International Journal of Pharmaceutics, 57 (1989) 163–168
Elsevier

IJP 01939

Gelrite®: A novel, ion-activated, in-situ gelling polymer for ophthalmic vehicles. Effect on bioavailability of timolol

A. Rozier, C. Mazuel, J. Grove and B. Plazonnet

Centre de Recherche, Laboratoires MSD-Chibret, Route de Marsat, 63200 Riom (France)

(Received 4 May 1989)
(Accepted 29 June 1989)

Key words: Timolol maleate; Gelrite; Phase transition system; In-situ gelling vehicle; Hydroxyethylcellulose; Ocular bioavailability; Gellan gum

Summary

Gelrite® solution, a novel ophthalmic vehicle, gels in the presence of mono or divalent cations. In the conjunctival sac 'ion-activation' of the sol/gel transition is accomplished by the lacrimal fluid. A 0.6% Gelrite® vehicle has been compared to an equiviscous solution of hydroxyethylcellulose (HEC) using timolol maleate as a drug probe. In vitro release rates of timolol from HEC and Gelrite® gel were similar. In vivo, the formation of the gel prolonged precorneal residence time and increased ocular bioavailability of timolol in the cornea, aqueous humor and iris + ciliary body of albino rabbits.

Introduction

Upon instillation of an ophthalmic solution, most of the instilled volume is eliminated from the precorneal area (Chrai et al., 1974; Zaki et al., 1986). This loss is mainly due to drainage of the excess fluid by the naso-lacrimal duct and dilution and elimination of the solution by tear turn-over. This results in a poor bioavailability of topically applied ophthalmic drugs.

Various ways to reduce the effect of drainage and the dilution of the instillate by the tears have been explored. They include the use of viscous and semi-solid vehicles (Zaki et al., 1986; Saettone et al., 1986). The corneal contact time has been increased to varying degrees by these preparations, but so far, no marked sustaining effect has been attained. Extended corneal contact times, as well as sustained release, have been obtained with solid forms such as the Ocusert® (Shell and Baker, 1974). However, poor patient acceptance and difficulties in administration have led ophthalmic researchers to seek other systems which would combine the ease of administration of liquid forms with the prolonged residence time of inserts.

Two leads that are currently being explored are bioadhesive and phase transition systems as follows:

(1) Bioadhesive systems can be either polymeric solutions (Gurny et al., 1987) or micro-particle suspensions (Hui and Robinson, 1985). They are retained in the cul-de-sac by adhesive bonds established with the mucins or the epithelium.

(2) Phase transition systems are instilled in a liquid form and shift to the gel or solid phase once in the cul-de-sac. Two such systems have been

Correspondence: A. Rozier, Laboratoires MSD-Chibret, Route de Marsat, 63200 Riom, France.

reported. Poloxamer 407 solution viscosity increases when its temperature is raised to the eye temperature (Miller and Donovan, 1982) and cellulose acetophthalate (CAP) latex (Gurny et al., 1985) coagulates when its native pH of 4.5 is raised by the tear fluid to pH 7.4. Both systems are characterized by a high polymer concentration (25% Poloxamer, 30% CAP). In addition, the surfactive properties of the former and the low pH of the latter may be detrimental to ocular tolerance.

A new phase transition system, Gelrite® solution, has been evaluated in our laboratory (Mazuel and Friteyre, 1987). Gelrite® is a polysaccharide, low-acetyl gellan gum, which forms clear gels in the presence of mono or divalent cations (Moorhouse et al., 1981). The ion concentration necessary to induce the gelation varies with the cation. The concentration of sodium in tears, 2.6 g/l (Milder, 1981), was particularly suited to cause gelation of the material when topically instilled into the conjunctival sac. The present study describes our investigations of the gelling properties of Gelrite® solutions and the evaluation of their influence on ocular drug bioavailability using timolol maleate.

Materials and Methods

Gelrite® (Kelco, Division of Merck, U.S.A.), hydroxyethylcellulose (HEC QP52000H) (Union Carbide, U.S.A.) and timolol maleate (Merck, U.S.A.) were used as received. Other formulation excipients were pharmaceutical grade and obtained from standard commercial suppliers.

Preparation of the formulations

(A) Gelrite® solution. 0.34% timolol maleate equivalent to 0.25% free base and 0.6% Gelrite® were dissolved in a 0.01 M Tris maleate buffer (pH 7.0). In order to keep the ion content below the gelation concentration, mannitol was used to achieve an isotonic solution. Sterilization was accomplished by terminal autoclaving.

(B) Hydroxyethylcellulose solution. A solution was prepared containing 0.34% timolol maleate, 0.5% HEC and the same Tris maleate and isotonizing agent as above. Sterilization was accomplished by autoclaving a 1% HEC solution and mixing it with a sterile filtered solution of the other ingredients.

(C) Reference solutions for diffusion studies. A solution was prepared containing 0.34% timolol maleate and the same Tris-maleate and isotonizing agent as above. Sterilization was accomplished by filtration through a 0.22 μm filter.

Viscosity

Measurements were made using a Rotovisco RV12 viscosimeter (Haake & Co.) equipped with an NV sensor system thermostated at $20 \pm 1°C$. A two-way recorder was attached for determinations of shear rate and shear stress. The shear rate was increased from 0 to 216 s^{-1} in 9.9 min and maintained at this level for 0.1 min. The viscosity was determined from the flow curve obtained at different values of the shear rate.

Sol-gel transition

The apparatus described above was employed with the two-way recorder connected to the viscosimeter for the shear stress measurements and to a digital thermometer for the measurement of the sample temperature. 7.8 g of solution A was mixed with 2.2 g of simulated tear fluid (Table 1). Other ratios (8.8 : 1.2, 7.2 : 2.8 and 4.4 : 5.6) were also tested. The mixture was heated to boiling, cooled to 50 °C and poured into the measuring cup pre-heated to 50 °C. The temperature was then decreased at a rate of 1.5 °C/min to 30 °C and the shear stress at 8 rpm measured during the whole process. The temperature at which a sharp break in shear stress occurred was taken as the sol-gel transition temperature (see Fig. 1).

In vitro diffusion test

Diffusion cells similar to those already described (Bottari et al., 1974) for the analysis of

TABLE 1

Simulated tear fluid composition

NaCl	0.67 g
$NaHCO_3$	0.20 g
$CaCl_2 \cdot 2H_2O$	0.008 g
H_2O qs	to 100 g

Fig. 1. Sol-gel transition temperature (see text for explanation). *x*-axis, *t* (°C); *y*-axis, stear stress (scale: 1 cm = 0.6 Pa).

semi-solid formulations have been used. The diffusion chamber is a 3.0 ml cylindrical reservoir, 32 mm in diameter, located in the cell bottom compartment (diffusion surface 8.04 cm^2).

Each cell was immersed in the flask of a USP rotating paddles apparatus II (model 72SL, Hanson Research, U.S.A.). For comparative studies of diffusion rates between Gelrite® and HEC formulations, the cells were covered with a cellophane membrane (PD 215, Dupont de Nemours) rinsed in acetone and soaked for 24 h in the diffusion medium. The formulations were also tested without membrane. In the latter case, the presence of a 30-mesh 316L stainless-steel screen cover was necessary to avoid disturbing the diffusion surface during the cell immersion and to hold the gel sample in place. The diffusion medium was 650 ml of simulated tear fluid (Table 1), prepared daily, degassed and equilibrated at 37 ± 0.5 °C. The paddle rotating speed was set at 50 rpm. Aliquots of medium were withdrawn at selected times and read directly, without dilution, by UV spectrophotometry at 295 nm.

Intraocular penetration

Bilateral instillation of 50 µl of the test solutions was made into the conjunctival sac of conscious albino rabbits. Groups of six animals were then killed at 10 min, 0.5, 1, 2 and 4 h post-instillation and timolol subsequently assayed in cornea, aqueous humor and iris + ciliary body by HPLC.

Results

Viscosity

Both the viscosity curves obtained for Gelrite® and HEC solutions (Fig. 2) are typical of pseudoplastic behavior. The curves are not superimposable, as expected with solutions of different polymers. However, they are comparable and would be expected to hinder drainage from the cul-de-sac to the same extent.

Sol-gel transition

In the present test, the gel forms after cooling of a hot solution of Gelrite® and cations. This is different from the in-vivo process where gelation occurs after diffusion of cations into Gelrite®. However, gel will form by neither mechanism if

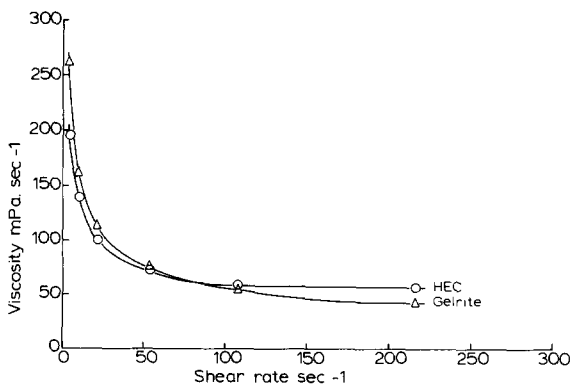

Fig. 2. Viscosity curves of Gelrite and HEC solutions.

TABLE 2

Sol-gel transition temperatures

Gelrite/simulated tears (g)	Corresponding ratio in μl	Transition temperature (°C)
8.8:1.2	50:7	36.0
7.8:2.2	25:7	38.0
7.2:2.8	25:10	39.0
4.4:5.6	25:32	42.5

the temperature of the solution exceeds that at which the sol-gel transition occurs. Because the sol-gel transition is sought in the precorneal area, the transition temperature of the system must be higher than 34°C, the surface temperature of the eye. This must be achieved for solution to tear fluid ratios of 8.8:1.2 (50 μl drop to 7 μl tear pool) or 7.8:2.2 (25 μl drop). Temperatures reported for different ratios in Table 2 are over 34°C, demonstrating that physiological conditions are suitable to the gelation process. Furthermore, they increase with increasing fraction of tear fluid, ensuring that the gel formed upon instillation will not liquify because of tear turn-over.

In vitro release

With dialysis membrane. When compared to the reference solution, timolol release is retarded at the early time points by the two viscous preparations (Fig. 3). Release from Gelrite® and HEC preparations is comparable, indicating that the

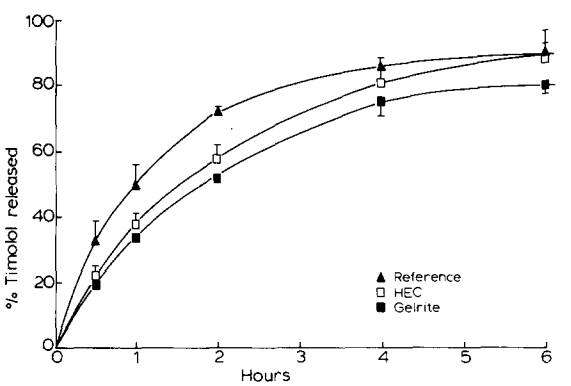

Fig. 3. In vitro diffusion test of timolol using cellophane membrane covered cells.

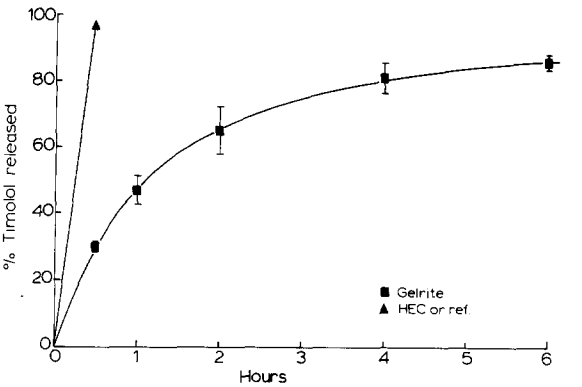

Fig. 4. In vitro diffusion test of timolol using screen covered cells.

interaction of the drug is the same with both polymers.

With stainless-steel screen. All timolol is released from the reference or HEC solution at 30 min (Fig. 4). The 30-mesh screen is not an effective barrier and allows the free passage of these samples and a complete mix with the diffusion medium. On the other hand, the surface of the Gelrite® samples gels as soon as it contacts the diffusion medium, preventing any escape of the bulk and entrapping the drug. The drug release pattern obtained for the gelled samples (Fig. 4) is characteristic of those already described for hydrophilic matrices (Shell and Baker, 1974). It is very rapid in the beginning and proceeds at a rate that declines with time. It is faster than with the dialysis membrane and no doubt reflects the in

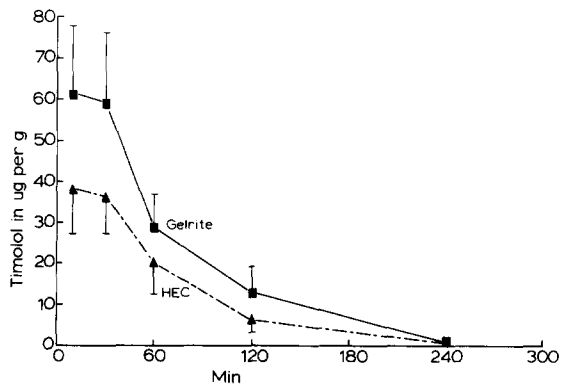

Fig. 5. Corneal concentration of timolol after instillation of 0.25% in Gelrite or HEC.

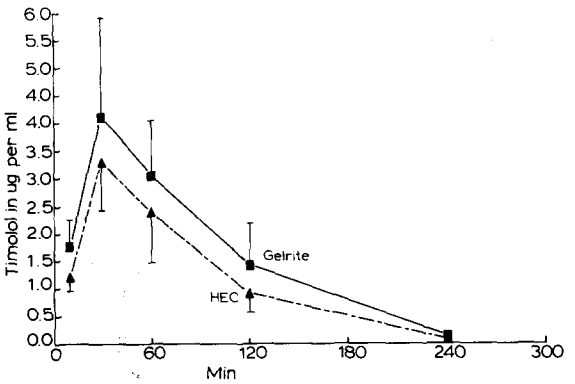

Fig. 6. Concentration of timolol in aqueous humor after instillation of 0.25% in Gelrite or HEC.

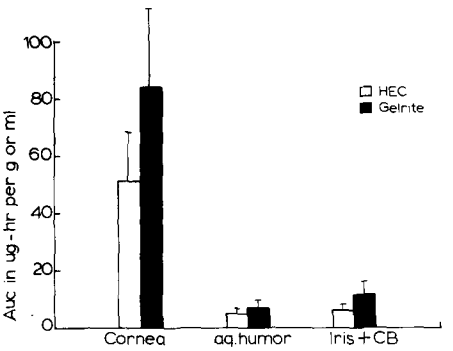

Fig. 8. AUC of timolol concentration vs. time plot after instillation of 0.25% in Gelrite or HEC.

vivo situation better than when a membrane is present to limit the diffusion rate. Nonetheless, a higher release rate should be expected in vivo, as the leaching action of the tears will tend to speed up the release process.

Intraocular penetration study

The corneal content of timolol (Fig. 5) was significantly higher (Student's t-test, $p \leq 0.05$) at all time points after administration of the Gelrite® formulation. Consequently, mean timolol concentrations in aqueous humor and iris + ciliary body were always higher with Gelrite® than with HEC (Figs. 6 and 7). These differences were significant ($p \leq 0.05$) at 10 min in the aqueous humor and at all time points in the iris + ciliary body. When AUC (0–4 h) was calculated for the various concentration/time profiles, ocular bioavailability increased in the cornea, aqueous humor and iris + ciliary body by 1.64-, 1.36- and 1.87-fold, respectively, for the Gelrite® vs the HEC formulation (Fig. 8).

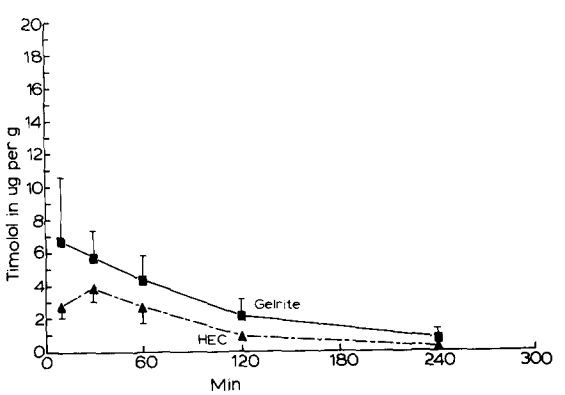

Fig. 7. Concentration of timolol in iris+ciliary body after instillation of 0.25% in Gelrite or HEC.

Discussion

Both HEC and Gelrite® solutions exhibit pseudoplastic flow, with comparable viscosities at different shear rates. The environment of the conjunctival sac and the ionic content of the lacrimal fluid favors the phase transition of the Gelrite® solution to the solid form. Drainage from the pre-corneal area would therefore be much more reduced in the case of Gelrite® than for HEC.

In vitro release studies have shown that timolol diffuses from Gelrite® at the same rate as from HEC formulations. This results in similar in vivo timolol concentration/time profiles of the two solutions; time to C_{max} and elimination half-life being of the same order for cornea, aqueous humor and iris + ciliary body. The enhancement of all concentrations with the Gelrite® formulation, at each of the three ocular sites examined, can be best explained by the longer residence time in the conjunctival sac. Evidence in support of an increased contact time was obtained by visual observation, but more recently gamma scintigraphy studies have suggested that the Gelrite® vehicle can, in certain animals, still be present at 45 min

following instillation (Wilson, C., personal communication).

Prospects for this new excipient are bright. It has an excellent ocular tolerance, low toxicity per os and it can be formulated as an isotonic neutral solution, all this making it a safe excipient for the ocular route. It presents the practical advantage over other polymers of withstanding sterilization by autoclaving. Above all, it has been shown by its unique ion-activated gelation to increase ocular drug bioavailability. In order to optimize its potential, further investigations are being directed to control and to prolong the release of different drugs from the gelled matrix.

References

Bottari F., DiColo G., Nannipieri E., Saettone M.F. and Serafini M.F., Influence of drug concentration on in-vitro release of salicylic acid from ointment bases. *J. Pharm. Sci.*, 63 (1974) 1779–1783.

Chrai S.S., Makoid M.C., Erikson S.P. and Robinson J.R., Drop size and initial dosing frequency problems of topically applied ophthalmic drugs. *J. Pharm. Sci.*, 63 (1974) 333–338.

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

Gurny R., Ibrahim H., Aebi A., Buri P., Wilson C.G., Washington N., Edman P. and Camber O., Design and evaluation of controlled release systems for the eye. *J. Contr. Release*, 6 (1987) 367–373.

Hui H.W. and Robinson J.R., Ocular delivery of progesterone using a bioadhesive polymer. *Int. J. Pharm.*, 26 (1985) 203–213.

Mazuel C. and Friteyre M.C., Composition pharmaceutique de type à transition de phase liquide-gel. *French Patent Application FR.2588189 A1* date 87-04-10. Filing Fr. 8514689 date 85-10-03.

Milder B., The lacrimal apparatus. In R.A. Mooses (Ed.), *Adler's Physiology of the Eye*, C.V. Mosby Co., London, 1981, pp. 16–37.

Miller S.C. and Donovan M.D., Effect of Poloxamer 407 gel in the miotic activity of pilocarpine nitrate in rabbits. *Int. J. Pharm.*, 12 (1982) 147–152.

Moorhouse R., Colegrove G.T., Sandford P.A., Baird J.K. and Kang K.S., PS-60: a new gel-forming polysaccharide. In D.A. Brandt (Ed.), *Solution Properties of Polysaccharides*, Washington, DC, 1981, pp. 111–124.

Saettone M.F., Giannaccini B., Guiducci A. and Savigni P., Semi-solid ophthalmic vehicles. III. An evaluation of four organic hydrogels containing Pilocarpine. *Int. J. Pharm.*, 31 (1986) 261–270.

Shell J.W. and Baker R.W., Diffusional systems for controlled release of drugs to the eye. *Ann. Ophthalmol.*, 6 (1974) 1037–1047.

Zaki I., Fitzgerald P., Hardy J.G. and Wilson C.G., A comparison of the effect of viscosity on the precorneal residence of solutions in rabbits and man. *J. Pharm. Pharmacol.*, 38 (1986) 463–466.