The active ingredient loss was only significant after three passes through the hammer mill.

Comparison of Fig. 1 with Fig. 4 shows that if the materials could be purchased in a fine form, it would probably be more economical to mix the preground fine material instead of grinding and mixing the coarser powders. The ordered mixture formation rate is slow when fine–fine components are mixed in a revolvo-cube mixer. With hammer mill mixture grinding, new surfaces are created by fracture of the coarse crystals. This procedure provides more area for drug particle adhesion. Furthermore, aggregates are broken down more rapidly in the hammer mill to facilitate mixing. Nevertheless, the cost of longer mixing time in the revolvo-cube mixer for fine–fine mixtures will be compensated by less handling of the mix than when the hammer mill is used.

Figure 4 also shows the mixing of a fine–coarse system in a revolvo-cube mixer where the desired degree of homogeneity is achieved after 10 min and the ordered mixture is stable and not prone to segregation (8). Even though homogeneity increases for fine particles, other problems such as fine powder flow (10) and storage effects (11) cannot be overlooked.

The sample size effect on the standard deviation for Preblend 3 is shown in Fig. 5. For a randomized mixture, the slope of the line will be −0.5. For an ordered mixture, it will approach zero. The 95% confidence limits for the slope were −0.554–−0.166. The mixture could not be classified as either completely randomized or completely ordered. In fact, it may have been a mixture of both systems, a description that may satisfy most powder mixtures used in pharmaceutical practice. A system with a large particle-size range is unlikely to be fully randomized due to size segregation. In practice, some degree of adhesional-type ordered mixture (12) may be formed. Due to the size differences of carrier particles, they will undergo ordered unit segregation (13, 14). Further mixing of this system will produce the randomized ordered unit powder mixture.

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Vehicle Effects on Ocular Drug Bioavailability III: Shear-Facilitated Pilocarpine Release from Ointments

JAMES W. SIEG * and JOSEPH R. ROBINSON ˣ

Received June 5, 1978, from the *School of Pharmacy, University of Wisconsin, Madison, WI 53706.* Accepted for publication November 29, 1978. *Present address: College of Pharmacy, University of Kentucky, Lexington, KY 40506.

Abstract □ Pilocarpine release from water-in-oil emulsion ointments was studied *in vitro* and *in vivo*, using albino rabbits. Pilocarpine release from the vehicle to the ocular fluids was dependent on shear, *i.e.*, blinking, and the dosing system emulsifying efficiency. A mechanical shearing component was vital for correlating corneal drug penetration and the *in vitro* pilocarpine release pattern. Simple diffusion studies with the vehicles did not predict drug *in vivo* release, but the ointment systems were all superior to an aqueous pilocarpine solution. Incorporation of a mechanical shearing component to mimic blinking gave good correlation of *in vitro* and *in vivo* results. Also, increasing the vehicle emulsifying efficiency by surfactant addition decreased shear-facilitated drug release and *in vivo* performance. Finally, increasing the internal aqueous phase volume fraction decreased *in vivo* performance and was linked to the influence of effective drug concentration in the vehicle.

Keyphrases □ Dosage forms, ocular—pilocarpine, shear-facilitated release, ointments □ Pilocarpine—release, shear facilitated, ocular ointments □ Shear—facilitation of pilocarpine release from ocular ointments □ Ointments—pilocarpine, shear-facilitated release, ocular

Important considerations in vehicle design are the anatomical and physiological aspects of the drug delivery site. To improve drug bioavailability, ocular drug delivery system designs are based on comfort, contact time, and dose volume, each of which recognizes an important anatomical/physiological constraint. An additional ocular limitation is blinking, which can both increase and decrease drug bioavailability. On the positive side, blinking spreads instilled solution or ointment across the cornea, thus promoting corneal contact and drug absorption. On the negative side, it forces drug away from the precorneal area and into the drainage apparatus.

An important blinking feature is the shear that occurs when a vehicle is placed in the eye and blinking occurs. The proximity of the eyelids to the eyeball exposes an instilled vehicle to a considerable shearing stress during blinking. Non-Newtonian fluid vehicles should undergo rheological changes during blinking, and ointments should have an altered drug release profile. This report describes an ointment drug release that is dependent on blinking and on the shear created by blinking.

Drug solubility, prolonged contact time, and modest sustained release are some positive features of ointments. Unfortunately, ointments sometimes create blurred vision and are apparently less accepted by the patient than simple aqueous solutions. Nevertheless, one generally has greater control over drug release from an ointment than from a corresponding aqueous based product in terms of drug solubility, emulsion *versus* nonemulsion form, and contact time. Generally, drug release from an ointment is attributed to partitioning and/or diffusion, depending on the drug properties and the specific vehicle selected. A third possibility for drug release, specifically from water-

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in-oil emulsions, is physical exchange of the internal emulsion phase with the neighboring tears. This report describes the properties of, and bioavailability from, such systems.

EXPERIMENTAL

Materials—Tritiated pilocarpine alkaloid (4.1 Ci/mmole) was obtained commercially[1] and purified by vacuum distillation immediately before each experiment to prevent problems with tritium exchange in the solvent. The ophthalmic ointment vehicles were commercially available, petrolatum-based products[2,3].

Male albino rabbits[4], 1.8–2.4 kg, were fed a regular diet with no restrictions on food and water consumption.

Pilocarpine Ointment Preparation—The 5% water-in-oil emulsion ointments were prepared by mixing a 0.05-ml aliquot of distilled water containing 2.08 mg of tritiated pilocarpine with sufficient ointment base to make 1 g of product. This procedure provided a 10^{-2} *M* pilocarpine ointment containing 5% water with a specific activity of 0.25 mCi/g, or approximately 160,000 cpm/mg.

For procedures in which the percent water in the ointment was increased, the ointments were prepared by adding more water in the initial step. The anhydrous ointment was prepared by geometrically mixing the tritiated pilocarpine alkaloid directly with the ointment vehicle. Surfactants were added directly to the ointment vehicles as required.

Drug distribution homogeneity was verified by the reproducibility of measured radioactivity among weighed ointment samples. Ointments were always prepared fresh prior to the experiment.

***In Vivo* Ointment Dosing Technique**—Individual ointment doses were weighed on an analytical balance. A standard 25-mg dose was used throughout the *in vivo* procedures. The weighed dose was carefully transferred to a microspatula and placed inside the center of the lower lid (cul-de-sac), with care being exercised not to irritate the eye or touch the corneal surface. The lower lid was gently moved once across the cornea to spread the dose uniformly and then released. No further manual action was performed, and normal precorneal ointment disposition was allowed to progress.

Aqueous Humor Drug Concentration–Time Profiles—The basic techniques used for monitoring aqueous humor drug levels after topical ocular dosing were described previously (1). Aqueous humor samples, 100 μl, were removed from the anterior chamber at specified times after dosing and analyzed for pilocarpine by liquid scintillation. Prior to sample removal, the precorneal area was rinsed thoroughly and the corneal surface was wiped gently with tissue to avoid contamination by any remaining ointment dose.

***In Vitro* Ointment Drug Release Study**—A 200-mg tritiated pilocarpine ointment sample was placed in the center of a 10-cm petri dish, covering a circular area 15 mm in diameter. The total pilocarpine was 416 μg, and the surface area figure was chosen to correspond to the average rabbit cornea. At the start of each release study, 35 ml of distilled water was added and the system was stirred using a magnetic stirrer and stirring bar positioned away from the ointment mass. One hundred-microliter samples were removed from the system at specified times and transferred to scintillation vials for liquid scintillation counting.

In studies where the ointment was sheared, the shearing action was accomplished manually. The ointment was positioned in the petri dish, as previously described; a microspatula, whose flat end was bent perpendicular to the rest of the handle, was used to shear the ointment against the bottom of the dish. The microspatula was rotated against the ointment, and an attempt was made to keep constant the ointment area exposed to the bathing fluid. Shear rates were predetermined and controlled by the operator. Despite the simplicity and crudeness of the system, very good reproducibility was obtained.

RESULTS

Effect of Ointment Vehicle Emulsifying Efficiency on Ocular Pilocarpine Penetration—In preliminary work (2), it was noted that the Absorption Base A[2] emulsifying efficiency was quite low compared with other hydrophilic absorption bases. Although this ointment vehicle can emulsify water to produce a relatively stable water-in-oil emulsion, 10% water is the limit. Since this vehicle was highly effective for intraocular pilocarpine penetration with a 5% water-in-oil emulsion system, this noteworthy *in vivo* success might be attributable to its low water emulsifying ability.

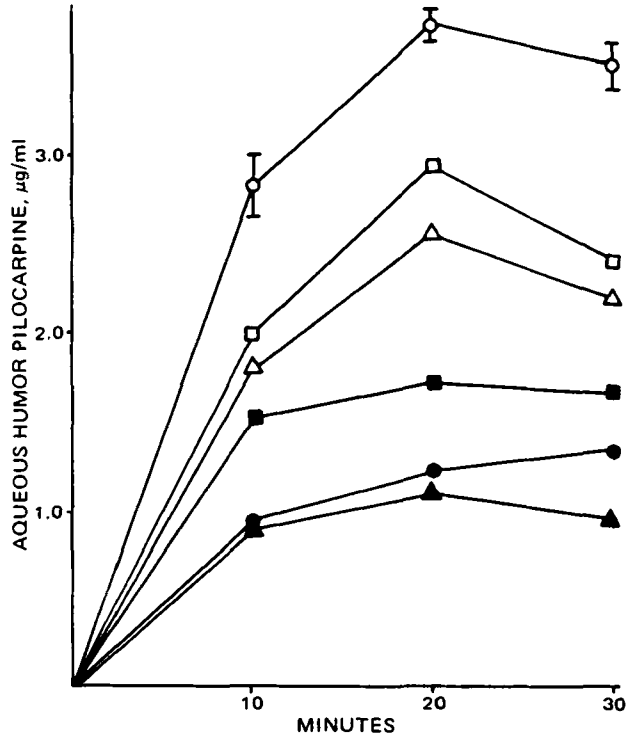

Figure 1—*Pilocarpine concentration in aqueous humor after dosing with various ointment vehicles and aqueous solution. Key:* ○, *Absorption Base A–5% water;* □, *Absorption Base A–2% sorbitan monooleate–5% water;* △, *Absorption Base A anhydrous;* ■, *Absorption Base B–5% water;* ●, *Absorption Base B–25% water; and* ▲, 10^{-2} M *aqueous solution, pH 6.24. Ointment doses were 25 mg, and the solution dose was 25 μl. At least 10 eyes were used for each data point, and representative variation in data is shown for only one system for clarity.*

Figure 1 presents current *in vivo* experiments performed to investigate this parameter. The superiority of the Absorption Base A–5% water system is readily apparent from the figure, especially when it is compared to the profile obtained from a 25-μl dose of 10^{-2} *M* pilocarpine aqueous solution (which contains the same total drug amount as the ointment). Peak aqueous humor pilocarpine levels occurred at 20-min postinstillation for both systems, but these levels are about four times higher for the ointment. The mechanism of transcorneal pilocarpine permeation and vehicle effects were extensively discussed in previous publications (3).

The next step was to incorporate 2% sorbitan monoleate[5] into the Absorption Base A vehicle to improve its emulsifying capability. A qualitative assessment of this parameter was made microscopically, and the base emulsifying efficiency was vastly improved[6] by surfactant addition. The *in vivo* experiments with this vehicle (Fig. 1) showed that aqueous humor pilocarpine levels decreased by about 25%.

At this point, the vehicle was changed to Absorption Base B[3] since its excellent emulsifying ability is well known. This base was mixed 50:50 with liquid petrolatum because the pure base did not melt at the rabbit body temperature. The mixture still possessed extremely good emulsifying efficiency, as indicated by microscopic inspection, while at the same time exhibiting melting and spreading characteristics in the eye similar to Absorption Base A. The data presented in Fig. 1 clearly indicated a further ocular drug penetration reduction when this vehicle was used.

The effect of increasing the vehicle percent water content was also investigated. Absorption Base B was used for these procedures since Absorption Base A would not accept more than about 10% water without serious "bleeding" problems. The 25% water addition decreased aqueous

[1] New England Nuclear, Boston, Mass.
[2] Lacri-Lube, Allergan Pharmaceuticals, Irvine, Calif.
[3] Aquaphor, Duke Laboratories, South Norwalk, Conn.
[4] Klubertanz, Edgerton, Wis.
[5] Span 80, Atlas Powder Co., Wilmington, Del.
[6] Homogeneity, size of internal phase, and ease of incorporation of water into the base were subjective guides in ranking bases as to their emulsifying efficiency.

Table I—*In Vitro* Shear-Facilitated Pilocarpine Release from Various Ointment Vehicles

Vehicle	Emulsifying Efficiency Ranking[a]	Maximum Amount[b] Released by Diffusion/ Partition	Maximum Amount Released by Shearing[c]
Absorption Base A–5% water	4 (poorest)	8	416
Absorption Base A–2% sorbitan monooleate–5% water	3	7	391
Absorption Base B–5% water	1 (best)	6	175
Absorption Base B–25% water	2	8	182
Absorption Base A anhydrous	NA[d]	4	216

[a] Emulsifying efficiency ranking was made by microscopic analysis. [b] All values represent micrograms of pilocarpine released. [c] Total amount of pilocarpine in each system was 416 μg. [d] Not applicable.

humor drug levels relative to the Absorption Base B–5% water system (Fig. 1).

To provide a clearer baseline for assessing relative water-in-oil emulsion vehicle effectiveness, an anhydrous pilocarpine ointment was prepared using Absorption Base A. This system was intermediate in its effectiveness for intraocular pilocarpine penetration. The pilocarpine alkaloid was not soluble in this vehicle, as discussed previously (2).

***In Vitro* Pilocarpine Release from Ointment Vehicles under Nonshear and Shear Conditions**—Shearing is an appropriate consideration for the present study with ophthalmic ointments owing to the natural shear forces that arise during blinking. These experiments were designed to determine if shear-facilitated drug release is a characteristic of such systems.

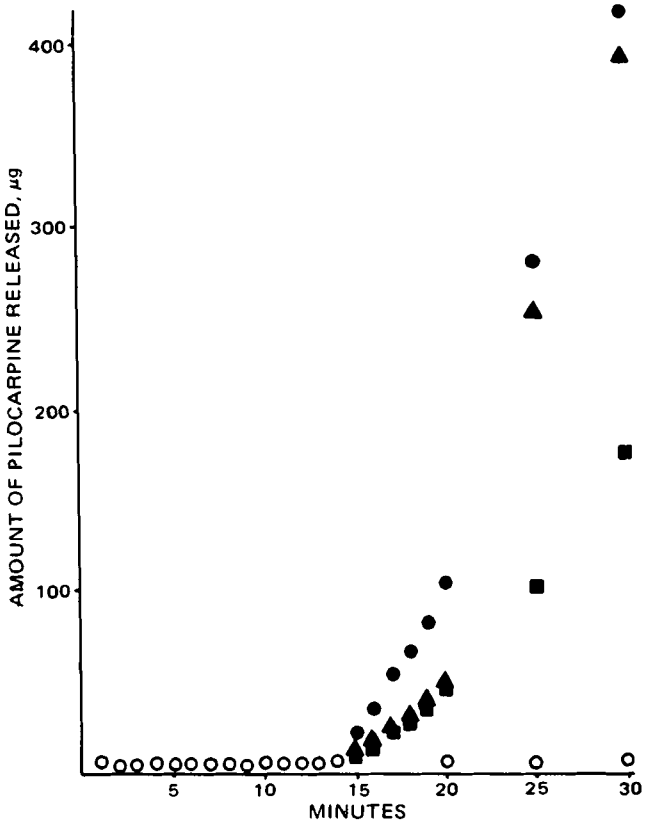

Figure 2—In vitro *pilocarpine release from various ointment vehicles under shear and nonshear conditions. Key:* ○, *Absorption Base A–5% water, no shearing;* ●, *Absorption Base A–5% water, shearing;* ■, *Absorption Base B–5% water, shearing; and* ▲, *Absorption Base A–2% sorbitan monooleate–5% water, shearing. The time interval from 0 to 15 min represents no shearing (simple diffusion), the time interval from 15 to 20 min represents a shear rate of 2/min, and the intervals from 20 to 25 min and from 25 to 30 min represent continuous shear conditions. Total pilocarpine in each system was 416 μg.*

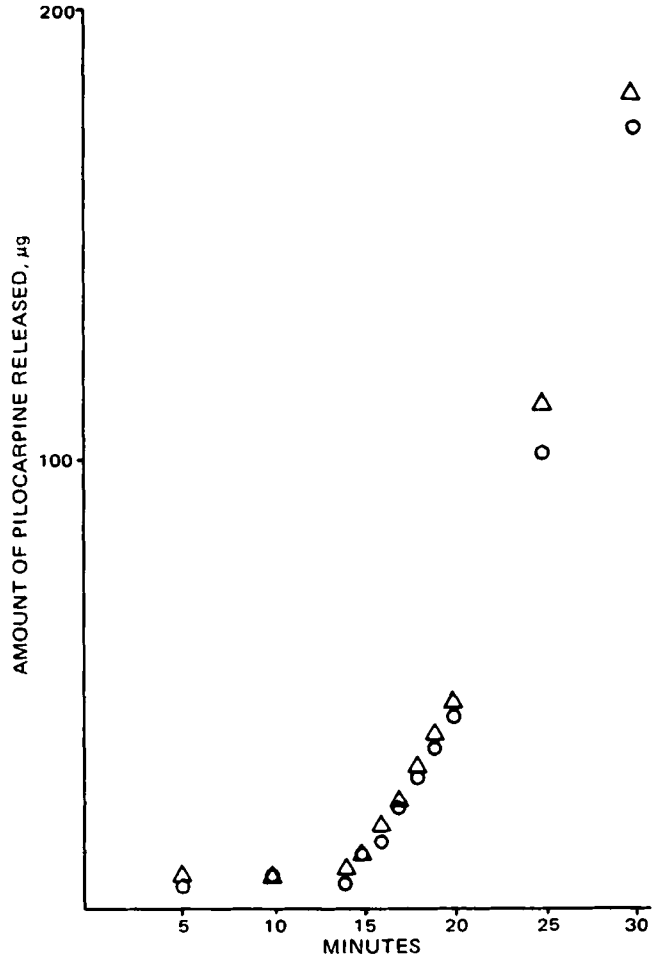

Figure 3—*Shear-facilitated pilocarpine release from absorption base B–5% water (○) and Absorption Base B–25% water (△). The time intervals representing specified shear conditions are the same as those indicated in Fig. 2. Total pilocarpine in each system was 416 μg.*

Figure 2 depicts the *in vitro* pilocarpine release patterns for three ointment vehicles. The data in each case represent an average of at least two determinations. The results of the nonshearing study using the Absorption Base A–5% water vehicle show that pilocarpine was not readily released by simple diffusion. In fact, the initial drug release, which was about 6–8 μg out of 416 μg, remained unchanged for the full 30 min of the studies.

A similar situation was observed with the other ointment vehicles. The interval from 0 to 15 min (Fig. 2) in the shearing experiments was actually a period of nonshear, or simple diffusion, drug release. The 15-min values for all systems are similar; values prior to this time point were omitted from the graph for clarity. The nonshear drug release for each system is presented in Table I.

At times beyond 15 min, addition of a mechanical shearing component to the *in vitro* release study dramatically changed the observed pilocarpine release pattern for each vehicle. The shear rate was 2/min in the interval from 15 to 20 min. The interval from 20 to 30 min represented continuous shear application during which time the operator attempted to liberate the maximum pilocarpine from the systems. The total drug released from each vehicle by the shearing procedures is listed in Table I.

Shearing caused immediate increased pilocarpine release from all vehicles (Fig. 2). The observed shear-facilitated release was consistent with microscopic assessment of the vehicle emulsifying ability. In each case, the shear-facilitated release rate was dependent on the shear rate, as indicated by the changes in slope at the 15- and 20-min points. In addition, within a given time interval with a specific shear rate, the release rate for each vehicle was a function of its emulsifying efficiency. The steepest slopes were observed for vehicles with the poorest emulsifying efficiency.

Figure 3 compares the shear-facilitated *in vitro* release patterns of the Absorption Base B–5% water and Absorption Base B–25% water vehicles.

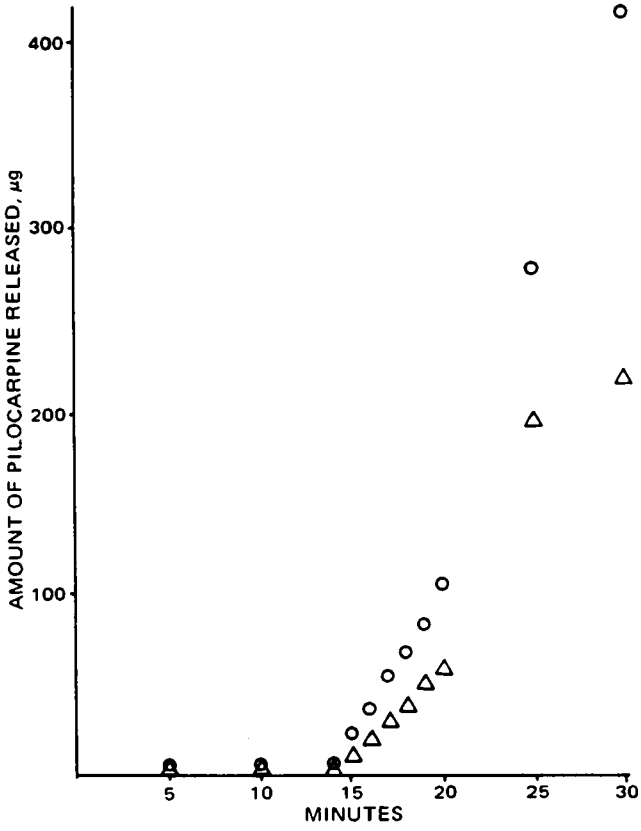

Figure 4—*Shear-facilitated pilocarpine release from Absorption Base A–5% water (○) and Absorption Base A anhydrous (△). The time intervals representing specified shear conditions are the same as those indicated in Fig. 2. Total pilocarpine in each system was 416 μg.*

These two systems showed nearly identical *in vitro* pilocarpine release. Figure 4 compares Absorption Base A–5% water and Absorption Base A anhydrous systems. The water-in-oil emulsion vehicle was clearly superior. This finding is in accord with the results of the *in vivo* experiments. A comparison of the data presented in Fig. 1 and in Table I shows that a good correlation between *in vitro* shear-facilitated drug release and *in vivo* corneal pilocarpine penetration was obtained for all of the vehicles studied. The Absorption Base A anhydrous vehicle also suffered a falloff of pilocarpine release during the terminal period of continuous shear application, a situation that is not apparent in the emulsion system for the time interval studied.

DISCUSSION

The results from *in vitro* and *in vivo* ointment studies show that pilocarpine release from the vehicles to the ocular fluids was dependent on shear and on the dosing system emulsifying efficiency. In particular, the mechanical shearing component was vitally important in correlating *in vitro* release patterns and *in vivo* performance. A literature search did not indicate that this approach had been used with *in vivo–in vitro* correlations for ophthalmic vehicles.

If the pilocarpine ointment studies are considered strictly in terms of simple partition–diffusion considerations, a number of apparent inconsistencies arise:

1. The simple *in vitro* diffusion study (without shearing) using the water-in-oil emulsion ointment vehicles did not indicate that drug release would occur *in vivo;* studies with albino rabbits, however, showed that these were excellent *in vivo* dosing systems and were as much as four times better than an aqueous solution containing the same pilocarpine amount.

2. Additional surfactant incorporation into the absorption vehicle improved the water-in-oil emulsion and increased the internal phase surface area. However, this result decreased the *in vivo* system performance.

3. Increasing the internal phase volume fraction, which decreased the oily diffusion barrier (external phase) thickness, also decreased *in vivo* performance.

A concise review of the parameters governing *in vitro* release of water-soluble drugs from water-in-oil emulsions using a simplified model was presented (4). In developing a qualitative interpretation, the following assumptions are commonly made: (*a*) the emulsion must be stable during the study time, (*b*) drug release occurs by simple diffusion or partition rather than by degradation or breaking of the emulsion, (*c*) the receptor medium acts as a perfect sink for the drug as it is released from the vehicle, and (*d*) the drug is dissolved in the aqueous phase.

Water-in-oil emulsion ointment vehicle stability was verified by checking the final product homogeneity during several storage days. Individually weighed ointment samples were analyzed for radioactivity distribution, and visual assessments of emulsion integrity were made using a microscope. No indications of instability were evident at any time for any of the vehicles studied.

The receptor medium for the *in vitro* release studies was also assumed to approximate a perfect drug sink. The relative amount of ointment and the medium volumes prevented pilocarpine concentration buildup during an individual experiment.

Pilocarpine solubility in the internal (aqueous) phase of the water-in-oil vehicles was verified previously (2). Pilocarpine resided almost totally within the aqueous component, and dissolution in the oleaginous portion of the base was highly unfavorable.

Drug release from ointment vehicles such as those under consideration is dependent upon several processes: (*a*) partitioning, (*b*) diffusion, and (*c*) "facilitated release," which refers to mechanical rupture of dispersed droplets in a water-in-oil emulsion system.

The mechanical shearing action affects each of these processes. The shearing force continually exposes new surface areas to the partitioning process between the vehicle and the receptor phase. Thus, the partitioning interface is altered by each successive shear input. The diffusion process may also be altered by a shearing component, since the diffusion layer thickness is changing. Finally, the mechanical shearing force greatly influences systems in which drug release is facilitated by coalescence and rupture of the dispersed droplets in a water-in-oil emulsion.

These considerations may be included in a discussion of the apparent inconsistencies regarding the *in vitro* and *in vivo* performance of the vehicles used in the present study. The first inconsistency can be eliminated by simply adding a mechanical shearing component to the *in vitro* drug release study. Good correlations between *in vitro* drug release and *in vivo* corneal drug penetration are thus achieved, whereas lack of such a component makes these correlations impossible. Such a shear component is intimately linked to the major drug release mechanism for the systems studied.

A qualitative measure of this shear release mechanism can be inferred from the slopes of the *in vitro* release rate profiles. The slopes are all very steep under shear conditions, suggesting that partitioning and diffusion are probably less important than shear-facilitated release. Undoubtedly, all three mechanisms operate simultaneously, but the shear-dependent mechanism is the more important and releases drug at a faster rate.

The second problem involves the addition of greater amounts of surfactant and the resulting increased internal phase surface area. While an increase in the internal surface area would be expected to increase drug release, this parameter is also a direct consequence of the emulsifying efficiency of the system and the "goodness" of the emulsion. The *in vivo* data correlate with this parameter in a unique manner. The best *in vivo* system (Absorption Base A–5% water) possessed the lowest emulsifying efficiency. Thus, surfactant addition decreased the *in vivo* performance. This observation must reflect the dependence of these systems on the shear component. Shear-facilitated drug release by emulsion rupture seems reasonable.

Finally, the decreased *in vivo* performance resulting from increasing the internal phase volume fraction may be explained on the basis of the effective pilocarpine concentration in the vehicle. A preliminary discussion of this parameter was reported (2). Since the pilocarpine is only soluble in the aqueous component of the water-in-oil emulsion vehicles, the drug will be present almost exclusively in the internal phase. However, since this phase comprises only a fraction of the total volume, the effective pilocarpine concentration in the ointments will be higher than an aqueous solution of the same volume. In other words, a 25-mg dose of 10^{-2} *M* pilocarpine in an Absorption Base A–5% water vehicle will possess an effective aqueous pilocarpine concentration 20 times higher than a 25-μl dose of 10^{-2} *M* aqueous pilocarpine solution. Similarly, the concentrations in the Absorption Base B–5% water and Absorption Base B–25% water vehicles differ by a factor of five due to different internal phase volumes.

The data in Fig. 3 show similar *in vitro* release patterns from these two vehicles, although the *in vivo* data presented in Fig. 1 show that the 5% water vehicle is superior. This finding has important implications re-

garding effective concentration influences. The *in vitro* system for measuring drug release could not distinguish between these two systems due to the large receptor medium volume. The effective concentration parameter was swamped by the excess receptor fluid. However, this result did not occur *in vivo* due to the limited volume (about 7 μl) of precorneal fluid present. This finding also points out problems that can arise when such *in vitro* tests are used to predict *in vivo* performance for systems containing high effective drug concentrations.

The *in vivo* data for these two vehicles (Fig. 1) may not reflect the fivefold difference in effective concentration since the aqueous humor levels produced by the Absorption Base B–25% water vehicle were decreased by less than one-half those values achieved by the Absorption Base B–5% water vehicle. However, these results were quite reasonable if the dilution factor arising from the normal resident tear pool volume (7.5 μl) was considered. The water volume in a 25-mg dose of the Absorption Base B–25% water vehicle was 6.25 μl, with one-fifth this amount (1.25 μl) being present in an equivalent dose of the Absorption Base B–5% water vehicle. If each volume were to be mixed with the tear pool, the calculated decrease in precorneal drug concentration would be no more than 40%. Of course, such mixing would not be instantaneous in either case, but these considerations do explain the observed results.

The data in Fig. 4 show that the Absorption Base A–5% water vehicle was able to release more pilocarpine *in vivo via* shearing than was the Absorption Base A anhydrous vehicle. Inspection of the *in vivo* data presented in Fig. 1 also shows the emulsion system to be superior. The reason for this result may be that as the percent water in the vehicle is reduced to zero, the rate-controlling process is switched from mechanical rupture of the emulsion to dissolution or diffusion control. Further work to determine this mechanism is indicated.

A shearing component is a necessary feature for *in vitro–in vivo* data correlations with ophthalmic ointments. Since some form of shearing action, such as inunction, is common to nearly every semisolid topical dosing system and to many parenteral products, this parameter should have almost universal importance.

These studies show that significant drug bioavailability increases can be achieved by careful design of a system that incorporates shear-facilitated drug release. More importantly, the results also demonstrate that such systems can be used to reduce the total drug amount required for topical ophthalmic dosing. This implies a reduced systemic drug load and decreased side effects, particularly important points for pediatric dosing.

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Enhanced Chartreusin Solubility by Hydroxybenzoate Hydrotropy

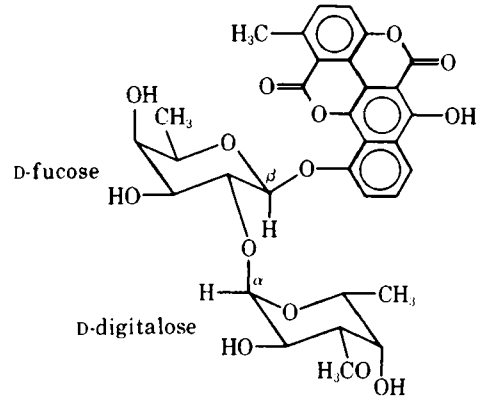
G. K. POOCHIKIAN ˣ and J. C. CRADOCK

Received October 6, 1978, from the *Analytical and Product Development Section, Pharmaceutical Resources Branch, National Cancer Institute, National Institutes of Health, Bethesda, MD 20014.* Accepted for publication November 30, 1978.

Abstract □ The apparent aqueous solubility of the water-insoluble cytotoxic agent, chartreusin, was increased at neutral pH in the presence of hydroxybenzoates. Water molecules play an important role in the chartreusin conformation. Studies included solubility and spectral examinations. The weakest and strongest interactants with chartreusin were sodium benzoate and sodium trihydroxybenzoate, respectively, while the effect of mono- and dihydroxybenzoates was intermediate. A plane-to-plane orientation of chartreusin and the ligand molecules brought together by electrostatic and hydrophobic interactions is postulated. The dramatic chartreusin aqueous solubility increase relative to its aglycone, chartarin, under similar conditions was best rationalized by micellization.

Keyphrases □ Chartreusin—aqueous solubility enhanced by hydroxybenzoates, conformation □ Hydroxybenzoates—electrostatic and hydrophobic interactions, stability constants □ Cytotoxic agents—chartreusin, aqueous solubility enhanced by hydroxybenzoates □ Antineoplastic agents, potential—chartreusin, aqueous solubility enhanced by hydroxybenzoates

Chartreusin[1] (I) produced by *Streptomyces chartreusis* was originally reported in 1953 (1), but its chemical structure was not fully elucidated until 1964 (2, 3). The chartreusin aglycone, chartarin (II), possesses essentially a planar ring system. The phenolic group at C-10 is glycosidically (β) bound to a D-fucose, which, in turn, is linked



I

by an α-glycosidic linkage to D-digitalose. Chartreusin has exhibited substantial chemotherapeutic activity in mice against the P-388 and L-1210 leukemias and, to some extent, against B16 melanoma (4). Biochemical studies demonstrated that I binds to DNA and inhibits RNA and DNA syntheses (5).

Low chartreusin solubility (15 μg/ml) inhibits preparation of reasonably concentrated aqueous solutions. Although the solubility may be increased to 2 mg/ml at a high pH (>9), these solutions may be irritating and incompatible with physiological fluids. More importantly, significant irreversible decomposition of these solutions is evi-

[1] Benzo[*h*][*l*]benzopyrano[5,4,3-*cde*][*l*]benzopyran-5,12-dione, 10-[[6-deoxy-2-*O*-(6-deoxy-3-*O*-methyl-α-D-galactopyranosyl)-β-D-galactopyranosyl]oxy]-6-hydroxy-1-methyl.

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