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Enhancement of the myotic response of rabbits with pilocarpine-loaded polybutylcyanoacrylate nanoparticles

T. Harmia [1,*], J. Kreuter [1], P. Speiser [1], T. Boye [2], R. Gurny [2] and A. Kubis [1]

[1] *School of Pharmacy, Federal Institute of Technology, Zürich (Switzerland)*
and [2] *University of Geneva, School of Pharmacy, CH-1211 Genève 4 (Switzerland)*

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Summary

In vivo studies of pilocarpine loaded to polybutylcyanoacrylate nanoparticles were carried out with albino rabbits. A prolonged pilocarpine-induced myosis was achieved with pilocarpine-nanoparticle adsorbates compared to a commercial pilocarpine solution. With nanoparticles containing incorporated drug no significant prolongation of the myotic effect was observed. A two-chamber diffusion cell with a cellophane membrane was found to be unsuitable for in vitro release studies.

Introduction

Sustained release of pilocarpine for the treatment of glaucoma is of therapeutic interest because of the poor bioavailability of this drug in conventional ocular dose forms (Harmia, 1984; Juslin et al., 1981; Smolen, 1981; Saettone et al., 1982). Due to their ultrafine solid nature, nanoparticles may be used successfully as drug carriers in ophthalmology. They are colloidal amorphous particles ranging in size from 10 to 1000 nm (Kreuter, 1983).

Biodegradable nanoparticles can be produced using alkylcyanoacrylates. These polymers have to date been used as tissue adhesives. Because of their biodegradability they have a potential as drug delivery systems (Couvreur et al., 1977, 1979; Lenaerts et al., 1984; Vezin and Florence, 1981). In ophthalmics, one of the main goals of nanoparticles is to achieve a controlled action of the encapsulated drug. The drug release may be controlled either by the desorption from the particles or by the degradation of the particle matrix (Chien and Lambert, 1974). Depending on the manufacturing method of the nanoparticles, different kinds of drug release characteristics may be achieved (Harmia, 1984). For in vitro drug release studies from colloidal particles such as nanoparticles and liposomes a two-chamber diffusion cell method with a cellophane membrane was suggested in literature reports (Harmia, 1984; Kreuter et al., 1981, 1983). The aim of this work was to evaluate the suitability of this method for in vitro release studies of pilocarpine nanoparticles and to de-

* *Present address*: University of Helsinki, School of Pharmacy, Fabianink. 35, SF-00170 Helsinki, Finland.
Correspondence: J. Kreuter, Inst. for Pharm. Technology, J.W. Goethe-Universität, D-6000 Frankfurt/Main, F.R.G.

termine their in vivo action by measuring the miotic response in rabbits.

Materials and Methods

Two different types of nanoparticles were studied in vitro and in vivo: the drug, pilocarpine, was bound to the nanoparticles either by adsorption or by incorporation (Harmia et al., 1986a and b).

Preparation of the nanoparticles

Adsorbates

Analytical grade chemicals were used throughout the experiments. For the preparation of polybutylcyanoacrylate (PBCA) nanoparticles, 0.5 g butylcyanoacrylate (BCA) (Sicomet, Sichel-Werke, Hannover, F.R.G.) was added drop by drop to a solution of 100 mg Pluronic F68 (Wyandotte Chemicals, Wyandotte, U.S.A.) in 50 ml 0.01 N HCl and stirred for 2 h at room temperature with a magnetic stirrer at 400 rpm. The resulting polymer suspension was neutralized with 0.1 N NaOH to pH 7, and stirring was continued for 6 h after neutralization. The resulting PBCA-nanoparticles had a diameter of 98 ± 4 nm, as measured by photon correlation spectroscopy (ALV-Laser, BBC Georz Laser Doppler velocimeter, LSO 01 ISC-Computer). The transmission electron micrograph of the nanoparticles shows their sperical shape (Fig. 1). The nanoparticles were then centrifuged at 30,000 rpm at 10°C for 1 h (Du Pont Instruments, Sorvall OTD 75). The sediment was then separated and dissolved in a mixture of water and alcohol (1 : 1). The procedure was repeated 3 times. The purified nanoparticles were freeze-dryed (Leybold-Heraeus-lyophilizator, Lyosystem I, GT3), and samples of 100 mg of nanoparticles were transferred to a series of glass tubes (20 ml) fitted with tight screw-caps. 10 ml of the appropriate concentration of pilocarpine (E. Merck, Darmstadt, F.R.G.), surfactants, and electrolytes in buffer solution (pH 5) were added. The tubes were shaken horizontally, wholely immersed, in a thermostated water bath (18 ± 1°C) at 200 cycles/min for 1 week.

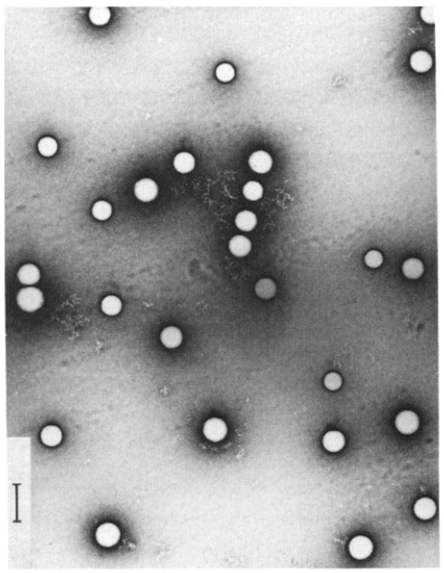

Fig. 1. TEM-photo of empty pBCA-nanoparticles produced at room temperature. Bar = 240 nm.

The adsorbed amount of pilocarpine was then determined by centrifugation of the samples and analysis of the pilocarpine content of the supernatant. The colorimetric method of Gibbs and Tuckermann (1970) was used for quantitative determination of pilocarpine. Two formulations were thus produced and tested.

Preparation I contained 2% pilocarpine hydrochloride and 0.15 M Na_2SO_4 in acetate buffer solution.

Preparation II. As preparation I but with an initial pilocarpine nitrate concentration of 6% in citrate buffer. A 2% pilocarpine hydrochloride solution was used as reference.

Nanoparticles with incorporated pilocarpine

Two different methods for the preparation of polybutylcyanoacrylate nanoparticles with incorporated pilocarpine were used.

(I) 0.5 g of BCA was added drop by drop to a solution of Pluronic F68 in 50 ml of 0.01 N HNO_3 containing pilocarpine nitrate. The dispersions were stirred for 12 h with a magnetic stirrer at 400 rpm. The resulting polymer suspension was neutralized with 1 N NaOH to pH 7 in order to

TABLE 1

NANOPARTICLE FORMULATIONS WITH INCORPORATED PILOCARPINE

Sample no.	Initial drug concentration (%)	Pluronic F68 concentration (%)	Manufacturing temperature (°C)	Manufacturing method	Incorporated amount of pilocarpine (%)	S.D. *
1	1.0	0.8	24	I	10.4	3.3
2	1.0	1.2	10	I	31.2	6.2
3	1.0	0.8	5	I	61.4	9.1
4	1.0	1.2	5	I	32.5	5.2
5	1.5	0.8	24	II	40.9	3.7

* S.D. = standard deviation (3 samples).

complete the polymerization. After that, the suspension was adjusted to pH 5 with citrate buffer because of the poor stability of pilocarpine at pH 7.

(II) 100–250 mg pilocarpine nitrate was dissolved in 1.0–1.5 ml 0.01 N HNO_3 and the solution was acidified to pH 1 with nitric acid (conc.) and carefully mixed with 0.5 g BCA. The resulting dispersion was added to a solution of 400 mg Pluronic F68 in 50 ml 0.01 N HNO_3. The system was polymerized and brought to pH 5 as described under I.

The in vivo experiments were carried out with preparation numbers 2, 3, 4 and 5 (Table 1).

In vitro drug release studies

The in vitro release of pilocarpine was studied using a two-chamber diffusion cell method (Harmia, 1984; Kreuter et al., 1981, 1983). The two compartments were separated by a cellulose membrane (Cuprofan dialysis membrane, m.w. 10,000, Diachema AG, Zürich, Switzerland). The chambers were filled with 7.0 ml of nanoparticle suspension (donor compartment) and 7.0 ml of the dispersion medium without nanoparticles (receiver compartment). Samples of 1.0 ml were drawn from the receiver compartment after 1, 2, 3, 4, 6, 8, 10 and 24 h. The samples were replaced by the same volume of the dispersion medium. The temperature was kept at $32 \pm 1°C$ and the stirring speed in both chambers was 50 rpm. Five parallel experiments were carried out.

In vivo experiments

The in vivo evaluation was carried out by determination of the miotic response of albino rabbits. Fifty μl of the nanoparticle suspensions were administered into the coul de sac. The pupillary diameter was measured every 30 min with the aid of a video camera and the response was evaluated (Gurny, 1981, 1982).

Results and Discussion

In vitro studies

One of the main unsolved problems with nanoparticle carriers is that so-far no meaningful in vitro release models have been developed. Since ocular drug delivery from the aqueous precorneal area through the cornea into the aqueous humor seemed to be quite similar to the transport events occurring in a two-chamber diffusion cell separated by a membrane, we tried to investigate the release and transport of the drug from nanoparticles in such a system, placing the nanoparticles in the donor chamber and measuring the appearance of the drug in the receiver chamber. The appearance curves with some nanoparticle and control preparations are shown in Fig. 2. Similar results were obtained with a wide variety of other samples, which were produced as described by Harmia et al. (1986a and b).

Two main observations can be made. (1) The drug transport in this model system occurs over a time period that is much too slow to have any

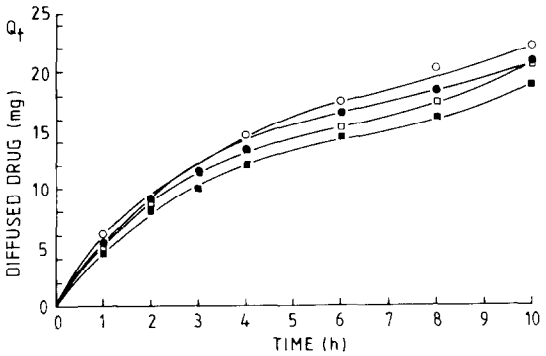

Fig. 2. In vitro drug release from a 1% pilocarpine solution (□); 1% pilocarpine solution containing 0.8% Pluronic F68 (●); adsorbate no. I (■); and nanoparticles with incorporated pilocarpine = preparation 1 (○).

significance for the times involved in ocular absorption. (2) No major differences in the rate constants between aqueous drug solutions and the different nanoparticle preparations were observed. In addition, no clear trends in the rate constants involving dependence on the preparation or on the additives used could be observed. A more hydrophobic membrane also did not improve our results. For this reason, we concluded that with the present membranes the above model is not useful for in vitro release and transport studies of pilocarpine nanoparticles.

In vivo studies

A prolonged pilocarpine induced myosis was achieved with the adsorbates compared to a commercial pilocarpine solution (Figs. 3, 4). Table 2 shows the maximum of the relative myotic response and the relative areas under the relative response versus time curve (AUC) of the adsorbates and of a control solution. The maximum

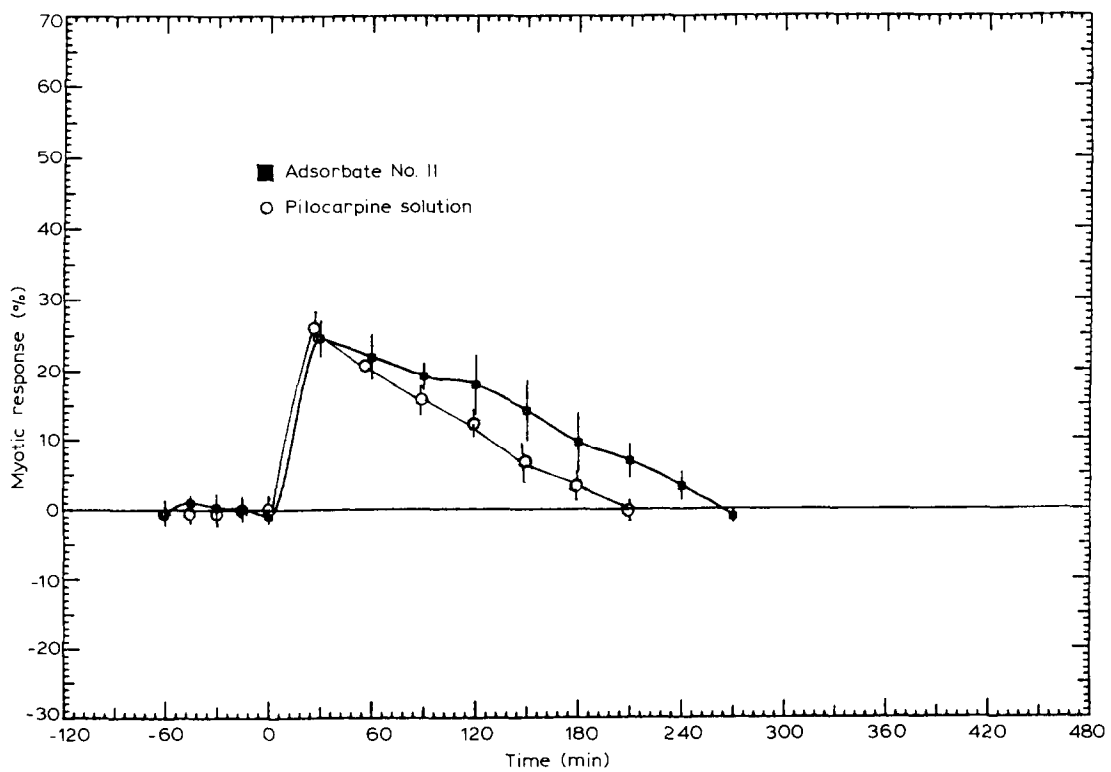

Fig. 3. The myotic response of a pilocarpine solution and adsorbate no. II.

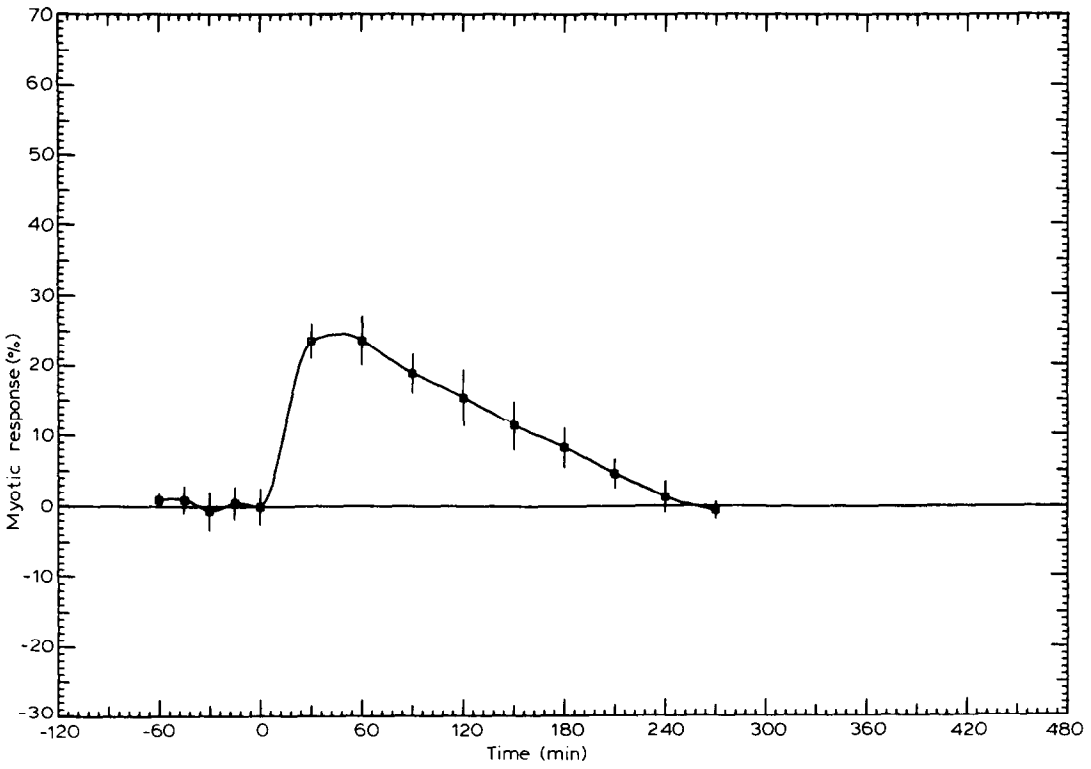

Fig. 4. The myotic response of adsorbate no. I.

relative response R (%) was calculated using Eqn. 1:

$$R = \frac{D_o - D_t}{D_o} \cdot 100 \quad (1)$$

TABLE 2

MYOTIC RESPONSE AFTER INSTILLATION OF PILOCARPINE ADSORBED TO NANOPARTICLES

	Preparation		
	I	II	Pilocarpine solution
Pilocarpine concentration	2%	6%	2%
Max. of myotic response	23.4%	23.9%	25.7%
AUC (myotic response vs time curve)	52.7	53.0	43.1
t_{max} (min)	30	30	30

where D_o is the average pupil diameter before administration and D_t is the pupil diameter after time t after administration of the pilocarpine preparation. The maximal effect was observed within 1 h, and no detectable pilocarpine action was present 5 h after instillation.

No remarkable prolongation of the myotic effect was observed with the nanoparticles with incorporated pilocarpine (Fig. 5). The maximum of the myotic response and the relative areas under the curve (AUC) of the preparations tested are shown in Table 3.

The adsorbates yielded a longer duration of the myotic effect (Figs. 3, 4) as well as a larger area of the myotic effect versus time curve (Table 2). They seem to be able to deliver the drug to the eye at a rate that favours drug absorption in comparison to the nanoparticles with incorporated drug. Possibly the pilocarpine adsorbates form a depot close to the tissues of the eye, whereby the drug may

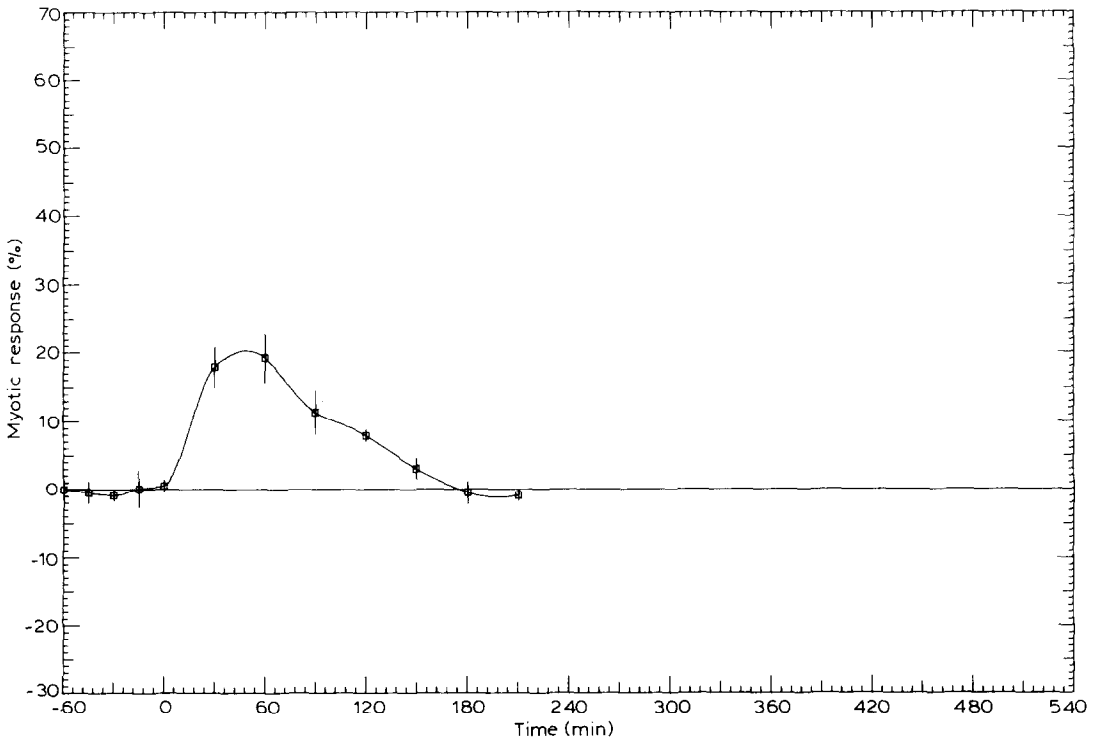

Fig. 5. The myotic response of preparation no. 3.

directly interact with the absorbing membranes of the eye. The release from the incorporation product may be too slow in that the nanoparticles are already eliminated from the precorneal area before sufficient drug absorption took place. The improvement of efficacy observed with the polybutylcyanoacrylate nanoparticles is comparable to other latex systems (Gurny, 1981; Gurny et al., 1985). Because of the possibility of altering the density, composition, and size of the nanoparticles and to increase the viscosity of the medium, polycyanoacrylate nanoparticles or microspheres hold promise as ocular delivery systems for pilocarpine.

Acknowledgements

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TABLE 3

MYOTIC RESPONSE AFTER INSTILLATION OF PILOCARPINE INCORPORATED INTO NANOPARTICLES

	Preparation			
	2	3	4	5
Pilocarpine concentration	1%	1%	1%	1.5%
Max. of myotic response	17.4%	19.2%	19.7%	14.6%
AUC (myotic response vs time curve)	19.2	29.5	26.3	13.3
t_{max} (min)	30	60	30	30

References

Chien, T.W. and Lambert, H.J., Controlled drug release from polymeric delivery devices II: Differentiation between partition-controlled and matrix-controlled drug release mechanisms. *J. Pharm. Sci.*, 63 (1974) 515–519.

Couvreur, P., Tulkens, P., Roland, M., Trouet, A. and Speiser, P., Nanocapsules: a new type of lysosomotropic carrier. *FEBS Lett.*, 84 (1977) 323–326.

Couvreur, P., Kante, B., Roland, M., Guiot, P., Baudhuin, P. and Speiser, P., Polycyanoacrylate nanocapsules as potential lysosomotropic carrier: preparation, morphological and biochemical properties. *J. Pharm. Pharmacol.*, 31 (1979) 331–332.

Gibbs, J.S. and Tuckermann, M.M., Optimal ferric hydroxamate method for determination of intact pilocarpine. *J. Pharm. Sci.*, 59 (1970) 395–396.

Gurny, R., Preliminary study of prolonged acting drug delivery systems for the treatment of glaucoma. *Pharm. Acta Helv.*, 56 (1981) 130–132.

Gurny, R. and Kloeti, W., A new device for measuring the miotic response to drugs. I. Preliminary communication. *Pharm. Acta Helv.*, 57 (1982) 274–275.

Gurny, R., Boye, T. and Ibraim, H., Ocular therapy with nanoparticulate systems for controlled drug delivery. *J. Controlled Release*, 2 (1985) 353–361.

Harmia, T., Nanopartikel als Trägersystem für Augenarzneien. Diss. ETH Nr. 7472 (1984) Zürich.

Harmia, T., Kreuter, J. and Speiser, P., Optimization of pilocarpine loading on to nanoparticles by sorption procedures. subm. for public. *Int. J. Pharm.*, 33 (1986a) 45–54.

Harmia, T., Kreuter, J. and Speiser, P., A solid colloidal drug delivery system for the eye: encapsulation of pilocarpin in nanoparticles. *J. Microencapsulation*, 3 (1986b) 3–12.

Juslin, M., Urtti, A. and Salminen L., Deliverial and pharmacokinetic aspects of ocular drug therapy. *Acta Pharm. Fenn.*, 90 (1981) 289–301.

Kreuter, J., Evaluation of nanoparticles as drug-delivery system. I: Preparation methods. *Pharm. Acta Helv.*, 58 (1983) 196–209.

Kreuter, J., Higuchi, W.I., Ganesan, M.G. and Weiner, N.D., Delivery of liposome membrane-associated sterols through silastic membranes. *Biochim. Biophys. Acta*, 676 (1981) 118–121.

Kreuter, J., Mills, S.N., Davis, S.S. and Wilson, C.G., Polybutylcyanoacrylate nanoparticles for the delivery of [^{75}Se]norcholesterol. *Int. J. Pharm.*, 16 (1983) 105–113.

Lenaerts, V., Couvreur, P., Christiaens-Leyh, D., Jorris, E., Roland, M., Rollman, B. and Speiser, P., Degradation of poly(isobutylcyanoacrylate)nanoparticles. *Biomaterials*, 5 (1984) 65–68.

Saettone, M.F., Giannaccini, B., Barattini, F. and Tellini, N., The validity of rabbits for investigations on ophthalmic vehicles: a comparison of four different vehicles containing tropicamid in humans and rabbits. *Pharm. Acta Helv.*, 57 (1982) 47–55.

Smolen, V.F., Non invasive pharmacodynamic and bioelectrometric methods for elucidating the bioavailability mechanisms of ophthalmic drug preparations. In *Progress in Drug Research, Vol. 25*, Birkhäuser Verlag, Basel (1981) 421–460.

Vezin, W.R. and Florence, A.T., Diffusive desorption of small solute molecules from amorphous polymers: poly(methyl methacrylate), poly(vinylacetate) and poly(*n*-alkyl-2-cyanoacrylates). *Eur. Polymer J.*, 17 (1981) 93–99.