Pilocarpine-Loaded Poly(DL-lactic-*co*-glycolic acid) Nanoparticles as Potential Candidates for Controlled Drug Delivery with Enhanced Ocular Pharmacological Response

K. Lekha Nair,[1] S. Vidyanand,[2] Jackson James,[2] G. S. Vinod Kumar[1]

[1]*Chemical Biology, Molecular Medicine Division, Rajiv Gandhi Centre for Biotechnology, Poojappura, Thiruvananthapuram 695 014, Kerala, India*
[2]*Neuro Stem Cell Biology Laboratory, Rajiv Gandhi Centre for Biotechnology, Poojappura, Thiruvananthapuram 695 014, Kerala, India*

Received 21 February 2011; accepted 10 July 2011
DOI 10.1002/app.35229
Published online 25 October 2011 in Wiley Online Library (wileyonlinelibrary.com).

ABSTRACT: The poor corneal residence time of pilocarpine, an alkaloid extracted from the leaves of the Jaborandi plant, limits its ocular application. The aim of this study was to develop, characterize, and evaluate the potential of pilocarpine entrapped by poly(DL-lactic-*co*-glycolic acid) (PLGA) nanoparticle carriers for ocular drug delivery. Pilocarpine-loaded nanoparticles were prepared with a double-emulsion (water in oil in water) method and characterized with transmission electron microscopy and X-ray diffraction analysis. The nanoparticles exhibited an average size of 82.7 nm with an encapsulation efficiency of 57%. Stability studies showed the absence of agglomeration and constancy in the amount of drug entrapped; this indicated the solidity of these particles for long-term use. The *in vitro* release studies conducted in simulated tear fluid showed the sustained release of pilocarpine. *In vivo* evaluation of the nanoparticles was done in a rabbit model with a miosis assay and compared to an equal dose of commercially available eye drops of pilocarpine (Pilocar drops). The *in vivo* miosis studies showed that the duration of miotic response increased by 40% for the nanoparticles and produced an almost 68% increase in total miotic response when compared to the eye drops. In conclusion, this study clearly demonstrated the potential of pilocarpine-loaded PLGA nanoparticles for multiplying the therapeutic effect of ophthalmic drug delivery with enhanced bioavailability and pharmacological response. J Appl Polym Sci 124: 2030–2036, 2012

Key words: biodegradable; compatibility; drug delivery systems; nanoparticle

INTRODUCTION

Pilocarpine, an alkaloid extracted from the leaves of the Jaborandi plant (*Pilocarpine mycrophyllus*), belongs to the small group of alkaloids having an imidazole ring in their structure. Its use has been reported in clinical eye applications as a topical miotic for the treatment of open-angle glaucoma and acute-angle-closure glaucoma.[1] However, the high hydrophilicity and low lipophilicity of pilocarpine results in its poor corneal penetration and extensive precorneal loss; this reduces its ocular bioavailability. Therefore, large quantities of pilocarpine have to be administered frequently into the eyes of patients to achieve effective therapeutic results; this results in undesirable side effects.[2]

Using a controlled release delivery system as a carrier has the potential of lessening the shortcomings of pilocarpine.[3–5] A variety of carriers, such as gels, polymer matrices, and hydrogels, have been reported to increase the topical effect and corneal residence time[6] of such hydrophilic drugs. In recent advances, the use of certain poly(amido amine)-based dendrimers as vehicles for controlled ocular drug delivery[7] has also been reported. However, Ocusert, a nonerodible insert that is considered as a technical breakthrough in this area, has also been shown to have certain limitations, including difficulty in retention, the requirement of frequent insertion, and the removal and rupture of the membrane causing a burst release of the drug.[8] Microemulsions, microspheres, nanoparticles, and nanospheres[9,10] have been reported to enhance the ocular residence time with prolonged pharmacological activity. Nanoparticles are considered as a breakthrough in the area of drug delivery[9] because, in contrast to the larger size of microspheres and liposomes, which are rapidly eliminated from the eye via rapid tear turnover,[11] nanoparticles provide longer residence time because of their smaller size. Recently, poly (ethylene glycol)-based hydrogels loaded with

Correspondence to: G. S. Vinod Kumar (gsvinod@rgcb.res.in).

Contract grant sponsors: Council of Scientific and Industrial Research, Government of India (for a research fellowship and financial assistance to K.L.N.).

pilocarpine for sustained release have been reported[12] with a similar goal. Although several polymers are used as biocarriers, poly(DL-lactic-*co*-glycolic acid) (PLGA) is a widely used carrier for ocular drug delivery because of its low ocular toxicity.[13]

The aim of this study was the formulation and characterization of a controlled ocular drug-delivery system for pilocarpine with PLGA as the carrier. We prepared pilocarpine encapsulated by PLGA nanoparticles using a double-emulsion method. These were characterized for their size and distribution with transmission electron microscopy (TEM), particle size analysis, and physicochemical analysis with X-ray diffraction (XRD). The stability of these nanoparticles in terms of variation in size, polydispersity index (PDI), and encapsulation efficiency (EE) was studied over a period of 1 year. To study the controlled release rate of pilocarpine from the nanoparticles, the *in vitro* release kinetics in simulated tear fluid (STF) were studied. These were further evaluated for controlled drug delivery by pupillary constriction in comparison to commercial eye drops of pilocarpine (Pilocar drops) in New Zealand white rabbits with a miosis assay.

EXPERIMENTAL

Materials

PLGA, with a lactide-to-glycolide molar ratio of 50 : 50 and a molecular weight range of 40,000–75,000 Da; pilocarpine nitrate (i.e., pilocarpine), and phosphate buffered saline were purchased from Sigma-Aldrich (Steinheim, Germany). Pilocar (2% pilocarpine nitrate ophthalmic solution) was purchased from FDC, Ltd. (Mumbai, India). Poly(vinyl alcohol) (PVA; molecular weight $\approx$ 125,000) was obtained from S. D. Fine Chemicals, Ltd. (Mumbai, India). All of the solvents were High-performance liquid chromatography (HPLC) grade.

Nanoparticle formulation

Pilocarpine-loaded PLGA nanoparticles were prepared with a double-emulsion (water in oil in water) method with some modifications.[14,15] Briefly, pilocarpine dissolved in water (10 : 1 w/v) was added to the organic phase consisting of PLGA (100 mg) dissolved in a dichloromethane–acetone (3 : 1 v/v) mixture and vortexed vigorously to form the primary emulsion. It was then poured into an aqueous phase containing a surfactant [poly(vinyl alcohol), typical concentration = 1% w/v, 20 mL] to form the secondary emulsion. This emulsion was then broken down into nanodroplets by application of external energy through a sonicator, and the organic phase was evaporated at room temperature with magnetic stirring overnight. This left behind a colloidal solution of pilocarpine containing PLGA nanoparticles. The nanoparticles were recovered after washing with distilled water to remove nonentrapped drug by centrifugation followed by freeze drying to obtain the dry powder. Similarly, polymer nanoparticles without pilocarpine were prepared with a single-emulsion method exclusive of aqueous solution of pilocarpine.

Characterization of the nanoparticles

Particle size and surface morphology

The size and size distribution of pilocarpine-loaded PLGA nanoparticles were measured with a particle size analyzer (Beckman Coulter Delsa nanoparticle analyzer). The images of the particles in the nanometer range were taken with a transmission electron microscope (JEOL 1011, Tokyo, Japan). For TEM, the sample of the nanoparticle suspension in Milli-Q water at 25°C was dropped onto formvar-coated grids without being negatively stained. Measurements were taken only after the sample was completely dried.

EE and drug loading

Calculations for EE and drug loading were done according to previous reports.[16,17] In brief, the nanoparticle suspension was centrifuged at 30,000 rpm for 30 min at 4°C, and the absorbance of the nonentrapped pilocarpine was measured with a UV spectrophotometer (PerkinElmer, Waltham, Massachusetts, USA) at a wavelength of 215 nm. The concentration of each sample was obtained by comparison of the absorption of the supernatant to the standard curve and the relationship of the absorption and pilocarpine concentration. The drug incorporation efficiency was expressed as the drug loading percentage:

$$\text{Encapsulation efficiency (\%)} = \frac{\text{Mass of drug in the nanoparticles}}{\text{Mass of drug used in the formulation}} \times 100$$

$$\text{Drug loading (\%)} = \frac{\text{Mass of drug in the nanoparticles}}{\text{Mass of nanoparticles recovered}} \times 100$$

$$\text{Yield (\%)} = \frac{\text{Mass of nanoparticles}}{\text{Mass of polymer and drug used in the formulation}} \times 100$$

XRD

The physicochemical state of the pilocarpine in the nanoparticle formulation was compared with the free drug by powder XRD patterns acquired at room temperature on an X-ray diffractometer (PANalytical X'pert PRO, Lelyweg, The Netherlands) with Cu Kα radiation at 5–40° in continuous mode with a step size of $2\theta = 0.02°$ and a step time of 5 s.[18]

Stability studies

Information on the stability of the drug entrapped in the nanoparticles was very important for determining the variation in parameters such as size, PDI, and amount of drug entrapped, which affected the therapeutic efficiency of the nanoparticles. Here, stability studies were done to analyze the extent to which these nanoparticles changed in their properties throughout their period of storage and use.[19] The change in the particle size of pilocarpine entrapped in nanoparticles and their distribution was studied with the particle size analyzer up to a period of 1 year. Also, the variation in the amount of pilocarpine entrapped inside the polymeric nanoparticles was analyzed with UV spectroscopy, as explained previously.

In vitro drug release

The release kinetics were studied as previously reported.[12,20] In brief, pilocarpine-loaded PLGA nanoparticles (2% w/v) were suspended in 4.0 mL of STF (NaCl, 0.67 g; $NaHCO_3$, 0.20 g; $CaCl_2{\cdot}2H_2O$, 0.008 g; and distilled deionized water to 100 g) and incubated at 37°C under continuous shaking at 60 rpm. Aliquots (200 μL) were collected from the vials at predetermined time intervals with equal volumes of tear fluid to maintain sink conditions throughout the study. The concentration of pilocarpine in the release medium was determined by UV spectroscopy at a wavelength of 215 nm. The cumulative amount of pilocarpine released was evaluated with a calibration curve. Similarly, the amount of pilocarpine released from Pilocar was analyzed to compare the release pattern with that of the nanoparticles.

Miosis studies

To assess the sustained release of the drug from the nanoparticles in comparison with commercial eye drops, Pilocar, a test of miosis, that is, the constriction of the pupil diameter, was conducted *in vivo*.[12,21] New Zealand albino rabbits of either sex, weighing 2.4–2.6 kg, were used without any special pretreatment diets. All animal experiments were carried out according to institutional animal ethics guidelines. A washout period of at least 3 days was kept between experiments. To accustom the rabbits to the environment of the laboratory, they were brought to the laboratory 1 h before the start of the experiment. All of the experiments were conducted in the same room with the same illumination conditions. To instill the solution into the eye, the lower eyelid was slightly pulled away from the globe, and the solutions were administered carefully into the cul de sac to avoid any direct contact with the eye. First, the biocompatibility of the polymer was assessed by observation of any ocular irritation, such as redness, tearing, or inflammation, on instilling the blank polymer nanoparticles. Once, the polymer showed no side effects, nanoparticle preparation buffered in phosphate buffered saline (pH 7.4) containing 2% w/v pilocarpine was tested in all of the animals by instillation of a dose of 25 μL in the left eye. To avoid experimental bias, the right eye received 25 μL of the nanoparticle solution having no drug and remained as a control. After 1 week, the aforementioned experiments were repeated with 2% w/v of the commercial ophthalmic solution Pilocar. The pupil diameters of both eyes were measured with a pupil gauge (Astrospace Co., Ltd., Sindian, Taiwan) at predetermined intervals by movement of the gauge close to the eye and with it kept at a fixed distance from the eyeball for some time. The constriction in pupil diameter was calculated from the difference in the pupil diameter of the normal eye from that of the experimental eye at a particular time.

Statistics

All of the measurements were done in triplicate, and the results were expressed as the arithmetic mean plus or minus the standard error on the mean. Statistical differences ($p < 0.05$) were calculated with GraphPad Instat 3 (GraphPad software Inc., San Diego, California, USA). To assess the extent of total pharmacological response of the nanoparticles compared to the eye drops, the area under the decrease in pupil diameter versus time curve (AUC) was calculated with GraphPad Prism 4 software.

RESULTS AND DISCUSSION

Characterization of the nanoparticles

Pilocarpine-loaded PLGA nanoparticles were successfully prepared by a double-emulsion solvent evaporation method. The nanoparticles obtained after freeze drying were easily redispersed in water and loaded for TEM. TEM images revealed that the pilocarpine-loaded PLGA nanoparticles were in the nanometer size range with a mean diameter of 82 nm (Fig. 1) and a low PDI of 0.09.

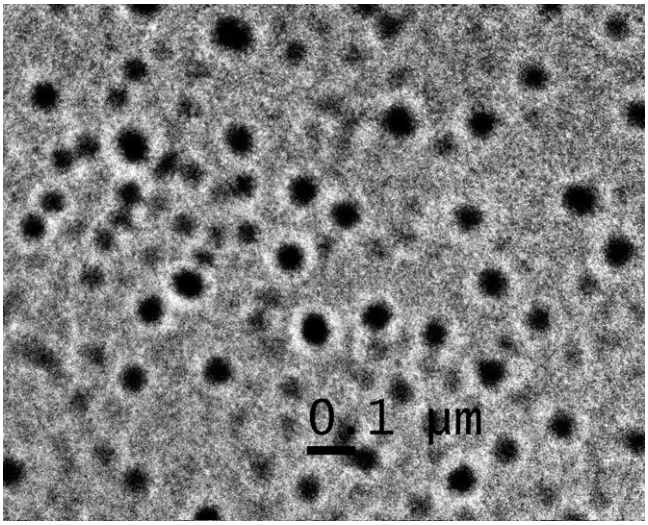

Figure 1 TEM image of the pilocarpine-loaded PLGA nanoparticles.

The nanoparticles exhibited an EE of 57%; this might have been due to the leaching of drug out of the polymer matrix during sonication[22] (Table I). The drug loading of particles is dependent on the size and shape of the carriers. The size of nanoparticles is a crucial factor in determining the drug delivery to the posterior site of ocular tissues. In a previous report, the authors stated the size dependency pattern with the *in vivo* corneal uptake of indomethacin-loaded polycaprolactone.[23] Particles in the size range of 100 nm are expected to exhibit the highest uptake and corneal penetration in comparison to larger size (800–1000 nm) particles.

The XRD patterns of pilocarpine, blank nanoparticles, and pilocarpine-loaded nanoparticles were obtained (Fig. 2) and compared to analyze the physicochemical state of the drug inside the polymer. In the case of pilocarpine, the diffractograms exhibited an intense peak at 2θ values near 19°; this showed its crystalline nature [Fig. 2(A)], whereas the nanoparticle formulations showed no characteristic peak of pilocarpine; this indicated that the drug was dispersed at a molecular level in the polymer matrix.[18]

Stability studies

The stability of nanoparticles at the biological level is a crucial issue because the size of these particles plays an important role in their ability to interact with the mucosal surfaces, in particular, with the ocular mucosa.[24] On analyzing the change in nanoparticle diameter over a period of 1 year after formulation, we observed that there was no significant increase in the diameter of the nanoparticles; this showed the absence of the formation of clusters (Fig. 3).

PDI also did not show any considerable change; this proved the monodispersed distribution of particles and the absence of agglomeration after storage (Fig. 4).

Because the amount of drug encapsulated in the polymer matrix should remain constant over a longer period, EE determination was done. The nanoparticles did not show any change in the amount of pilocarpine loaded (Fig. 5); this proved that these nanoparticles were stable and could be stored for long-term use.

In vitro drug-release profile

The main aim of this study was to develop a controlled drug-delivery system for increasing the ocular residence time of pilocarpine. Thus, *in vitro* release studies were conducted in STF to show the sustained release of pilocarpine from the PLGA nanoparticles. The pilocarpine-loaded PLGA nanoparticles followed a biphasic pattern, showing an initial burst release of 28% in the first 2 h, which was followed by sustained release for up to 24 h.[25,26] The initial burst release might have been due to the presence of drug present on the surface of the particles, and the sustained release, which is a characteristic of nanoparticles,[27,28] was a consequence of matrix erosion.[29] On the contrary, the ophthalmic solution was readily dissolved, releasing almost 100% pilocarpine in the media because of the absence of any coating for controlling the release (Fig. 6). More than 85% of the pilocarpine was released from the eye drops in the first 2 h, compared to a release of only 28% drug from the nanoparticle suspension. Thus, this study indicated a change in the release pattern on the formulation of the drug from free form to the nanoparticle form.

In vivo studies

After administration of the drug-free PLGA nanoparticles, the eyes were checked frequently, and no adverse effects such as swelling, tearing, redness, or inflammation of the eye were observed; this proved

TABLE I
Characterization of the Nanoparticles

Sample	Mean size (mm)	PDI	EE (%)	Yield (%)	Drug loading (%)
Pilocarpine nanoparticles	82.7 ± 5.4	0.095 ± 0.04	56.7 ± 5.9	71.7 ± 2.2	3.4 ± 0.76

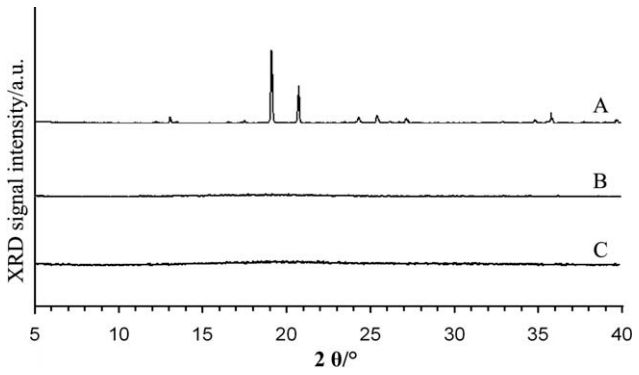

Figure 2 X-ray diffractograms of (A) pilocarpine, (B) blank PLGA nanoparticles, and (C) pilocarpine-loaded PLGA nanoparticles.

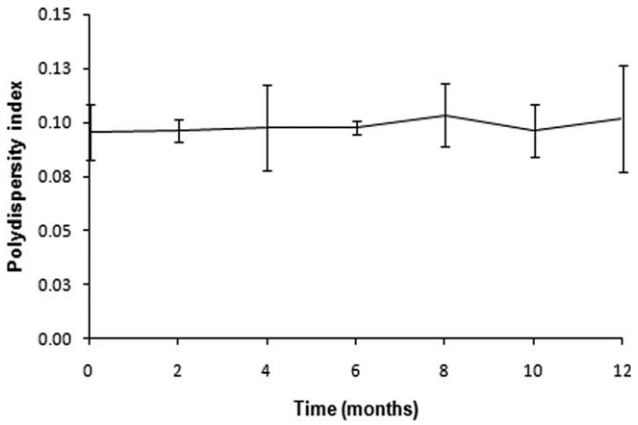

Figure 4 Variation in PDI of the nanoparticles with time.

the potential of PLGA as a carrier for ocular drug delivery.[24,30] The persistent release of pilocarpine for sustained pharmacological response was studied as the change in pupil diameter as a function of time after the instillation of equivalent amounts of the pilocarpine nanoparticle formulation compared to the pilocarpine ophthalmic solution Pilocar. The time points that showed a statistically significant difference ($p < 0.05$ or $p < 0.01$) are indicated by single and double asterisks, respectively, in Figure 7. The instillation of Pilocar resulted in a fast miotic response, which soon returned back to normal. However, in the case of the nanoparticle-based drug-delivery system, the miosis response was significantly more substantial and prolonged ($p < 0.01$) compared to the pilocarpine eye drops (Fig. 7). This was in agreement with previous reports indicating the potential of nanoparticles for ocular drug delivery.[23,31,32]

The efficacy of the pilocarpine-loaded ocular drug formulations were assessed on the basis of following four parameters: (1) the peak miosis intensity (I_{max}), (2) the time to reach peak miosis response (t_{max}), (3) AUC, and (4) the duration of the miosis response. The duration of miosis was the duration for which a change in pupil diameter of 1 mm or more was observed. The diameter of 1 mm was arbitrarily chosen as the reference for the calculation of the duration of miosis response.[21] I_{max}, t_{max}, and duration of miosis response were calculated by linear interpolation between the data points, whereas AUC was calculated with Graph Pad Prism 4 software. As shown in Table II, the pilocarpine-loaded PLGA nanoparticles produced similar I_{max} values, that is, the maximum constriction in the pupil diameter. However, t_{max} was greater for the nanoparticle formulation ($t_{max} = 60$ min) when compared to the 30 min taken by the eye drops; this was due to the controlled release of pilocarpine from the nanoparticles. On treatment with the nanoparticles, the constriction was sustained for 420 min compared to the 250 min effect of the eye drops. The duration of miotic response (the time taken by the pupil diameter to return to its normal value as an untreated control) increased by 40% for the nanoparticles; this indicated the sustained release of pilocarpine from the nanoparticles, as shown *in vitro*. Also, from the AUC values, it was seen that the nanoparticle formulation

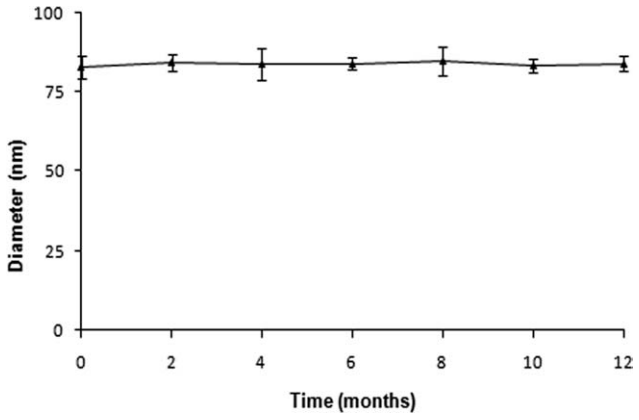

Figure 3 Variation in the size of the nanoparticles with time.

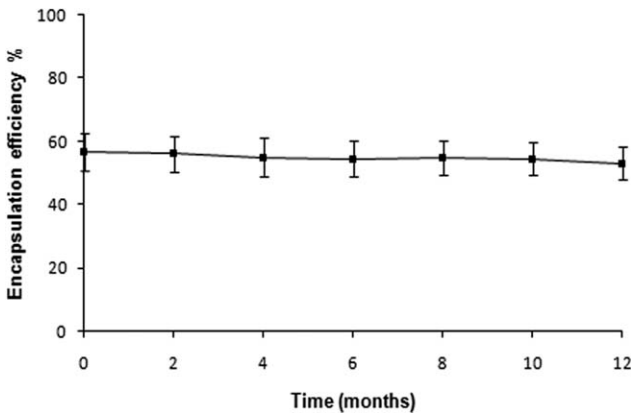

Figure 5 Variation in EE of the pilocarpine-loaded PLGA nanoparticles with time.

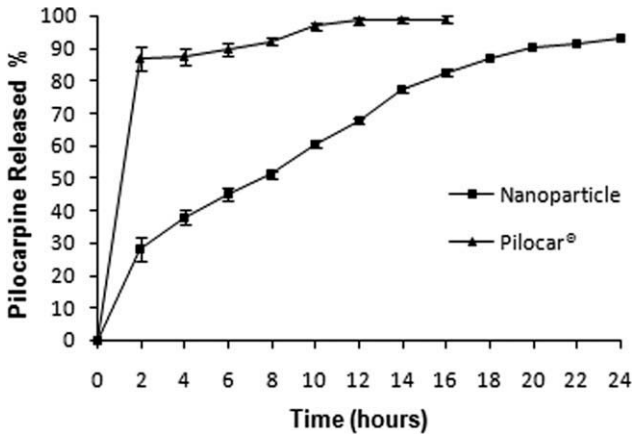

Figure 6 Release pattern of pilocarpine from the PLGA nanoparticles when compared to the ophthalmic pilocarpine solution, Pilocar.

produced a 68% better total miotic response (AUC was proportional to the amount of drug reaching the target site) relative to the eye drops; this proved that pilocarpine entrapment in the PLGA nanoparticles increased the bioavailability of the pilocarpine. Although the *in vitro* release continued for 24 h, in the case of the *in vivo* experiment, that much prolonged constriction in the pupil diameter was not seen (this might have been due to the loss of a fraction of the nanoparticle formulation due to lachrymal fluid release); from pharmacokinetic point of view, the increase was considerably important. The higher efficacy of the pilocarpine-loaded PLGA nanoparticles when compared to the eye drops was due to the sustained release of the drug, as already shown from the *in vitro* kinetic studies.

The results here indicate the efficiency of uptake of the PLGA nanoparticles smaller than 100 nm in size to deliver pilocarpine for the enhancement of ocular drug absorption and controlled release.[33] This

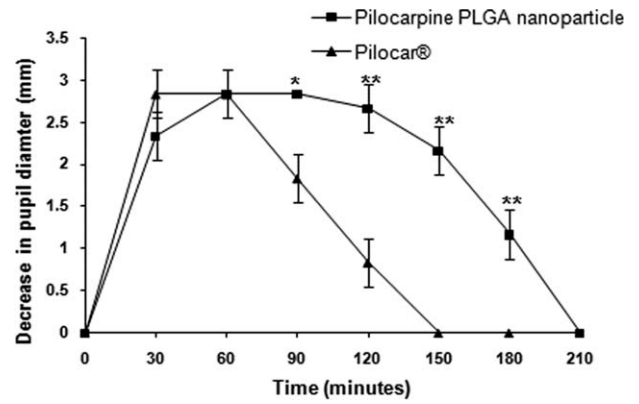

Figure 7 Plot of the miosis response (in millimeters) as a function of time comparing PLGA pilocarpine nanoparticles and commercial ophthalmic solution, Pilocar ($n = 6$, $^{*}p < 0.05$, $^{**}p < 0.01$).

TABLE II
Comparison of the Pharmacological Efficacy of the Pilocarpine Entrapped in PLGA Nanoparticles and Pilocar Commercial Eye Drops

Ocular delivery system	I_{max} (mm)	t_{max} (min)	AUC (mm/min)	Duration of miosis (min)
Nanoparticles	2.83	60	420	210
Pilocar	2.83	30	250	150

prolonged ocular residence time with enhanced miosis with nanoparticles could reduce the frequency of administration of the drug and add to patient compliance. The *in vivo* results, when coupled with the *in vitro* studies, clearly demonstrated the enhanced bioavailability and pharmacological response of nanoparticles and provided a strong base for the controlled delivery of pilocarpine.

CONCLUSIONS

Pilocarpine encapsulated in PLGA nanoparticles were successfully prepared with a double-emulsion method, for sustained ocular delivery. The nanoparticles obtained were small and uniformly dispersed, with a diameter of 82 nm. The prolonged sustained release of pilocarpine *in vitro* was further supported by the increase in the ocular residence time of the drug *in vivo* when compared to commercial eye drops (Pilocar). This was accompanied by an enhancement in miotic response, which showed an improvement in the bioavailability of pilocarpine. Thus, the results support the rationale behind the use of pilocarpine PLGA nanoparticles for ophthalmic drug delivery.

The authors gratefully acknowledge the National Institute of Interdisciplinary Sciences and Technology, Kerala, for carrying out the XRD studies.

References

1. Rosin, A. Oftalmologia 1991, 35, 53.
2. Mazor, Z.; Ticho, U.; Rehany, U.; Rose, L. Br J Ophthalmol 1979, 63, 48.
3. Costa, V. P.; Braga, M. E. M.; Guerra, J. P.; Duarte, A. R. C.; Duarte, C. M. M.; Leite, E. O. B.; Gil, M. H.; de Sousa, H. C. J Supercrit Fluids 2010, 52, 306.
4. Hsiue, G. H.; Guu, J. A.; Cheng, C. C. Biomaterials 2001, 22, 1763.
5. Nadkarni, S. R.; Yalkowsky, S. H. Pharm Res 1993, 10, 109.
6. Peng, C. C.; Kim, J.; Chauhan, A. Biomaterials 2010, 31, 4032.
7. Vandamme, T. F.; Brobeck, L. J Controlled Release 2005, 102, 23.
8. Ali, Y.; Lehmussaari, K. Adv Drug Delivery Rev 2006, 58, 1258.
9. Sahoo, S. K.; Dilnawaz, F.; Krishnakumar, S. Drug Discovery Today 2008, 13, 144.
10. Vandamme, T. F. Prog Retinal Eye Res 2002, 21, 15.

11. Ding, S. Pharm Sci Technol Today 1998, 1, 328.
12. Anumolu, S. N. S.; Singh, Y.; Gao, D.; Stein, S.; Sinko, P. J. J Controlled Release 2009, 137, 152.
13. Yoncheva, K.; Vandervoort, J.; Ludwig, A. Pharm Dev Technol 2009, 16, 1.
14. Cohen-Sela, E.; Chorny, M.; Koroukhov, N.; Danenberg, H. D.; Golomb, G. J Controlled Release 2009, 133, 90.
15. Zambaux, M. F.; Bonneaux, F.; Gref, R.; Maincent, P.; Dellacherie, E.; Alonso, M. J.; Labrude, P.; Vigneron, C. J Controlled Release 1998, 50, 31.
16. Govender, T.; Stolnik, S.; Garnett, M. C.; Illum, L.; Davis, S. S. J Controlled Release 1999, 57, 171.
17. Yan-Yu, X.; Yun-Mei, S.; Zhi-Peng, C.; Qi-Neng, P. Int J Pharm 2006, 319, 162.
18. Jain, A.; Jain, S. K. Eur J Pharm Sci 2008, 35, 404.
19. Missirlis, D.; Kawamura, R.; Tirelli, N.; Hubbell, J. A. Eur J Pharm Sci 2006, 29, 120.
20. Kao, H. J.; Lo, Y. L.; Lin, H. R.; Yu, S. P. J Pharm Pharmacol 2006, 58, 179.
21. Simamora, P.; Nadkarni, S. R.; Lee, Y. C.; Yalkowsky, S. H. Int J Pharm 1998, 170, 209.
22. Gómez-Gaete, C.; Tsapis, N.; Besnard, M.; Bochot, A.; Fattal, E. Int J Pharm 2007, 331, 153.
23. Calvo, P.; Sanchez, A.; Martinez, J.; Lopez, M. I.; Calonge, M.; Pastor, J. C.; Alonso, M. J. Pharm Res 1996, 13, 311.
24. Nagarwal, R. C.; Kant, S.; Singh, P. N.; Maiti, P.; Pandit, J. K. J Controlled Release 2009, 136, 2.
25. Agnihotri, S. A.; Mallikarjuna, N. N.; Aminabhavi, T. M. J Controlled Release 2004, 100, 5.
26. Wilson, B.; Samanta, M. K.; Santhi, K.; Kumar, K. P. S.; Paramakrishnan, N.; Suresh, B. Brain Res 2008, 1200, 159.
27. Brannon-Peppas, L. Int J Pharm 1995, 116, 1.
28. Jalil, R.; Nixon, J. R. J Microencapsulation 1990, 7, 297.
29. Heller, J. Biomaterials 1980, 1, 51.
30. Ludwig, A. Adv Drug Delivery Rev 2005, 57, 1595.
31. Bourges, J. L.; Gautier, S. E.; Delie, F.; Bejjani, R. A.; Jeanny, J. C.; Gurny, R.; BenEzra, D.; Behar-Cohen, F. F. Invest Ophthalmology Visual Sci 2003, 44, 3562.
32. de Campos, A. M.; Diebold, Y.; Carvalho, E. L. S.; Sánchez, A.; Jose Alonso, M. Pharm Res 2004, 21, 803.
33. Qaddoumi, M. G.; Ueda, H.; Yang, J.; Davda, J.; Labhasetwar, V.; Lee, V. H. L. Pharm Res 2004, 21, 641.