[Chem. Pharm. Bull. 33(3)1242—1248(1985)]

Controlled Release of Pilocarpine Hydrochloride from Ethylene-Vinyl Alcohol Copolymer Matrices[1)]

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(Received September 13, 1984)

An ethylene-vinyl alcohol (EVAl) copolymer was evaluated as a new carrier for a long-acting delivery system of pilocarpine–HCl. The release characteristics of pilocarpine-EVAl copolymer matrix (film or beads) systems were investigated in *in vitro* and *in vivo* test models. The results of the *in vitro* study suggested that the drug release rate could be easily controlled by modifying the proportions of ethylene and vinyl alcohol in the copolymer. Sustained release can be obtained by using EVAl copolymer containing more ethylene. The results of the *in vivo* study indicated that the use of the pilocarpine-EVAl copolymer bead system is more effective than that of the conventional liquid dosage form for prolonging the duration of a desired pupillary response. Its biocompatibility, flexibility, and heat processability suggest the EVAl copolymer to be a good candidate for pilocarpine delivery into the eye.

Keywords——ethylene-vinyl alcohol copolymer; pilocarpine–HCl; drug delivery; controlled release; sustained release; matrix system; film form; beads form; pupillary response; rabbit

The usefulness of a biomedical polymer, ethylene-vinyl alcohol (EVAl) copolymer, as a new carrier for drug delivery has been examined.[2,3)] The EVAl copolymer has a hydrophilic character and good mechanical properties.[4)] The safety and biocompatibility of the copolymer are reflected in its use as hemodialysis membrane.[5)] The EVAl copolymer can be used to provide controlled release of hydrophilic drugs because of its hydrophilic character.[6)]

In previous studies,[7,8)] the EVAl copolymer was evaluated as a carrier for controlled release of a potent anticancer agent, 5-fluorouracil (5-FU). It was demonstrated that the release rate of 5-FU could be easily controlled by modifying the ethylene/vinyl alcohol ratio in the copolymer matrices.[7)] An *in vivo* study also indicated that implantation of EVAl copolymer matrices containing 5-FU may be effective in cancer chemotherapy.[8)]

Many attempts have been made to modify the response to drugs which are delivered topically to the eye. In the development of a controlled-release drug delivery system for ophthalmological applications, the use of the Ocusert® system based on ethylene-vinyl acetate (EVAc) copolymer has been reported to prolong the action of pilocarpine in the eye.[9)] However, disadvantages reported by patients included problems of insertion or retention, leakage, and discomfort.[10)] The Ocusert® is also more expensive than eye drops. In order to circumvent some of these problems, a variety of polymer membranes have been employed as rate-controlling barriers.[11)] However, it is still desirable to develop less costly, more convenient and more comfortable systems. We previously reported the entrapment of pilocarpine in a fibrin film and suggested this to be a good candidate system for pilocarpine delivery in the eye.[12)]

In the present study, the possible use of EVAl copolymer as a new carrier for controlled release of pilocarpine hydrochloride (pilocarpine–HCl) was examined. *In vitro* release of pilocarpine–HCl dispersed in copolymer matrices composed of different ratios of ethylene and vinyl alcohol was investigated. The effect of EVAl copolymer matrices containing the drug on the pupil size in the rabbit eye was also studied.

Experimental

Materials——EVAl copolymers ranging from 15 to 81 mol% of ethylene content were gifts from Kuraray Co. They were prepared from EVAc copolymer solution by saponification.[13] Pilocarpine–HCl was obtained from Wako Pure Chemical Industries.

Preparation of Pilocarpine-EVAl Films——Weighed amounts of pilocarpine–HCl and EVAl copolymers were mixed and ground thoroughly in a mortar. Then, this mixture was passed through a 48 mesh sieve. The EVAl copolymer films containing the drug were prepared by melting the fine mixture at 160—180 °C under 500 kg/cm^2 pressure for 1 min between two polyester films.

The thickness of the films, determined by using a micrometer (Mitsutoyo 102-230), was 0.30—0.32 mm. The films used in the *in vitro* study were 1 × 1 cm squares. The drug content was calculated from the weight ratio of drug and copolymer used.

Preparation of Pilocarpine-EVAl Beads——Pilocarpine-EVAl film with 2.3 mm thickness was prepared according to the above-mentioned method. Two kinds of small squares, approximately 10 and 20 mg in weight, were obtained by cutting the 2.3 mm film with a razor blade. Spherical beads, 2.2 and 3.2 mm diameter, were prepared by transferring each square in a Teflon molder at 150—160 °C.

***In Vitro* Study**——The drug-EVAl copolymer matrix was placed in 20 ml vials containing 2 ml (beads) or 5 ml (films) of distilled water. The drug release was followed during shaking at a rate of 100 strokes/min on a laboratory shaker at 37 °C. Each film or bead was successively transferred to another vial containing the same volume of fresh water. The amount of the drug released was measured by the colorimetric method.[14] Release studies were done at least in triplicate, and the average values were plotted.

***In Vivo* Study**——Miotic studies were conducted using female albino rabbits weighing 2.8—3.2 kg. Lighting and temperature in the test room were constant throughout the study. A solution (8% (w/v), 25 μl) of pilocarpine–HCl in saline was instilled into the lower cul-de-sac of the left eye of four rabbits with a syringe. EVAl copolymer beads containing 2.0 mg of pilocarpine–HCl were placed in the lower sac of the left eye of another four rabbits. Only one eye of each animal was used, and the other eye served as a control. The size of each pupil was measured with vernier calipers held always at the same distance from the eye, by the same operator.

Results

Controlled Release of Pilocarpine–HCl from EVAl Copolymer Matrix

i) Release from the Film——In order to study the effect of monomer ratio changes on the drug release pattern, the release of pilocarpine–HCl dispersed in matrices composed of different ratios of ethylene and vinyl alcohol was investigated. In this study, both the initial drug content in the matrix (15% (w/w)) and the temperature (37 °C) were held constant, while

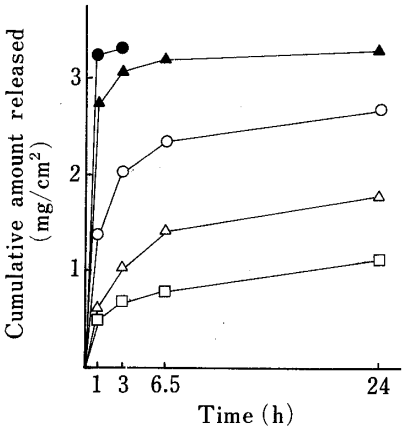

Fig. 1. Effect of Ethylene Content on Pilocarpine–HCl Release from EVAl Copolymer Films at 37 °C

●, 15; ▲, 31; ○, 54; △, 60; □, 81 mol% ethylene. Each preparation contained 15% drug by weight.

the ethylene content of the EVA1 copolymer film was varied (15, 31, 54, 60, and 81 mol%).

Figure 1 shows plots of the data, expressed as the cumulative amount of drug release, *versus* time. It is apparent that an increase in ethylene monomer content decreased the drug release from the copolymer films. The 15 or 31 mol% preparations released the drug very rapidly, and the preparations with more than 54 mol% ethylene content released the drug much more slowly. The total amount of pilocarpine–HCl released during the 24 h test period was 100.0, 94.0, 68.5, and 47.5% of the dose for the films prepared with copolymers containing 31, 54, 60, and 81 mol% ethylene, respectively. Thus, the release rate could be controlled by modifying the ethylene/vinyl alcohol ratio in the copolymer film.

Factors determining the rate of drug release are particularly important in the design and formulation of controlled-release preparations. Thus, other fabrication parameters (initial drug content) that affect the drug release were studied. Matrix films with copolymers having 60 and 81 mol% ethylene contents were used in this study.

The effect of drug content on the release patterns was examined at three concentrations of pilocarpine–HCl (15, 20, and 25% (w/w)). As shown in Fig. 2, the initial drug content of the film affects the drug release; increasing the drug content increases the drug release rate. As much as a 2-fold increase in the cumulative drug release was caused by increasing the drug content from 15 to 25% (w/w) for both 60 mol% ethylene-EVA1 and 81 mol% ethylene-EVA1

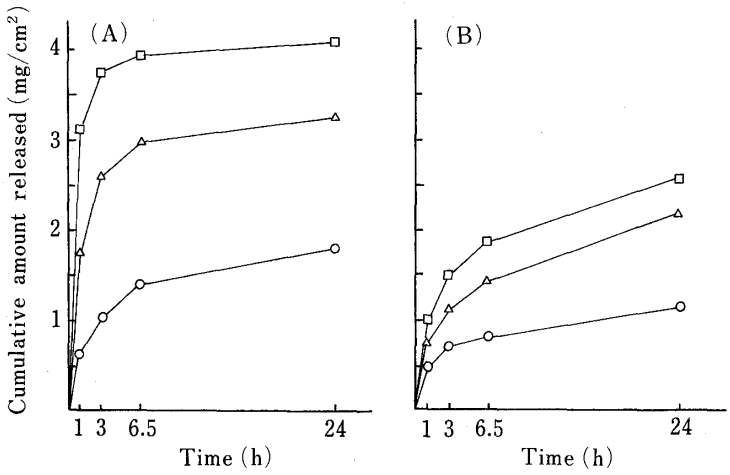

Fig. 2. Effect of Drug Content on Pilocarpine–HCl Release from EVA1 Copolymer Films with 60 (A) and 81 (B) mol% Ethylene Content at 37 °C

○, 15; △, 20; □, 25% (w/w).

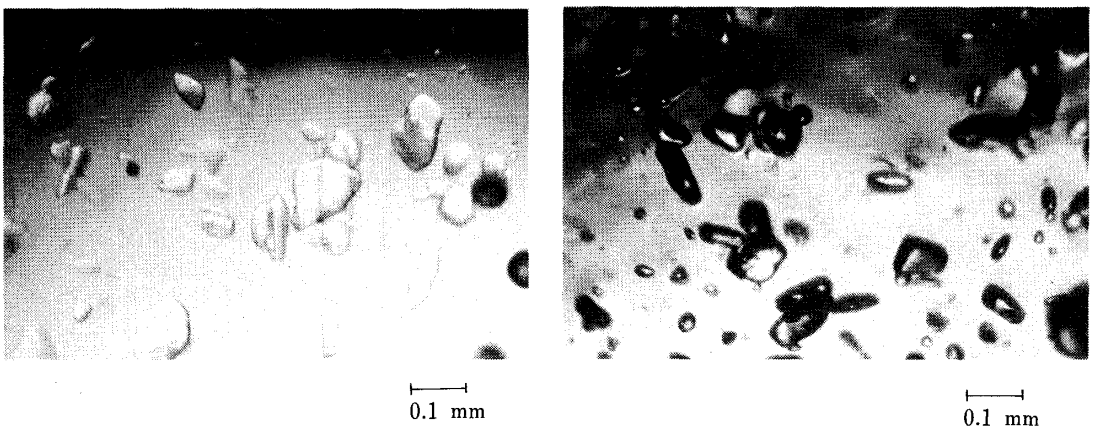

Fig. 3. Micrographs of EVA1 Copolymer Films Containing Pilocarpine–HCl before Release (Left) and after (Right) Release for 24 h (× 100)

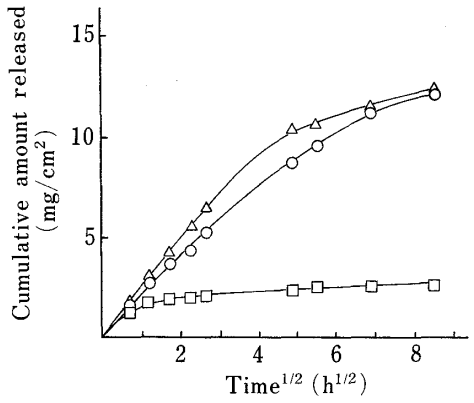

Fig. 4. Effect of Ethylene Content on Pilocarpine–HCl (○) and EVA1 Copolymer Copolymer Beads, 3.2 mm in Diameter, at 37 °C

△, 54; ○, 60; □, 81 mol% ethylene. Each preparation contained 20% drug by weight.

copolymer films.

In order to elucidate the mechanism of drug release from the EVAl copolymer matrices, the surface of the matrix before any release had occurred and after release for 24 h was viewed under a microscope. The EVAl copolymer film used had 60 mol% ethylene content and 20% (w/w) drug content.

Before release, the matrix appeared as transparent, nonporous sheets (Fig. 3-left), and the drug existed as discrete microcrystals within the copolymer matrix. After the drug had been released, the matrix became very porous and holes large enough to permit drug diffusion could be discerned (Fig. 3-right). The EVAl copolymer is hydrophilic in nature, and the matrix film is capable of imbibing adequate quantities of water.[7] The dissolution of the drug crystals in this water may therefore produce holes through which the dissolved drug can diffuse.

ii) Release from the Beads——EVAl copolymer matrices containing pilocarpine–HCl could be clinically used as insert drug delivery devices and placed in the conjunctional cul-de-sac of the lower eyelid. Therefore, it is necessary to ensure that the size, form, and composite structure are suitable for insertion. Spherical beads were also prepared for study of the drug release into aqueous solution.

The dependency of the drug release profile on the ethylene content in the beads is illustrated in Fig. 4. Preparations containing pilocarpine–HCl at 20% of the total weight were formulated into 3.2 mm diameter beads using EVAl copolymers with 54, 60, and 81 mol% ethylene content. The cumulative amount of drug released (Q) was plotted *versus* the square root of time ($t^{1/2}$).[15] After an initial period of rapid release of the drug (*i.e.*, burst effect), the release was approximately linear with respect to $t^{1/2}$. The steady-state rate of drug release (k) was estimated from the slope of the linear $Q-t^{1/2}$ profile during periods from 0.5 to 7 h (54 mol% ethylene) and 1.5 to 30 h (60 and 81 mol% ethylene). The k values for the beads with 54, 60, and 81 mol% ethylene content were 2.52, 1.60, and 0.17 mg/cm^2/h$^{1/2}$, respectively. An increase in ethylene monomer content decreased the drug release rate from the copolymer beads. With this system, beads with various release patterns could be easily obtained by changing the proportions of ethylene and vinyl alcohol monomer.

The effect of the relative concentration of pilocarpine–HCl in the drug/polymer preparation is illustrated in Fig. 5. In this experiment, copolymer beads with 60 mol% ethylene content containing 15, 20, and 25% pilocarpine–HCl by weight were formulated at 2.2 and 3.2 mm diameter. It is evident that the greater the drug concentration, the more rapidly the drug was released.

Miotic Activity of EVAl Copolymer Beads Containing Pilocarpine–HCl in the Rabbit Eye

On the basis of *in vitro* release studies, it was expected that the beads would release the drug more slowly than the films. The effect of the beads containing the drug on the pupil size

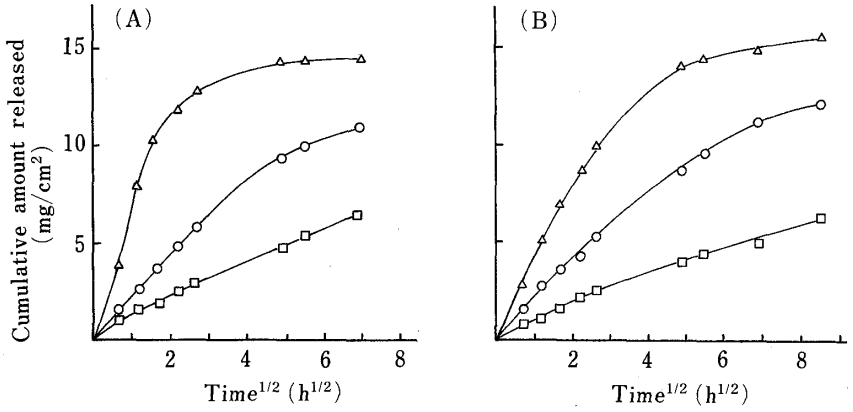

Fig. 5. Effect of Drug Content on Pilocarpine–HCl Release from EVA1 Copolymer Beads, 2.2 mm (A) and 3.2 mm (B) in Diameter, with 60 mol% Ethylene Content at 37 °C

□, 15; ○, 20; △, 25% (w/w).

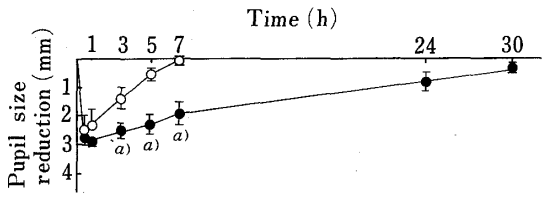

Fig. 6. Miotic Response Curve for Aqueous Pilocarpine–HCl (○) and EVA1 Copolymer Beads with 60 mol% Ethylene Content, 2.2 mm in Diameter, Containing Pilocarpine–HCl (●)

Each point represents the mean of 4 determinations and is shown with the standard error. Each preparation contained 20% (w/w) (2.0 mg) pilocarpine–HCl.
a) Denotes a significant difference at $p < 0.005$ from the liquid dosage form (*t*-test).

of rabbit eye was also studied.

Figure 6 shows typical data obtained in the rabbit *in vivo* system. The EVAl copolymer matrix containing 20% pilocarpine–HCl by weight (2.0 mg) was prepared from 60 mol% ethylene and inserted into the eye in a bead form (2.2 mm diameter). Pupillary responses showed that the duration of pupil size reduction was greatly increased by delivery of pilocarpine–HCl *via* the EVAl copolymer beads as compared with the liquid dosage form. Restoration of normal pupillary diameter in the case of the ophthalmic solution was observed to occur between 5 and 7 h, in contrast to more than 30 h for the EVAl-drug bead system. No inflammation was seen in the treated eyes and no pupillary constriction was noted in the control eyes. The copolymer beads did not slip out of place for at least 5 d.

Discussion

The concept of an intraocular drug delivery system was recently realized in the form of the pilocarpine-releasing Ocusert® system, based on EVAc copolymers, for glaucoma treatment.[9,10] In recent years, a number of publications have described the use of soft contact lenses (hydrogels) or similar matrices presoaked in dilute pilocarpine solutions to prolong therapeutic actions.[16–19] A hydrogel is defined as polymeric material that is capable of imbibing large quantities of water (>20%) without dissolving, and thus shows good biocompatibility.[20] However, such approaches produce release rates that decline rapidly, *e.g.*, 90% of the drug is released within 1/2 h.

The EVAl copolymer prepared from EVAc copolymer is nontoxic, flexible, and heat-processable. The unique characteristic of this copolymer, in contrast to hydrophobic EVAc copolymer, is its hydrophilicity.[6] Since it is capable of imbibing an adequate amount of water,[7] it should be well adaptable to the eye. Our biocompatibility study of inserts made of

this copolymer showed no gross adverse effects in the rabbit eye after a period of 2 weeks. The feasibility of using EVAl copolymers as a polymer matrix for the controlled release of pilocarpine–HCl has been confirmed in this work.

The variation of initial drug content and, to an even greater extent, the variation in monomer ratio, affects the drug release rate from both films (Figs. 1 and 2) and beads (Figs. 4 and 5). An increase in the ethylene content of the copolymer decreases the drug release rate. The results of the *in vitro* study suggest that EVAl copolymers can be used as a controlling membrane for the release of pilocarpine–HCl. The rate of release can be easily adjusted by using EVAl copolymers having different ethylene content and drug content.

The use of the pilocarpine–EVAl copolymer beads was more effective than that of the conventional liquid dosage form for prolonging the duration of a desirable pupillary response (Fig. 6). This result indicates that sustained drug release does occur in the eye and that effective drug concentrations may be maintained by insertion of the EVAl beads. The pilocarpine–EVAl bead systems as tested *in vitro* did release the drug in a manner consistent with observed prolonged biological activity *in vivo*. Its biocompatibility, flexibility, and heat processability suggest the EVAl copolymer to be a good candidate for pilocarpine delivery into the eye.

Most ocular inserts have been designed in the form of a film, but Abraham[11,21] proposed a capsule having a semipermeable shell and containing a medicament which is gradually released through the shell. The diameter of such a capsule should be sufficiently small that there is no irritation or discomfort when the capsule is situated in the conjunctival sac of the eye. A diameter of less than about 1 mm is preferred (although in this work we used beads of 2.2 mm diameter). The method has the merits of simplicity, non-irritability, ease of insertion and removal of the small beads, and the fact that the beads are resistant to expulsion by movement of the eye for at least 5 d. Thus, for short-term delivery (up to 5 d) small spherical beads may be a practical dosage form for humans.

ALZA's Ocusert®, which is a membrane control device that is placed in the cul-de-sac of the eye, delivers pilocarpine at a precisely controlled rate for the treatment of glaucoma. Of course, linear $t^{1/2}$ diffusional delivery systems are not ideal, and a "zero order" delivery would be better. We are attempting to prepare membrane-controlled systems with zero-order kinetics based on EVAl copolymers.

Acknowledgement This work was supported by the Japan International Cooperation Agency (JICA). The authors are grateful to Mr. Shigeyuki Harita of Kuraray Co. for supplying ethylene-vinyl alcohol copolymers. We are also indebted to Miss Chizuko Yokouchi and Mr. Norio Hashiguchi of Higashi-Nippon-Gakuen University for their technical assistance.

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