

**STUDY ON OCULAR ABSORPTION OF DICLOFENAC SODIUM
NIOSOME IN RABBITS EYE**

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Summary

Though the topical and localized applications are still an acceptable and preferred way to achieve maximum therapeutic level of drugs used to treat ocular disorders, but the conventional dosage form of diclofenac sodium for ocular therapy has poor bioavailability mainly due to tear production, nonproductive absorption, transient residence time and impermeability of corneal epithelium. To overcome this, the non-ionic surfactant vesicles were prepared by lipid film hydration method using span 60 and cholesterol with various molar ratios and characterized for *invivo* drug release study. In addition, ocular irritation test was carried out to evaluate its safety in rabbit's eye. The molar ratio of 100:60 showed higher entrapment of drug and released 79.34 % ± 1.04 at 10th h was used for the *invivo* drug release study. The availability of drug in the tear fluid and aqueous humor in rabbits eye was estimated at various time interval which was confirmed by HPLC method. The ocular irritation test of niosome containing diclofenac sodium was found to be safe which was confirmed by histopathological study. Study may be concluded that the non-ionic surfactant vesicles formulated with span 60: cholesterol in a molar ratio of 100:60 showed potential approach to improve the ocular bioavailability of diclofenac sodium for the prolonged period of time.

Keywords: Diclofenac sodium, Niosomes, Ocular delivery and Non-ionic surfactant vesicles.

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Introduction

Diclofenac sodium is widely accepted for its safety and efficacy in the treatment of post operative inflammation in patients who have undergone cataract extraction, and temporary relief of pain and photophobia in patients who have undergone corneal refractive surgery (1, 2). However, the major problems encountered with the topical delivery of ophthalmic diclofenac sodium is the rapid and extensive precorneal loss caused by the high tear fluid turnover as well as the relatively large volume of the administered eye drop (~50 µl versus 7 µl of corneal tear film), lead to a high rate of lacrimal drainage. Due to the resulting elimination rate, the precorneal half life of drugs applied by these pharmaceutical formulations is considered to be between about 1-3 min. As a consequence, only the very small amount of about 1-3% of the dosage actually penetrates through the cornea and is able to reach intraocular tissues. The poor productive absorption, on the other hand, results in a high amount of drug that is drained into the nose or into the gut. Especially the nose and also the gut are very efficient absorption organs of the body. This in turn leads to an extensive systemic absorption and may result in unwanted side effects and toxicity of the drug (3-5). Lacrimation and blinking are actually efficient protective mechanisms which keep the eye free from foreign substances, but they prevent efficient ocular therapy. The drug is mainly absorbed systemically via conjunctiva and nasal mucosa, which may result in some undesirable side effects (6). Patient compliance may also occur due to the local irritation of most of the topical applications. Although these problems have been recognized for a long time, surprisingly little effort has been made by the researchers. To overcome these problems, various ophthalmic vehicles such as ointments, suspensions, micro- and nanocarrier systems, inserts, and liposomes have been investigated (6).

Niosomes have been reported as a possible approach to improve the low corneal penetration and bioavailability characteristics shown by conventional ophthalmic vesicles. Niosomes are formed from the self assembly of non-ionic amphiphiles in aqueous media resulting in closed bilayer structures which can entrap both hydrophilic and lipophilic drugs either in an aqueous layer or in vesicular membrane (7).

The niosomes are preferred because they are chemically stable, they have low toxicity because of their non-ionic nature, handling and storage of surfactants require no special precautions or conditions, they can improve the performance of the drug via better availability and controlled delivery at a particular site and they are biodegradable, biocompatible and non-immunogenic (8).

The aim of the work was to develop a suitable non ionic surfactant vesicle of diclofenac sodium by lipid film hydration technique to be applied topically and to carry out the entrapment efficiency (9), *invitro* release pattern (10), *invivo* drug release study (13-14) and ocular irritancy test (15).

Materials and Methods

Materials

Diclofenac sodium was obtained as a gift sample from Novartis Limited, India. Sorbitan monostearate (span 60), cholesterol, chloroform, and sodium chloride were purchased from Loba chem. Pvt ltd, Mumbai. Methanol, ethyl acetate, potassium di-hydrogen phosphate and disodium hydrogen phosphate were purchased from S.D fine chem. Ltd, Mumbai, India. Sodium hydroxide from Nice chem. Ltd, Mumbai, Xylocaine from Astra zenica Ltd, India. Distilled water from Leo scientific, Erode,T.N, India and HPLC water was purchased from Qualigens, India.

Animals

Male albino rabbits 10-12 weeks old weighing 2.5-3.5 kg was used in this study. The animals were purchased from animal house, IRTT Perundurai Medical College, Perundurai, Tamilnadu. On arrival, the animals were housed individually and fed with standard pellet diet with free access to water. The temperature was maintained at $28\pm 2^{\circ}\text{C}$ through out the study. The study protocol was approved by Institutional Animal Ethical Committee (688/2/C/CPCSEA - NCP/IAEC/02/2006-2007) for the use of animals in the research work.

Preparation of Diclofenac sodium niosomes

The niosomes were prepared by lipid film hydration method (16-17). Accurately weighed quantity of Diclofenac sodium, and surfactant, cholesterol in different molar ratios such as 100: 30, 100:40, 100: 50, 100:60, 100: 70 and 100: 100 were dissolved in chloroform/methanol (2:1, v / v) in a 100 ml round bottom flask. A thin lipid film was formed under reduced pressure in a rotary flash evaporator at 60° C. After the removal of last trace of organic solvent, the film was then hydrated by 10 ml of Phosphate buffer saline pH 7.4 at 60° C for one hour. The prepared niosomal suspensions was shaken for one hour using horizontal mechanical shaker at 60 rpm and 40°C leading to the formation of multilamellar niosomes. The niosomal suspension was left to mature overnight at 4°C.

***In vivo* study**

Male albino rabbits were used in the study. The optimized niosomal suspension of diclofenac sodium containing span 60: cholesterol in molar ration of 100:60 was instilled in the lower cul-de-sac of right eye. The upper eyelids were gently held closed for 10 s to maximize the corneal contact. The aqueous humor samples were collected at various time intervals between 40 - 360 mins of post dose. Aqueous humor samples were collected, by anesthetizing the eyes using 4% xylocaine solutions topically, with the help of 28 gauge needle between the junction of sclera and cornea of right eye (test) whereas left eye was treated as control (Difenic[®], Intas). After the extraction, the eyes were treated with ciprofloxacin eye drops for the prevention of infection. Sampled aqueous humor was then mixed with 100 µl of ethyl acetate and kept in the refrigerator for one hour. The mixture was then centrifuged at 3000 rpm for 20 mins and the supernatant obtained was analyzed for the presence of Diclofenac sodium by HPLC.

Assessment of ocular irritancy of niosome

Male albino rabbits were used for this ocular irritancy test. The potential ocular irritancy and/ or damaging effects of the formulations under test were evaluated by observing them for any redness, inflammation, or increased tear production. The right eye of each rabbits received niosomal suspension of span 60: cholesterol in a molar ratio of 100:60 by single instillation for a period of 40 days. The left eyes were considered as a control in all the

experimental rabbits. After 40 days, the rabbits were sacrificed, the eyes were removed and the cornea was separated followed by the histological examination.

Statistical analysis

Results are expressed as the mean \pm S.D. Statistical analysis was performed by student 't' test. P value of < 0.05 is considered statistically significant.

Results

Various niosomal formulations of diclofenac sodium using span 60: cholesterol was prepared with different ratios. The selection of surfactant, cholesterol and the ratios were based on the report of Yongmei Hao et al., (9). The prepared niosomal suspension of diclofenac sodium in molar ratio of 100:60 showed the maximum entrapment of 79.67 % and released 79.34 % *invitro* at 10th hour. Based on the above entrapment and drug release study, the span 60: cholesterol in 100:60 molar ratio was selected for the further study. The *in-vivo* drug release study of diclofenac sodium niosomes in rabbit eye is presented in Fig. 1. The concentrations of diclofenac in aqueous humor instilled with niosome were higher than that with eye drop solution. The retention time was 13.10. The C_{max} of 4.731 $\mu\text{g}/\text{ml}$, T_{max} 200 mins and AUC 513.46 were observed for diclofenac sodium niosome whereas control produced the C_{max} at 3.155 $\mu\text{g}/\text{ml}$, T_{max} 80 min and AUC 351.74. The representative chromatogram of diclofenac sodium of blank, standard, control and test were given in Fig. 2 – 5 respectively.

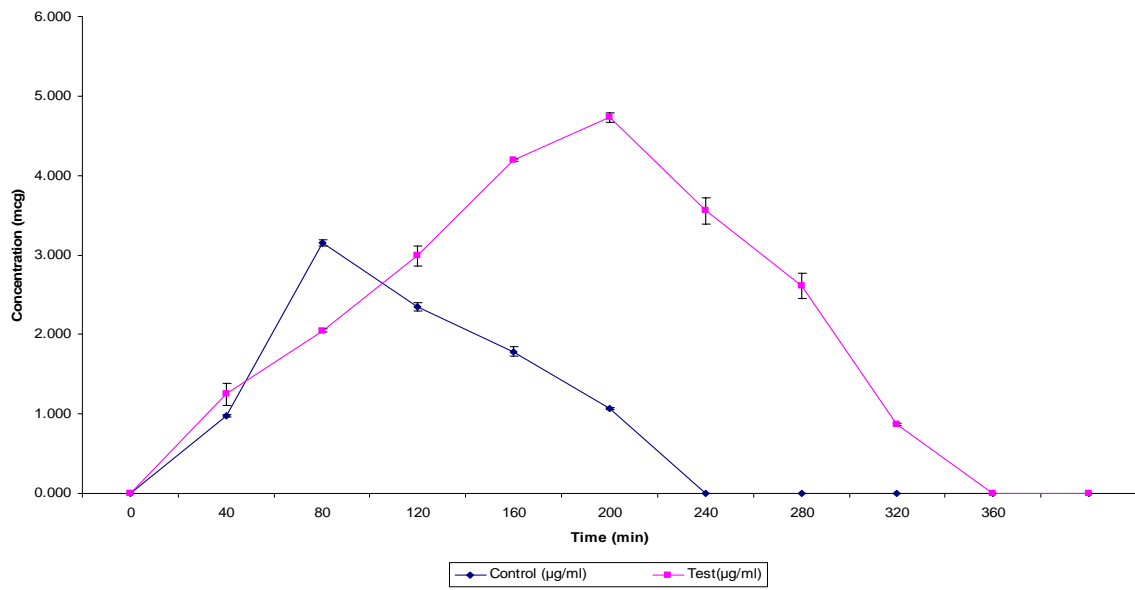


Fig.1 Concentration of diclofenac sodium niosome and control in aqueous humor of rabbits eye at various time interval

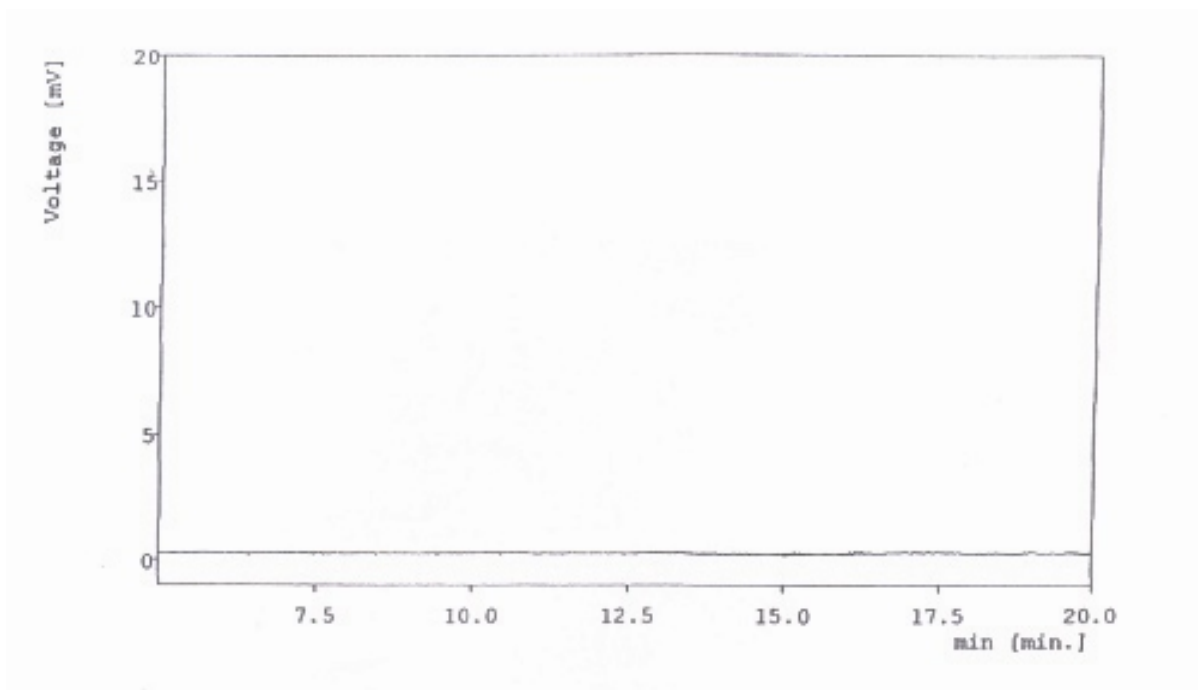


Fig.2. The representative HPLC graph of blank

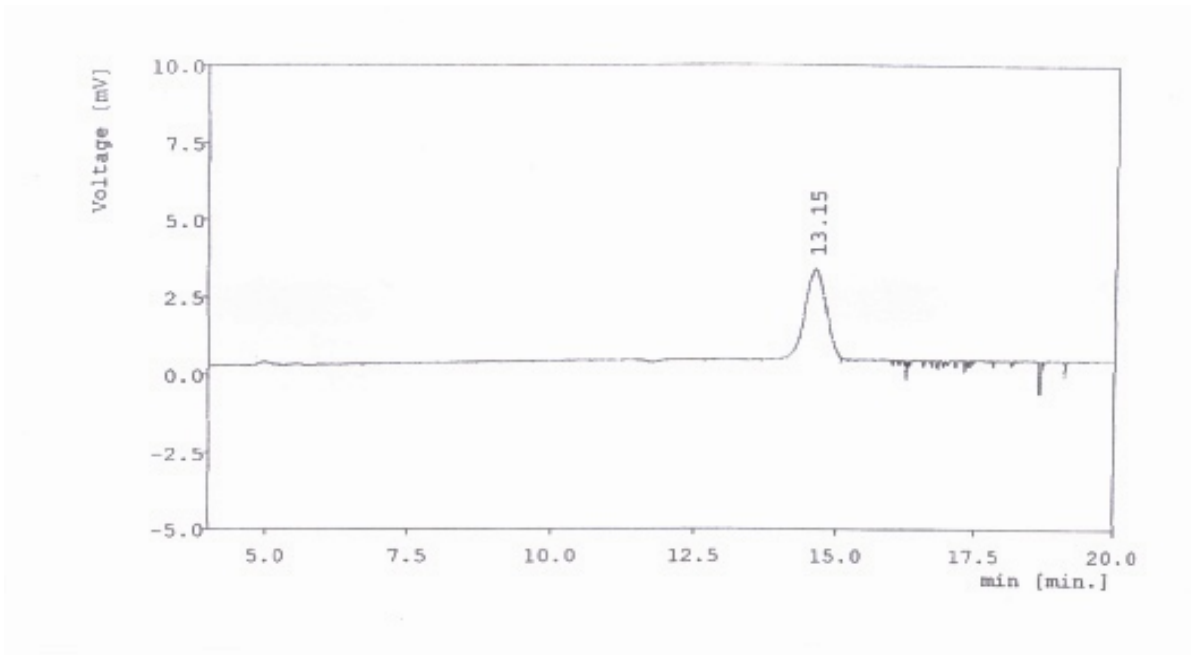


Fig.3. The representative HPLC graph of standard.

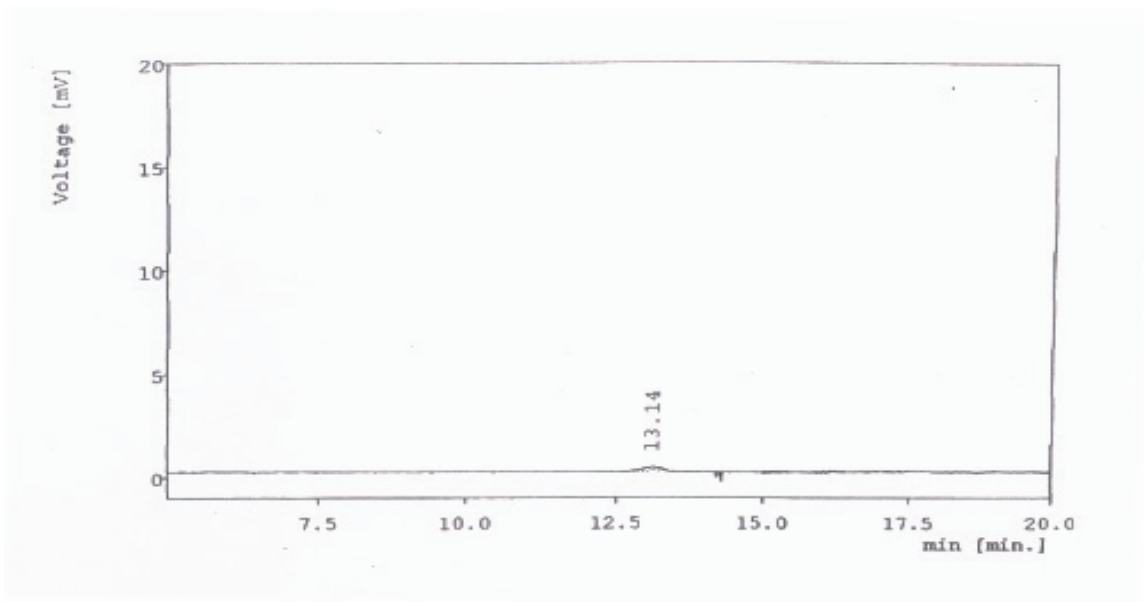


Fig.4. The representative HPLC graph of control in aqueous humor.

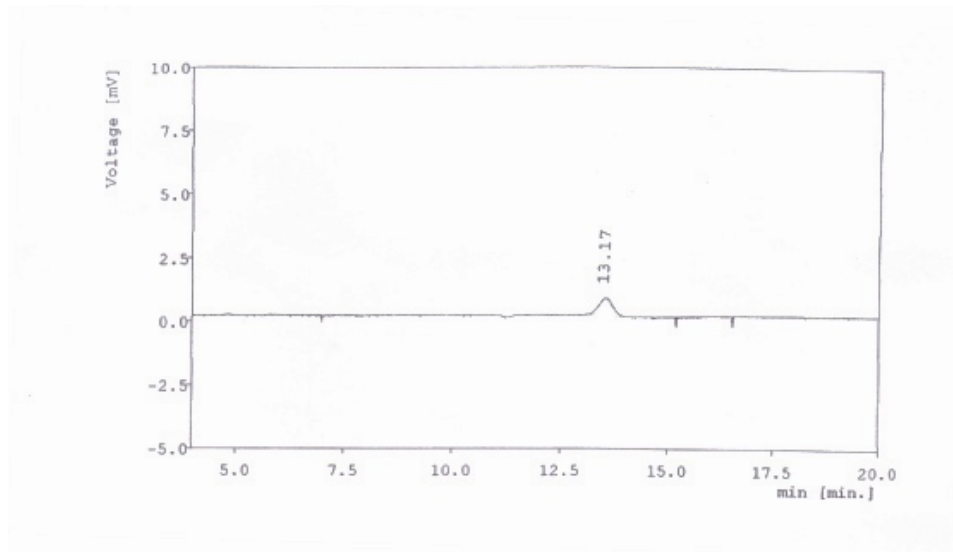


Fig.5. The representative HPLC graph of diclofenac sodium niosomes in aqueous humor of test animal.

The potential ocular irritancy effects of the formulations under test were evaluated by observing them for any redness, inflammation, or increased tear production. The control and test corneal tissues, following instillation of multi-lamellar niosomal formulation composed of span 60: cholesterol in a 100:60 molar ratio on six rabbits was observed. Both eyes of the rabbits under test were examined for any signs of irritation. When compare with control, the suspension under test did not show any sign of redness, inflammation or increased tear production. Fig.6 showed histopathology of rabbits cornea exposed to diclofenac sodium niosome.



Fig. 6. Histological examination of control and test cornea

Discussion

Diclofenac sodium niosomes prepared with span 60: and cholesterol in 100:60 molar ratios were selected for *invivo* studies as they showed higher entrapment efficiency and percentage of drug release compared with other molar ratios. In this study, the obtained C_{max} of test was higher and T_{max} was also more than the control and it may be due to the retention of drug in the aqueous humor, high corneal contact time and permeability of diclofenac sodium niosomes. Surfactants are the chief constituents of niosomes, act as penetration enhancer (18). Moreover, span 60 has a longer saturated alkyl chain which is a crucial factor in permeability (10). The viscosity of diclofenac sodium niosomes in 100:60 molar ratio had the viscosity of 1.29cp which may also influence the high corneal contact time and retention time of drug in the cul-de-sac of rabbits eye. The more prolonged effect obtained with diclofenac sodium multilamellar niosomes may be due to the numerous lipid bilayers found in multilamellar vesicles which play a very important role in retarding drug release over a prolonged period of time (10). The t-test revealed significant differences at $P < 0.001$.

Histopathological examination of control and test treated cornea of rabbits eye was shown in Fig. 6. Histopathology of control and test treated cornea showed slightly oedema of the substantia propria especially in the deep stroma where the collagen fibers were separated from each other. This change may be due to slight irritation caused by the nonionic surfactant, span 60. This type of irritation is reversible where the slight oedema clears over time (19).

In conclusion, Post operative inflammation in patents, temporary relief of pain and photophobia in patients under gone corneal refractive surgery requires a continuous and chronic administration of drug in eye. Conventional dosage forms of Diclofenac sodium will not meet/satisfy above needs. Niosomal suspension formed with span 60: cholesterol in a molar ratio of 100:60 showed highest drug entrapment of $79.67\% \pm 1.53$ and produced $79.36\% \pm 1.04$ drug release *invitro* over the period of 10 h. Viscosity was 1.29 cp which showed suitable consistency for ocular preparation and did not produce blurred vision or drainage. The niosomal formulation also found to ensure a good ocular bioavailability of the drug *in-vivo* with $4.731 \mu\text{g}$ in aqueous humor.

By these facts, study can be concluded by saying niosomes formed with span 60: cholesterol in a molar ratio of 100:60 is a promising approach to improve the bioavailability of diclofenac sodium for an extended period of time. From histopathological study, the preparations were considered as safe for short and long term treatments.

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