous humor. Topically delivered drugs are usually administered in the form of drops onto the surface of the cornea, from which they diffuse into the aqueous humor. Systemically delivered drugs can be administered by injection and enter the aqueous humor via the ciliary body. Most drugs can be recognized and quantified through a characteristic absorbance or fluorescence spectrum. Alternatively they may be labeled with fluorophores to aid identification. In order to measure the bioavailability of a drug compound in the eye during the evaluation of a new formulation, the current practice is to extract a sample of aqueous humor from the eye of a rabbit, and use a laboratory spectrophotometer to measure the absorption or fluorescence spectrum of the compound. Our approach is designed to offer ocular pharmacologists an alternative method for the determination of the drug concentration in the anterior chamber. This will substantially reduce the number of animals used in experimentation as explained in the following paragraph.

The current technique for sampling the aqueous humor of the eye is invasive, and involves the application of anaesthetic eye drops to the eye followed by paracentesis. Paracentesis, in this context, involves the insertion of a hypodermic needle into the anaesthetized eye to extract a sample of the aqueous humor. Since paracentesis is a procedure that carries a risk of endophthalmitis, it is a procedure rarely used for investigation in man, while for research involving animals (typically rabbits) it is usually restricted to single point sampling. This

Address all correspondence to D. Uttamchandani. Tel: +44 141 548 2211; Fax +44 141 553 1955; E-mail: d.uttamchandani@eee.strath.ac.uk

1083-3668/2002/$15.00 © 2002 SPIE

leads to the need for very large numbers of animals in the definition of a drug concentration-time profile,[2] and the numbers are further increased when a set of formulations is tested. This is a significant consideration in the development of implant devices to control opportunistic infections such as cytomegalovirus which may develop as a consequence of AIDS or other conditions in which the immune system is severely depressed. From this real and immediate clinical need, we initiated the development of an optical, minimally invasive method for drug measurement in the eye without the need for paracentesis.

The concept of using a contact lens to measure the concentration of components of the aqueous humor was first proposed by Rabinovitch, March, and Adams.[3,4] In their papers they explored the possibility of determining the glucose concentration within the aqueous humor via polarimetry. Glucose is a chiral molecule and a solution containing glucose will rotate the plane of polarization of a beam of light as it is transmitted through the solution. Rabinovitch, March, and Adams described the construction of a bulk optics bench for measurement of the concentration of glucose in rabbit aqueous humor using polarimetry measurements performed on samples extracted from rabbit eyes via parcentesis. They go on to propose the concept of a scleral contact lens which would allow *in situ* measurements of glucose in the anterior chamber. The rotation of polarization produced by glucose in the anterior chamber is very small and a sensitive polarimeter is required to detect this rotation. Cote et al., King et al., and Cameron et al.[5–7] developed a polarimeter with the required sensitivity. They reported the construction of a device which allows light to be transmitted across the anterior chamber of a rabbit's eyes that allows successful transmission of monochromatic light across rabbit's eyes.[8,9] As will be explained below, our objectives and hence our achievements are different from those of these authors and result in our design and manufacture of a solid contact lens.

The focus of the work reported here and also that of previous work reported by our group is not polarimetry, but the use of absorption spectroscopy to determine the concentration of drugs which are present in the anterior chamber of the eye. To this end we have successfully coupled light across the anterior chamber of an isolated, arterially perfused bovine eye and, using absorption spectroscopy, measured the concentration of test compounds introduced into the anterior chamber.[10,11] An arterially perfused eye is an eye that has been removed from a freshly slaughtered animal and kept viable by a supply of nutrients via a main artery. The isolated bovine eyes were perfused using the method of Wilson et al.[12] and the eye was placed cornea side down into a water filled cuvette which was just large enough to enclose the entire cornea. The water acted as a refractive index matching fluid and allowed light to be transmitted laterally across the anterior chamber of the eye.[10,11] More recently Cameron et al.[8,9] have constructed the light coupling device mentioned above. This device enables monochromatic light to be coupled across the anterior chamber of New Zealand White rabbits. The device was constructed from a glass tube with two 45° prisms attached to the base of the tube; when the saline filled tube is placed over the cornea light can be coupled laterally across the anterior chamber of the eye via the two 45° prisms. Unfortunately polarimetry precludes the use of optical fibers as light guides (transmitting light down optical fibers alters its polarization) and the whole rabbit has to form part of the optical table, making it extremely difficult to decouple the optical setup and the rabbit.

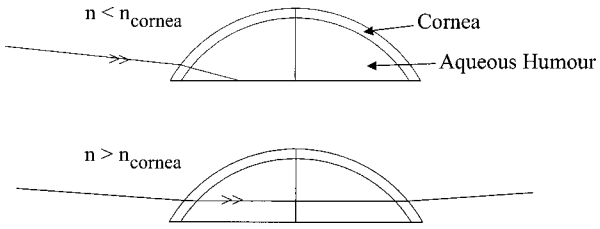

Fig. 1 Effect of altering the refractive index adjacent to the anterior corneal surface on the path of light entering the side of the cornea.

The results from our initial studies convinced us that the use of fluid as an optical coupling medium is unsatisfactory for an *in situ*, *in vivo* measurement, therefore a solid contact lens design was considered. The use of such a lens to transmit light across the anterior chamber was first demonstrated by our group.[13,14] A prototype perspex lens was constructed and when placed over an isolated perfused ovine eye allowed monochromatic light to be laterally transmitted across the anterior chamber. This light was not recovered for analysis and the lens material and design were not optimized. It is our purpose here to take that concept one stage further by demonstrating the following.

1. The design and manufacture of an optimized contact lens which when placed on the surface of the cornea allows light to propagate across the anterior chamber of a rabbit eye.
2. The use of a complete instrument which is capable of demonstrating the detection of pharmaceutically interesting drugs, in real time, in a model anterior chamber.

2 Design of the Contact Lens

As already stated, our approach to the problem of obtaining *in situ* optical access to the aqueous humor was to design a contact lens which, when placed in contact with the cornea, effectively turns the whole of the anterior chamber into a cuvette. This is achieved by altering the refractive properties of the anterior corneal surface. Figure 1 shows two ray traces to demonstrate the point. In both cases a beam of light enters the cornea from the side of the eye, almost normal to the visual axis. The upper trace corresponds to the anterior surface of the cornea in contact with a medium of lower refractive index, for example, air. The lower trace corresponds to the cornea in contact with a medium of higher refractive index, for example, synthetic fused silica (SFS). In the upper trace the light incident on the cornea is refracted toward the pupil/lens of the eye, making it difficult (if not impossible) to retrieve for analysis via absorption spectroscopy. If, as shown in the lower trace, the air/cornea boundary is replaced by a SFS/cornea boundary the light is refracted away from the lens of the eye and the beam travels laterally across the anterior chamber and out through the cornea on the opposite side of the eye where it can be collected for spectroscopic analysis

Table 1 Data used in the optical model of the anterior chamber of a rabbit's eye (Ref. 16).

Refractive indices	
Cornea	$n_c = 1.376$
Aqueous humor	$n_a = 1.337$
Corneal dimensions (mm)	
Anterior corneal radius of curvature	7.5
Posterior corneal radius of curvature	7.1
Corneal thickness	0.4
Cornea to lens distance	2.9

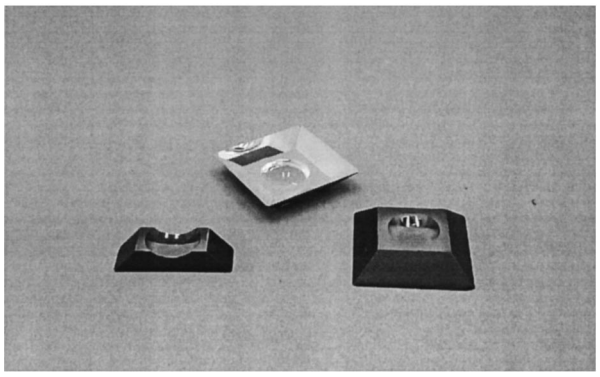

Fig. 3 Photograph of three typical contact lenses. The flat mirrored surfaces are coated with protective (black) coating.

The long term goal of the project is to develop an instrument which is suitable for studying drugs in the human aqueous humor. However in the first instance the instrument was developed to enable light to be directed across the anterior chamber of a rabbit's eye. Rabbits were chosen for two reasons. First, they are animals that are commonly used in pharmaceutical studies and, second, the anterior chamber of a rabbit's eye has similar dimensions to those of the human eye.[15,16] It must be noted that the rest of the rabbit's eye is not nearly as similar to the human eye. To facilitate the design of the contact lens a model of a rabbit's cornea and anterior chamber was constructed using Zemax optical design software. The data for the model are obtained from a paper by Huges[16] and are summarized in Table 1.

Using the principle demonstrated in Figure 1, we have designed a contact lens which allows light to be directed across the anterior chamber of the eye as shown in Figure 2. The two mirrored surfaces allow the light entry and exit points to be located on the front (as opposed to the side) of the contact lens, making the mounting of the associated optics and positioning equipment easier to engineer. The orientation of the two mirrored surfaces controls the angle at which the light beam strikes the surface of the cornea. This in turn determines the direction and position of the beam as it traverses the anterior chamber. This angle needs to be accurately controlled if the beam is to be directed horizontally across the anterior chamber as in the lower trace of Figure 1. It is desirable to maximize the length of travel through the aqueous humor, but care has to be taken to avoid the possibility of the beam striking the iris or lens of the eye. The compromise was to direct the 1 mm diam beam horizontally across the anterior chamber midway between the posterior corneal surface and the anterior surface of the lens. In a typical rabbit this will give a path length through the aqueous humor of approximately 7 mm, while leaving a 1 mm clearance between the beam path and the anterior surface of the eye lens. Figure 3 shows a photograph of three of the prototype contact lenses. The angled surfaces are coated with an aluminum reflective layer which is in turn coated with a protective layer. The lenses were manufactured to our design by Spanoptec (Glenrothes, Scotland) using computer numerical control (CNC) lens manufacturing equipment. Synthetic fused silica (Spectrosil 2000, TSL, Tyne

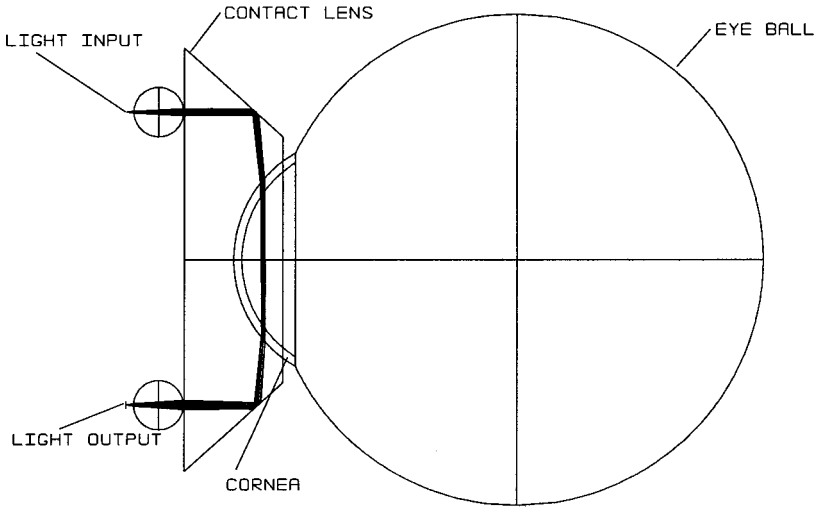

Fig. 2 Zemax optical design software used to design a contact lens which, when placed against the cornea, effectively turns the front chamber of the eye into a cuvette. This allows light to be directed in, across and out of the anterior chamber of the eye, enabling absorption spectroscopy measurements of the aqueous humor.

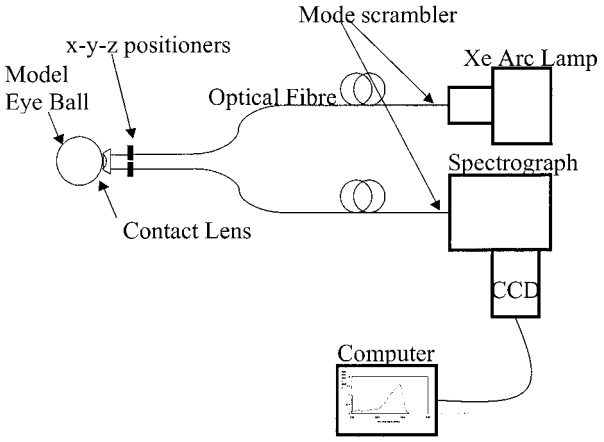

Fig. 4 Schematic of the equipment used.

& Wear, England) was chosen as the lens material.

In any population of rabbits and humans there will be variation in the corneal curvature; when using our contact lens this will result in variation of the optical path length through the aqueous humor. It is our intention to directly measure the individual path length for each eye measured by using a digital camera to image the front surface of the cornea as the light beam passes through. The entry and exit points of this light beam can be detected from the scatter produced as the beam traverses the cornea. The path length through the aqueous humor can then be directly measured. The viability of this technique was previously demonstrated by our group, using perfused eyes.[10,11,14]

The ultraviolet/visible (UV/vis) transmission of the eye needs to be considered to determine the spectral region which will be measured using this instrument. The anterior chambers of humans and rabbits are relatively transparent between 300 and 1400 nm.[17] The tear film transmits light well into the ultraviolet,[18] however both the cornea and the aqueous humor of rabbits and humans have a transmission cutoff between 290 and 300 nm.[17,19,20] Many of the drugs of pharmaceutical interest will have detectable absorption peaks in the ultraviolet or visible regions of the spectrum. It is therefore the spectral region below 700 nm but above the corneal cutoff of around 290 nm which we have initially chosen to characterize our measurement system. Synthetic fused silica was chosen as a lens material because it has good transmission characteristics throughout this spectral region.

3 Equipment Configuration

The contact lens was incorporated into the equipment setup shown in Figure 4. Light is taken from the xenon arc lamp to the contact lens and from the contact lens to the spectrograph using silica cored optical fibers. These fibers were chosen for their good transmission characteristics in the ultraviolet and visible parts of the spectrum. The lamp and spectrograph were supplied by Oriel Ltd (Leatherhead, England). A key part of this equipment is the sensitive open-electrode charge coupled device (CCD) array (supplied by Oriel Ltd. and originally manufactured by Andor Technology, Belfast, Northern Ireland). This arrangement allowed a complete spectrum to be measured in under 1 s. A short measurement time was considered an essential requirement for the intended use of this instrument to make *in situ* spectroscopy measurements of the aqueous humor.

For absorption measurements knowledge of the path length and molar absorption constant of the drug is sufficient to determine the concentration of the drug using Lambert–Beer law,

$$\log 10\left(\frac{I_0}{I}\right)=\varepsilon cL. \tag{1}$$

Here I_0 is the initial light intensity and I is the intensity after the light has traveled a distance L through an absorbing solution which has a concentration c. ε is the molar extinction coefficient and is a constant for a particular substance at a particular wavelength. The absorbance (A) of a material is defined as

$$A=\log 10\left(\frac{I_0}{I}\right). \tag{2}$$

The sensitivity of the measurement needs to be considered. The limiting factor to resolution of this single beam absorption spectroscopy arrangement is the temporal drift of the light source intensity. The long term temporal drift is kept to a minimum by a light intensity controller. It measures the output of the xenon arc lamp, via a photodiode, and adjusts the lamp output to minimize any long term drift in the intensity of the lamp output. This gives a stable average intensity over a period of days or even weeks if required. This leaves short term temporal variations as the main source of noise, and it results in a signal which has a noise of $\pm 0.001-\pm 0.002$ absorption units (AU) across a typical baseline spectrum. The lower limit for quantitative determination of absorption peaks can be taken as approximately 10 times this noise, giving a lower limit of sensitivity of 0.01 and 0.02 AU, respectively. This sensitivity should enable the instrument to detect micromolar concentrations of reasonably absorbing compounds that have an absorption band in the UV/vis part of the spectrum. Drugs which are topically applied to the cornea achieve concentrations of a few μM to a few tens of μM.[2,21] These concentrations should be detectable using the current equipment setup.

4 Results and Discussion

The detection resolution of the equipment was determined for three different sets of test solutions. To achieve this the contact lens was turned upside down so that its concave hollow acted as a well into which the test solutions were introduced. The test solutions acted as a crude substitute for the cornea and the aqueous humor of the eye. Although not nearly an exact structural substitute, the small refractive index differences between the test solutions and those of the cornea/aqueous humor of the eye did not significantly alter the path of the light through the eye as shown by the ray trace in Figure 5(a).

Fluorescein solution (fluorescein sodium in de-ionized and distilled water) was used as a test solution because of its high molar absorptivity. It also has the added advantage of being fluorescent which, for concentrated solutions, allows the observer to visually track the light path through the solution. In

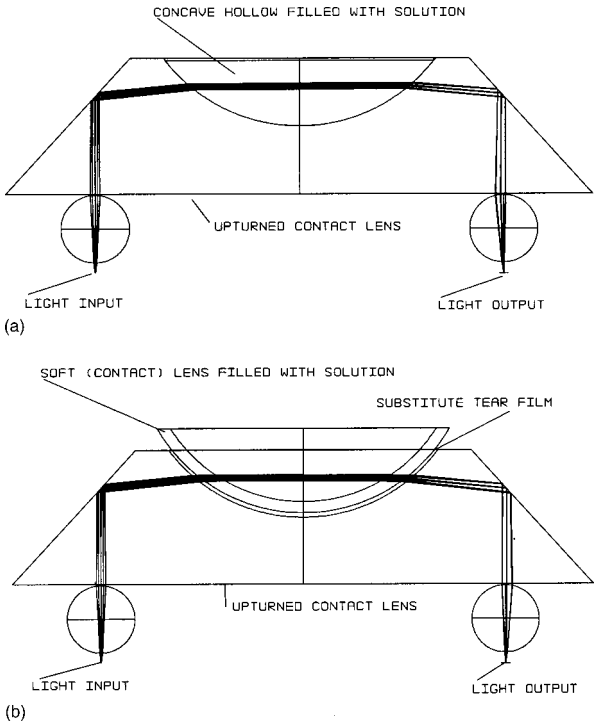

Fig. 5 Physical model of the cornea and anterior chamber initially obtained with (a) a contact lens turned upside down that is filled with the test solution and (b) a more accurate model constructed using a solution filled soft contact lens.

the black and white picture shown in Figure 6 the beam path is seen as the white streak traversing the solution filled contact lens. In this photograph the top right-hand part of the contact lens also appears white; this is due to the authors' lack of photographic skill and is part of the lens which was not successfully placed in the shade of the room lighting. Dilute con-

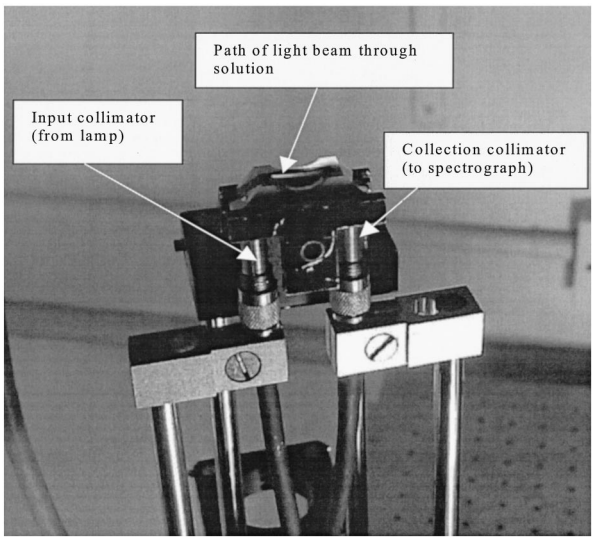

Fig. 6 Photograph of a contact lens turned upside down that is filled with fluorescein solution. The fluorescent solution is relatively concentrated and the beam path through the solution can clearly be seen as the horizontal white streak.

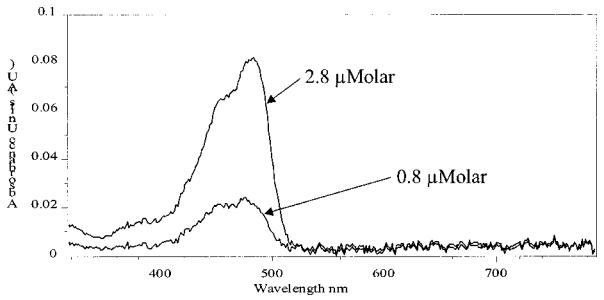

Fig. 7 Absorption spectra obtained for two dilute fluorescein solutions using the configuration shown in Figure 5(a).

centrations of fluorescein were easily detected using our equipment. Figure 7 shows the absorption spectra obtained for 2.8 and 0.8 μM concentrations of fluorescein solution. These spectra were always obtained by first obtaining a reference spectrum from a water filled upturned contact lens (I_0). This was stored in the computer after which the spectra were obtained for the solution filled upturned contact lens (I). The absorption spectrum (in AU) was calculated by taking the log of the ratio of the two spectra, as in Eq. (2). All the absorption spectra described were obtained in this manner.

Tetracycline and brimonidine, two pharmaceutically interesting drugs, were also used as test solutions. Tetracycline is one of the most widely prescribed antibiotics for general applications and is occasionally used to treat eye infections by means of topical application. Brimonidine is an α_2 agonist which is used to treat open-angle glaucoma in the eye. It works by reducing the rate of formation of the aqueous humor and hence the intraocular pressure.[22] Absorption spectra for 30 and 10 μM solutions of brimonidine tartrate are shown in Figure 8. Table 2 shows a summary of the detection limits obtained for the three test solutions. Submicromolar concentrations of fluorescien were detectable and absorption peaks for both brimonidine and tetracycline were clearly resolvable at concentrations of 3 μM. This sensitivity should be just enough to detect the presence and peak concentrations of many drugs which have been topically applied to the cornea.[2,21] Acheampong et al.[2] have reported C^{14}-brimonidine concentrations of 4.88 μM in the aqueous humor of New Zealand White rabbits after topical application of the radiolabeled brimonidine solution to the cornea. Acheampong's study highlights the large number of rabbits used to accurately study ocular drug pharmacokinetics. For each of the nine sampling times at least five rabbits were killed to obtain a statistically relevant sample of the time course of one specific drug

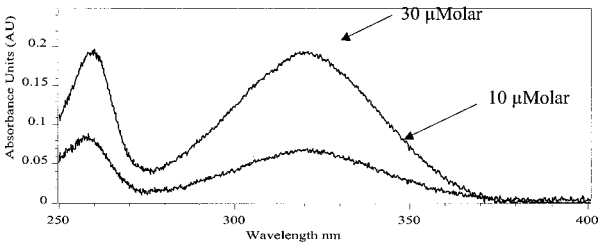

Fig. 8 Absorption spectra obtained for two dilute brimonidine solutions using the configuration shown in Figure 5(a).

Table 2 Minimum detection resolution obtained for the three test compounds using the configuration shown in Figure 5(a).

Compound	Detection limit (μM)
Fluorescein	0.3
Tetracycline	3.0
Brimonidine	3.0

formulation in one specific breed of rabbits. It must be stated that the detection sensitivities we report could be matched or bettered by many bench top spectrometers, the novelty is the acquisition speed combined with the use of the contact lens to allow detection of samples within the anterior chamber of the living eye. The sensitivity of our instrument could be improved by switching to a two beam arrangement. We are considering setting up such a system for future measurements. In two beam systems the second beam acts as a permanent reference beam, thus reducing noise caused by temporal variation of the light source intensity.

A physically more realistic model of the anterior chamber of the eye was placed in the upturned contact lens. A soft (disposable) contact lens filled with tetracycline solution was used as a model cornea and aqueous humor. This solution filled soft contact lens was placed in the upturned contact lens [as shown in Figure 5(b)], with a thin film of water acting as a substitute tear film. The absorption spectrum derived from this arrangement for a solution of 30 μM tetracycline is shown in Figure 9. It was found that this spectrum had approximately 18% less absorption than that measured for an identical concentration measured using the configuration shown in Figure 5(a). This can be directly attributed to a reduction in the path length of the light beam through the solution as a result of the inclusion of the soft lens and substitute tear film in the beam path.

To test the performance of the contact lens in a real eye the propagation of light across the anterior chamber of a rabbit's eye was studied. A dead rabbit was placed in a standard restraining holder and the contact lens was positioned against the rabbit's eye. The rabbit was not put down specifically for this project, but had been used in an unrelated procedure. A live rabbit would have been preferable, and we are in the process of obtaining a licence which will allow us to perform future measurements on live rabbits. The equipment setup was

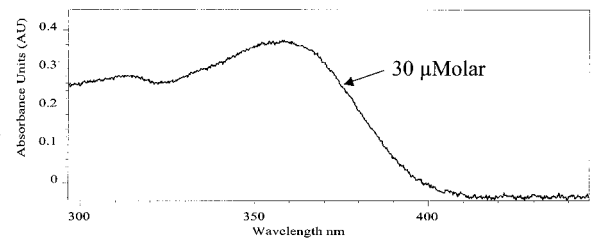

Fig. 9 Absorption spectra obtained for 30 μM tetracycline solution using the configuration shown in Figure 5(b).

Table 3 Comparative powers of light transmitted through the rabbit's eye and reference solution of water placed in a contact lens turned upside down.

Anterior chamber of rabbit's eye	0.009
Anterior chamber replaced with water	0.014

altered from the arrangement shown in Figure 4. A low power HeNe laser was used as the light source and the spectrograph was replaced with an optical power meter. The power of the light after it had passed through the rabbit's eye was measured, the contact lens was then removed from the vicinity of the rabbit's eye, upturned, filled with water as in Figure 5(a) and another reading taken. The two readings are shown in Table 3. Using the corneal transmission of 91% reported by McLaren and Brubaker[20] the power reading taken for the light which passed through the rabbit's anterior chamber should be 83% of that through water. However, as can be seen from Table 3 it is only 64% of this value. The rabbit had been dead for approximately 3 h when the readings were taken and the cornea appeared slightly cloudy. A lot of scattering was observed at the points where the light entered and exited the cornea and there appeared to be no other obstructions to the light path as it passed through the anterior chamber. It was concluded that the difference between the 83% expected and the 64% measured can be explained by the clouding over of the cornea after death and that the light path through the anterior chamber of the eye was as expected. One further observation to come from this measurement was that miniaturizing the optical head of the system would enable the optical head to be hand held, allowing greater flexibility in positioning the contact lens on the surface of the cornea. The optical head comprises the contact lens and the collimated terminations of the two fibers.

5 Conclusions

The type of minimally invasive ophthalmic measurement described here has the potential to be used to detect pharmaceutically useful drugs in the anterior chamber of the living eye. The equipment was used to successfully guide light into, across and out of the anterior chamber of an *in situ* (but *ex vivo*) rabbit's eye. The sensitivity of this technique was established by detecting the presence of micromolar concentrations of drugs in solution within a realistic time frame. Two of the drugs detected had a pharmaceutical function within the eye and are used to treat specific eye disorders. This type of measurement has the potential to replace sampling via paracentesis in ophthalmic drug studies with a minimally invasive measurement which requires only a short period of contact between the eye and a contact lens.

Acknowledgments

The authors wish to thank Wee Kuan Kek and Blythe Lindsay of the Department of Pharmaceutical Science, University of Strathclyde, for their assistance and guidance with the practicalities of the pharmaceutical aspects of the experimentation and J. Brown of the BPU, University of Strathclyde. This

project was funded by a grant from the Engineering and Physical Sciences Research Council of the UK.

References

1. R. L. Stamper, ''Aqueous humor: secretion and dynamics,'' in *Physiology of the Human Eye and Visual System*, R. E. Records, Ed., Chap. 6, pp. 156–182, Harper & Row, New York (1979).
2. A. A. Acheampong, M. Shackleton, and D. D.-S. Tang-Liu, ''Comparative ocular pharmacokinetics of brimonidine after a single dose application to the eyes of albino and pigmented rabbits,'' *Drug Metab. Dispos.* **23**(7), 708–712 (1995).
3. B. Rabinovich, W. F. March, and R. L. Adams, ''Non-invasive glucose monitoring of the aqueous humor of the eye, *Part* I,'' *Diabetes Care* **5**(3), 254–258 (1982).
4. W. F. March, B. Rabinovich, and R. L. Adams, ''Non-invasive glucose monitoring of the aqueous humor of the eye, *Part* II,'' *Diabetes Care* **5**(3), 259–265 (1982).
5. G. L. Cote, M. D. Fox, and R. B. Northrop, ''Non-invasive optical polarimetric glucose sensing using a true phase measurement technique,'' *IEEE Trans. Biomed. Eng.* **39**(7), 752–756 (1992).
6. T. W. King, G. L. Cote, R. McNichols, and M. J. Goetz, ''Multispectral polarimetric glocose detection using a single Pockels' Cell,'' *Opt. Eng.* **33**(8), 2746–2753 (1994).
7. B. D. Cameron and G. L. Cote, ''Non-invasive glucose sensing using a digital closed-loop polarimetric approach,'' *IEEE Trans. Biomed. Eng.* **44**, 1221–1227 (1997).
8. B. D. Cameron, H. W. Gorde, and G. L. Cote, ''Development of an optical polarimeter for *in vivo* glucose monitoring,'' *Proc. SPIE* **3599**, 43–49 (1999).
9. B. D. Cameron, H. W. Gorde, B. Satheesan, and G. L. Cote, ''The use of polarised laser light through the eye for noninvasive glucose monitoring,'' *Diabetes Technol. Therapeut.* **1**(2), 135–143 (1999).
10. R. Blue, D. Uttamchandani, B. Culshaw, and C. G. Wilson, ''Development of a non-invasive technique using fibre optics for optical measurements of anterior chamber aqueous humor in the eye,'' *Proc. OFS-11*, Hokkaido, pp. 442–445 (1996).
11. R. Blue, D. Uttamchandani, and C. G. Wilson, ''Optical system for drug detection in the anterior chamber of the eye,'' *IEE Colloq. Biomedical Applications of Photonics*, Savoy Place, London (April 1997).
12. W. S. Wilson, M. Shahidullah, and C. Millar, ''The bovive arterially-perfused eye: An *in vitro* method for study of drug mechanisms,'' *Curr. Eye Res.* **12**, 609–620 (1993).
13. R. Blue, D. Uttamchandani, and C. G. Wilson, ''Non-invasive optical interrogation of the ocular anterior chamber,'' *Proc. SPIE* **3483**, 114–115 (1998).
14. R. Blue, D. Uttamchandani, and C. G. Wilson, ''Minimally invasive optoelectronic sensing technique for chemical analysis of aqueous humor,'' *IEE Proc.: Sci., Meas. Technol.* **146**(1), 41–46 (1999).
15. S. Patel, J. Marshall, and F. W. Fitzke III, ''Refractive index of the human corneal epithelium and stroma,'' *J. Refract. Surg.* **11**, 101–105 (1995).
16. A. Huges, ''A schematic for the rabbit eye,'' *Vision Res.* **12**, 123–138 (1972).
17. E. A. Boettner and J. Reimer Wolter, ''Transmission of the ocular media,'' *Invest. Ophthalmol.* **1**(6), 776–783 (1962).
18. P. Michalos, E. N. Avilia, G. J. Florakis, and P. S. Hersh, ''Do human tears absorb ultraviolet light?'' *CLAO J.* **20**(3), 192–193 (1994).
19. W. Ambach, M. Blumthaler, T. Schopf, E. Ambach, F. Katzgraber, F. Daxecker, and A. Daxer, ''Spectral transmission of the optical media of the human eye with respect to keratitis and cateract formation,'' *Doc. Ophthalmol.* **88**, 165–173 (1994).
20. J. W. McLaren and R. F. Brubaker, ''Measurement of transmission of ultraviolet and visible light in the living rabbit cornea,'' *Curr. Eye Res.*, 411–421 (1996).
21. C. I. Phillips et al., ''Penetration of timolol eye drops into human aqueous humor: The first hour,'' *Br. J. Ophthamol.* **69**(3), 217–218 (1985).
22. British National Formulary (No. 35), British Medical Association, London (1998).