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Characterization of the Novel Ophthalmic Drug Carrier Sophisen in Two of Its Derivatives: 3A Ofteno™ and Modusik-A Ofteno™

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ABSTRACT Sophisen, a new ophthalmic drug carrier, was characterized using physicochemical and morphological criteria. Diclofenac belongs to a nonsteroidal anti-inflammatory molecule group and its ophthalmic use avoids side effects produced by steroid drugs. Cyclosporine-A is a cyclic peptide used as an immunosuppressive when administrated systemically. Its application in ophthalmology has been reported, but it is a very poor soluble drug. Diclofenac sodium and Cyclosporine-A were mixed with Sophisen to render two new ophthalmic solutions that were named 3A Ofteno™ and Modusik-A Ofteno™, respectively. Based on transmission electron microscopy and dynamic light scattering studies, we concluded that Sophisen is a polydisperse solution with a molecular weight of 413±122 kDa, whereas 3A Ofteno™ and Modusik-A Ofteno™ are monodisperse solutions with molecular weights of 169±44 and 153±10, respectively. Sophisen was shown to be a good carrier for diclofenac sodium as evaluated by passive diffusion through the cornea. A comparative study suggests that diclofenac applied as eye drops was better tolerated when associated with Sophisen. In addition, Modusik-A Ofteno™, a new aqueous solution of Cyclosporine-A, improved tear production in patients with moderate or severe dry eye condition.

KEYWORDS Drug carrier, Diclofenac, Cyclosporine-A, Ophthalmic solution

INTRODUCTION

The eye is an organ of easy access for topical application of drugs. However, determinations of optimal drug concentrations are difficult and easily affected by the biological properties of the organ. Bioavailability on the corneal surface is chiefly affected because of its relative impermeability to foreign substances and tears dynamics. Corneal drug absorption is a slow process as compared to drug removal. It has a low efficiency of absorption under 1% (m/v) and additional problems when the active drug is insoluble in water, therefore requiring a nonaqueous formulation (Kaur & Kanwar, 2002). Strategies to

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produce effective drug doses include increasing both frequency of application and drug retention time through the use of suitable carriers (Seattone et al., 1982). Topical ocular administration for treatment of external diseases is more appropriate because it is relatively painless; additionally, only minor modifications are exerted in the liver, as smaller doses of drugs reach the systemic circulation (Nigel, 2000). Preferably, a topical eye formulation must exclude factors such as annoyance, burning sensation, and high ionic concentrations. There are important factors that determine a drug's ocular residence time and bioavailability, including drainage rate, drug binding to tear proteins, metabolic degradation, increase of lacrimation, and physicochemical properties of the tear (Lee, 1993). Liquid-dosage forms are rapidly drained from the conjunctiva to the nasolacrimal duct (Mishima, 1981). The residence time of installed dose varies from 4 to 23 min due to the tendency of the eye to maintain a residence volume of 7 to 10 μL. It has been reported that irritation increases the drainage rate and reduces the residence time of topical instillation (Baeyens & Gurny, 1997). For example, in rabbits, an application of 50 μL of ophthalmic drops can be cleared in 2 min. However, if the instillation volume is decreased, the residence time could be extended; in addition, an increase in the viscosity of the formula should extend its residence time (Patton & Robinson, 1975). But viscosity is not the only property in formulations that result in a longer residence time. To date, drug delivery systems for the eye have included the use of a number of polymers and surfactants as vehicles to make the medications more comfortable and to keep them in place for an extended period of time. Certain polymers, such as polyoxyl 40 stearate (POE40), chitosan, and hyaluronic acid, interact with the mucous layer of the tear film and with the outermost layers of the cornea and the conjunctiva. The adhesive capability provided by these polymers improves the retention of the polymer solution on the eye (Davis et al., 1991). In addition, polymers in ophthalmic solutions could play an important role in protecting the product quality and use of the drug. Excipients have been considered to be inert, but sometimes their degradation pathways involve the formation of toxic side-products that affect the stability of the formulation. Also, residues and impurities in excipients can affect the quality of the preparation by interacting with the drug or other key components (Dubost et al., 1996).

Diclofenac, an inhibitor of prostaglandin biosynthesis (Mitchell et al., 1994), is considered to be a nonsteroidal anti-inflammatory drug (NSAID) and has shown to be highly effective in the treatment of joint disorders (Insel, 1990). It is used to reduce swelling after cataract removal surgery, inflammation, and pain in the eyes. Using diclofenac in ophthalmology became accepted because it produces less adverse effects than steroidal drugs, but its ophthalmologic use has been limited due to annoying sensations such as intense burning and stinging when it is applied on the eye surface. On the other hand, Cyclosporine-A (CsA), an antibiotic, has been used to decrease the immune response after transplant surgery (Theng et al., 2002). However, the systemic use of CsA may cause serious side effects such as high blood pressure and kidney problems. At the present time, CsA is thought to increase tear production in people with certain eye conditions when applied topically. However, its poor solubility in aqueous systems has limited its use in eye drops (Jiménez-Bayardo et al., 2000; Lallemand et al., 2003). Well-tolerated ophthalmic solutions and drugs in aqueous solutions are issues to be addressed in developing new products.

We describe the physicochemical and morphological characterization of Sophisen (US patent 6,071,958), a new carrier for ophthalmic application and the clinical evaluation of its derivatives 3A Ofteno™ (1.0% diclofenac sodium w/v) and Modusik-A Ofteno™ (0.1% Cyclosporine-A w/v).

MATERIALS AND METHODS

Sorbic acid was obtained from Syntorgarn (Mexico City); sodium chloride and boric acid were from Merck (Whitehouse Station, NJ); other inorganic salts were purchased from Sigma (St. Louis, MO). POE40 was bought from Canamex Especialidades (El Salto, Mexico); diclofenac sodium was purchased from Zhejinag Medicine (Hangzhou, China); and Cyclosporine A (CsA) was obtained from Ivax Pharmaceuticals (Opava, Czech Republic). Sophisen is the carrier solution. Modusik-A Ofteno™ is 0.1% (w/v) CsA dissolved in Sophisen and 3A Ofteno™ is 0.1% (w/v) diclofenac sodium in Sophisen. The manufacturing process for Sophisen and 3A Ofteno™ were as

indicated in the US patent 6,071,958 and Modusik-A Ofteno™ was prepared as established in the Mexican patent PCT/MX03/00040.

Dynamic Light Scattering (DLS)

Polydispersity and molecular mass were determined by dynamic light scattering (DLS). Brownian motion in the solution was measured through the diffusion coefficient (Jena & Bohidar, 1993). Data were collected at 18°C over approximately 4 hours in a DynaPro-801 apparatus (Protein Solutions, Inc., Charlottesville, VA) and analyzed with the Dynamic 4.0 software. Samples were injected through 0.02-μm Anotop filters (Whatman, Clifton, NJ) and multiple measurements were done for the different samples (Juárez-Martínez et al., 2001).

Transmission Electron Microscopy (TEM)

For negative staining, 30-μL aliquots of the different samples were deposited on nickel grids previously covered with a Formval film and stabilized with a carbon coating. Grids were floated on a drop of 2% (w/v) uranyl acetate, dried, and viewed through a JEOL 2000EX (JEOL Inc., Tokyo, Japan) transmission electron microscope at 80 kV. For replicates, grids were transferred into the evaporation chamber of a Balzers 400F unit where they were fix-angle shadowed with a platinum-carbon gun and then rotatory shadowed with a carbon gun under vacuum conditions. Grids were observed and micrographed as described (Moradian-Oldak et al., 1998).

In Vitro Cell Diffusion

Horizontal Side-Bi-Side (Crow Glass Co., Somerville, NJ) diffusion cells were used to monitor the transport of either diclofenac sodium or CsA. Fresh corneas were dissected from New Zealand white rabbits and subsequently mounted in diffusion cells, with epithelial tissue facing the donor chamber and the endothelial side facing the receptor chamber. Two milliliters of the sample to be analyzed were loaded into the donor chamber at a concentration of 0.1% (w/v), and 2 mL of phosphate buffer saline (PBS) were added to the receptor chamber. The contents of each chamber were continuously stirred at 600 rpm with magnetic stir bars in the diffusion system (Hayat, 1972). Solutions from both chambers were recovered after 6 hours and quantitatively assayed for diclofenac and CsA by HPLC as described in the following section.

Determination of Diclofenac Sodium and CsA by High-Performance Liquid Chromatography

The content of diclofenac sodium in the sample was determined by reverse-phase high-performance liquid chromatography (HPLC) method in a Waters 2690 (Alliance Analytical Inc., Auburndale, MA) apparatus equipped with a 996 Photodiode array detector. The sample was injected in to a C18 column (Waters, Milford, MA) and separation was performed by using a mixture of acetonitrile:0.1 M Phosphate pH 2.3 (70:30 v/v) as the mobile phase. Diclofenac sodium was monitored by A_{229}. CsA was assayed by a reverse-phase HPLC method using acetonitrile:water:methanol (70:25:5 v/v/v) as the mobile phase in a 25-cm C18 column (Waters, Milford, MA) at 60°C.

Clinical Evaluation

A comparative tolerance study between Voltaren™ (CIBA Vision, Switzerland) and 3A Ofteno™ was performed in 120 healthy volunteers in a randomized double-blind clinical study of patients at the Hospital San José de Monterrey, affiliated with the Instituto Tecnológico de Estudios Superiores de Monterrey, Nuevo Leon, Mexico. The hospital's ethics committee approved the protocol used in the study. One drop was applied to volunteers every 30 minutes in each eye over a 90-min period. Symptoms were recorded as absent, mild, moderate, or severe for pain, burning sensation, photophobia, lachrymal secretion, and sensation of strange bodies. An additional prospective and multicentric study was carried out for Modusik-A Ofteno™. In this study, the subjects were 42 women who had been diagnosed with severe or moderate dry eye syndrome. Modusik-A Ofteno™ was topically administered every 12 hours for a maximum of 98 days, and the increase of tear production was evaluated using the Schirmer I test. Tear production at different times was compared to basal values.

TABLE 1 Physical Characterization of Sophisen and Derivatives

Compound	Polydispersion index	MW (kDa)
Sophisen	4.36	413±122
3A Ofteno™	1.57	169±44
Modusik-A Ofteno™	1.34	153±10

RESULTS

Polydispersity, molecular mass, hydrodynamic radius, and diffusion coefficient of a molecule can be determined by DLS. In this case, the polydispersity index indicates the distribution of individual molecular weights and is a measure of homogeneity of the solution. Based on our DLS studies, we concluded that Sophisen was present as a polydisperse solution of particles with molecular mass of 413±122 and a polidispersity index of 4.36. However, the addition of 0.1% (w/v) CsA to Sophisen (Modusik-A Ofteno™) induced the formation of particles with an average molecular mass of 153±10 kDa and a polydispersity index of 1.34. Based on the hydrodynamic radius of 3A Ofteno™, a molecular mass of 169±44 kDa and a polydispersity index of 1.57 were observed (Table 1). These results suggest that there is a strong interaction of CsA and diclofenac with the carrier Sophisen. The resultant aggregates for both drugs appear to be monodisperse solutions, based on their hydrodynamic radius that matched those of compounds in a true solution. These results explain why Sophisen and its derivatives are kept clear and transparent during storage. Under TEM, Sophisen was observed as spherical structures of variable sizes (10 to 600 nm) distributed on the grid (Fig. 1). The most abundant population consisted of small particles of about 10 nm (Fig. 1D). Some of these electron-dense vesicles were

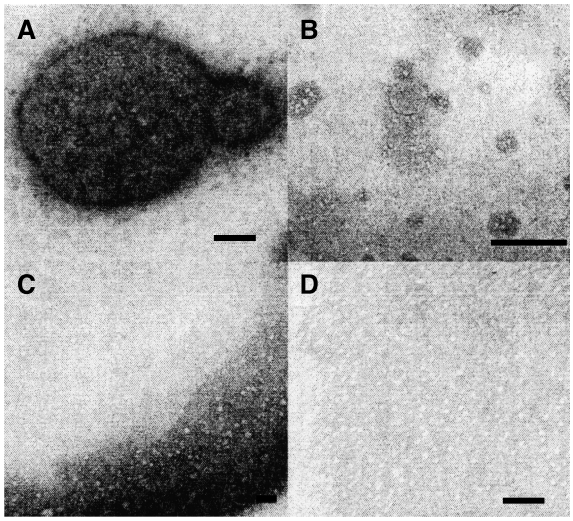

FIGURE 1 Ultrastructure of Sophisen. Sophisen Particles were Deposited on Nickel Grids and Processed for Negative Staining. Panel A: High Magnification of Sophisen Particles Showing a Big Structure Fused to Another Vesicle with Smaller Particles on the Surface. Panel B: Other Structures are Liposome-Like, with a Size of 50 nm. Panel C: Small Particles Associated to a Membrane-Like Structure. Panel D: Low-Magnification Micrographs of Sophisen Showing Dispersed Particle Structures. Scale Bar: 200 nm.

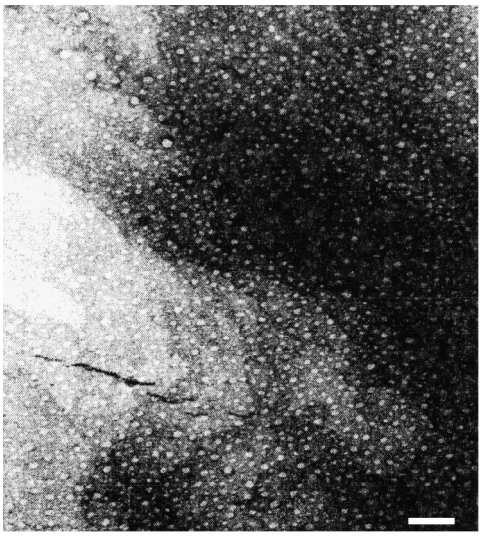

FIGURE 2 Ultrastructure of 3A Ofteno™. Structures were Visualized by Replication. Small Particles were Uniformly Distributed Over All the Grids and Found to be Associated to Membrane-Like Structures. Scale Bar: 200 nm.

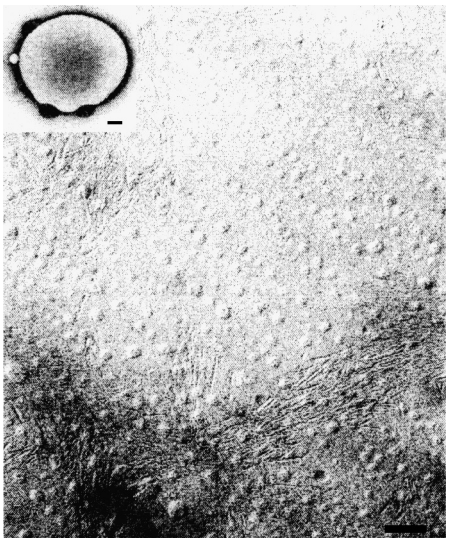

FIGURE 3 Ultrastructure of Modusik-A Ofteno™ (Cyclosporine A Plus Sophisen). Samples were Negatively Stained and Viewed by TEM. Small Homogeneously Distributed Particles were Observed with a Size of About 10 nm, but Occasionally, Intimate Associations Between Different Size Particles were Observed (Insert). Scale Bar: 200 nm.

TABLE 2 Diffusion of Diclofenac Sodium Through Fresh Rabbit Corneas

	Initial (mg/mL)	Donor (mg/mL)	Receptor (mg/mL)	% Recovered
Diclofenac in PBS	0.9747 ± 0.017	0.8040 ± 0.044	0.0895 ± 0.022	9.181 ± 2.32
Diclofenac in Sophisen	0.9972 ± 0.009	0.8392 ± 0.0259	0.0047 ± 0.0009	0.476 ± 0.10

observed as small particles fused to a bigger one (Fig. 1A), whereas others could be seen as membrane-like aggregates (Fig. 1B, C). This distribution matches the polydispersity index data as determined by DLS (Table 1).

The addition of diclofenac sodium to Sophisen (3A Ofteno™) induced the formation of dense structures with a size of 10 nm (Fig. 2). When CsA was added to Sophisen at 0.1% (w/v) (Modusik-A Ofteno™), 10-nm particles were observed (Fig. 3) in freshly prepared solutions, as well as in those stored for 1 year. Noticeably larger structures (500 nm) with regular shapes were occasionally seen in these preparations. These larger structures could be the result of an interaction between small vesicles with the larger ones (Fig. 3 insert). In diffusion experiments with fresh rabbit corneas, about 89.5 μg/mL of diclofenac sodium was recovered in the receptor solution, or 9% of the initial concentration (0.9747 mg/mL in PBS) of diclofenac sodium placed in the donor chamber (Table 2). However, when 3A Ofteno™ was the donor solution, 20-times less diclofenac (4.7 μg/mL) was recovered in the receptor chamber as compared to the original concentration (0.9972 mg/mL). Therefore, we suggest that Sophisen could contribute to the maintenance of diclofenac sodium outside the eye surface (Table 2).

In order to evaluate the tolerance of diclofenac sodium, a comparative study between 3A Ofteno™ and Voltaren™ was assessed in 120 healthy volunteer. Results shown in Table 3 suggest less annoying sensation in patients who used 3A Ofteno™. In the case of Cyclosporine-A, it was possible to dissolve it in Sophisen within a range of 0.5 to 5 mg/mL, and previous clinical studies demonstrated that 1 mg/mL (Modusik-A Ofteno™) was effective in the treatment of dry eye. The Schirmer I test was used to evaluate the clinical effectiveness of Modusik-A Ofteno™ in 42 women with severe to moderate dry eye syndrome. Schirmer values were compared between the beginning and after 98 days of topical instillation. Subjects who received Modusik-A Ofteno™ increased from baseline 5 to a value of 11 mm in the Schirmer I test after treatment (pair t-test, $p<0.001$). This indicates an improvement of tear production (Fig. 4). Also, Modusik-A Ofteno™ corneal performance was evaluated for passive diffusion using the experimental conditions described above. CsA did not pass through the cornea significantly, but consistently we found 2.0–2.5 ng of CsA/mg of tissue.

TABLE 3 Comparison of Tolerance of 3A Ofteno™ and Voltaren™ After Three Topical Applications

	Absent (%)	Low (%)	Mild (%)	Severe (%)
First application				
3A Ofteno™	78.3	11.7	8.3	1.7
Voltaren™	32.5	26.7	25.8	15.0
Second application				
3A Ofteno™	65.0	18.3	11.7	5.0
Voltaren™	35.0	11.7	41.7	11.7
Third application				
3A Ofteno™	63.3	28.3	8.3	0.0
Voltaren™	33.3	21.7	26.7	18.3

DISCUSSION

The effectiveness of a drug will depend on its presence on the action site and the residence time. For years the interest of many pharmaceutical companies has been to develop controlled drug delivery systems to reach the right tissue to maintain a therapeutic concentration and residence time of a drug. Using

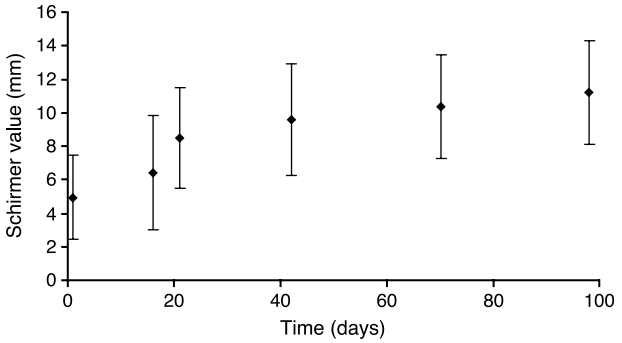

FIGURE 4 Improvement of Tear Production in Patients Treated with Modusik-A Ofteno™ Evaluated by Schirmer I Test. A Pair t-test was Significant ($p<0.001$).

polymers is an option to overcome problems related to maintain therapeutic concentrations. Thus, they are an important part of the design of drug delivery systems (Ahmed et al., 1987). However, effective drug delivery is still a challenge for efficacy and safety. In this study we report some of the physical and structural properties of Sophisen and its derivatives. Sophisen was evaluated as a possible carrier for ocular topical application. The Sophisen formulation was based on the physicochemical properties of polyoxyl 40 stearate (POE40). Due to the amphipatic nature of POE40, it has been considered as a part of the vehicle in several pharmaceutical preparations. It can form several structures when mixed with other substances under controlled temperatures and pH conditions (Kohudic, 1994). Molecules of pharmaceutical interest such as diclofenac and Cyclosporine A can bind to Sophisen, rendering stable and effective solutions. Some of the resultant commercial formulations were 3A Ofteno™, which is prescribed for ocular inflammation (Steinberg & Kinoshita, 2002), and Modusik-A Ofteno™, prescribed for dry eye syndrome (Kuner et al., 2000; Reer et al., 1994).

For Sophisen, independent TEM experiments suggest the formation of a variety of particles with different sizes and shapes. The vast majority of particle population corresponds to vesicles of 10 nm in size, although other characteristic membrane- and liposome-like structures were present as well (Fig. 1). This explains its polydispersity when DLS was applied to freshly prepared solution. When either diclofenac sodium or CsA was added to Sophisen, the solution became monodisperse, possibly due to a strong association between the drug and the Sophisen preparation, which contributed to the overall stability of the system. Addition of diclofenac to Sophisen resulted in a new preparation that was given the brand name 3A Ofteno™, which was evaluated for its capacity for diffusion across fresh rabbit corneas in comparison with the same concentration of diclofenac dissolved in PBS. According to the amount recovered in the receptor chamber, we suggest that Sophisen associates to diclofenac, but only a limited amount of the drug passes by the cornea to the anterior chamber (Table 2). This phenomenon could be related to the decrease of the annoying sensation of 3A Ofteno™ as compared to Voltaren™ (Table 3). Diclofenac as an NSAID exhibits anti-inflammatory, analgesic, and antipyretic activity (Schalnus, 2003; Stevenson et al., 2000). It acts by inhibiting the cyclooxygenase isozymes involved in the endogenous prostaglandin biosynthetic pathway, COX-1 and COX-2. These proteins are also referred to as prostaglandin G_2/H_2 synthase-1 (PG G_2/H_2 -1) and -2 (PG G_2/H_2 -2), respectively, although the precise mechanisms for the anti-inflammatory and analgesic effects of NSAIDs are still under investigation (Flach, 1992).

Diclofenac has been shown to be effective against other diseases such as joint disorders with excellent results (Fitzgerald, 2003). However, its use in ophthalmology has been limited because it causes annoying sensations, such as intense burning and stinging when topically applied on the eye surface.

As confirmed by TEM, Modusik-A Ofteno™ (an association of CsA with Sophisen) rendered a monodisperse solution predominantly with particles of about 10 nm in size. The Modusik-A Ofteno™ solution also had some particles associated with larger structures (Fig. 3 insert). A clinical study was performed in which Modusik-A Ofteno™ was applied for 98 days to patients with severe or moderate dry eye syndrome and comparing the initial tear production as evaluated by the Schirmer I test (Fig. 4). Our results suggest a consistent improvement of tear production based on the Schirmer I test and are in agreement with previous reports, which show that CsA was effective in improving some problems related to Sjögren syndrome and dry eye disease (Tsubota, 1998; Zacher et al., 2003).

Both 3A Ofteno™ and Modusik-A Ofteno™ are benzalkonium–chloride-free formulations. However, both products, 3A Ofteno™ and Modusik-A Ofteno™, passed the test described in the USP 27 for antimicrobial effectiveness; therefore, the preservative system was efficient to protect the solution of contamination.

Stability studies were conducted in three batches of the same formulation and in manufacturing processes that were identical to large-scale batch production. These studies helped us to characterize the variation of the quality of the drug product to remain within specifications in terms of strength quality and purity. Samples were stored under stress conditions, included 30°C and 40°C during 1 to 6 months. Modusik-A Ofteno™ and 3A Ofteno™ fulfilled the requirements, such as sterility, completeness and clarity of solution, pH range, and osmolarity. The drug products maintained the same properties and characteristics that they possessed at the time of their manufacture.

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