

## OCULAR DELIVERY OF FLURBIPROFEN FROM OPHTHALMIC LIPOSOMES DISPERSED IN THERMOSENSITIVE GEL

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يتكرر تطهير المستحضرات الرمدية في العين للمحافظة على مستوى كاف من العقار عند علاج الأمراض الرمدية. فلربيبروفن عقار فعال من مجموعة العقاقير الغير سترويدية التي تستخدم في علاج بعض الأمراض الرمدية سواء الحادة منها أو المزمنة. تم صوغ هذا العقار في صورة صيدلانية وذلك بحبس جسيماته داخل حويصلات دقيقة وعمل انتشار لهذه الحويصلات في وسط هلامي ملائم للعين وتم تحضير هذه الحويصلات من الفوسفاتيديلكولين والكوليسترول بنسبة ٣:٧ مول بتقنية معينة. تم قياس حجم الحويصلات ومقدار الشحنة التي عليها قبل الانتشار وبعده في الوسط الهلامي. تم إجراء التجارب العملية لانطلاق العقار من المستحضر وقد وجد أن انتشار الحويصلات المقيدة للعقار في الوسط الهلامي يزيد من فترة نصف عمر انطلاقه بوقت يعادل ٤ مرات للعقار الحر في نفس الوسط الهلامي. تم إجراء التجارب الحية على عيون الأرانب وذلك بتقدير تركيز العقار في الأنسجة المختلفة للعين مثل القرنية، الرطوبة العينية، الصلب الأبيض والجسم الزجاجي وذلك للمستحضرين (العقار المقيد والحر) وقد وجد أن تركيز العقار في هذه الأجزاء المختلفة من العين يكون كبيرا بدلالة إحصائية عالية جدا للعقار المقيد مقارنة بالمستحضر ذو العقار الحر. وتمت هذه المقارنات بحساب المساحة تحت المنحنى وأعلى تركيز في هذه الأنسجة المختلفة من العين. ويتضح من الدراسة أن استخدام هذا النوع من الصواعغات الرمدية له مستقبل واضح في تهديف أدوية إلى أجزاء محددة في العين لعلاجها.

*In order to maintain adequate drug levels in ocular tissues, frequent drug instillation is necessary in treatment of ophthalmic disorders. Flurbiprofen, NSAIDs, was entrapped in EPC liposomes dispersed in thermosensitive poloxamer gel. The size and Zeta-potential of the vesicles were analyzed. The in-vitro leakage of the entrapped and free drug were carried out under sink condition, the  $t_{1/2}$  for entrapped drug was 4.1 fold when compared to that of the free drug. The concentrations of Flurbiprofen in the ocular tissues of the rabbits for both formulations (entrapped and free drug) were determined by HPLC measurement. The in-vivo study depicted that entrapment of the drug in liposomes enhanced the drug precorneal retention, drug penetration and consequently increased the drug levels in ocular tissues compared to free drug formulation. The study also revealed that, the highest drug level deposited in the cornea and sclera followed by aqueous humor and vitreous body. The pharmacokinetic parameters (AUC and  $C_{max}$ ) and the significance t-test levels (P values) for the difference between entrapped and free drug were calculated. It was found that the majority of differences were very highly to highly significant. These findings indicate that entrapment of drug in liposomes may be promising formulation, particularly when dispersed in highly viscous system, for ophthalmic therapeutic use.*

### INTRODUCTION

The anatomical structure and the protective physiological process of the eye exert a strong

defense against ocular drug delivery. Limited absorption of the drug through the lipophilic corneal barrier is mainly due to short precorneal residence time related tear turnover, rapid

nasolacrimal drainage of instilled drugs and non-productive absorption through the conjunctiva. For these factors a small proportion (1-3%) of the applied drug penetrates the cornea and reaches intraocular tissues.<sup>1,2</sup>

Phospholipid vesicles (liposomes) have also been used to formulate a variety of hydrophobic, poorly soluble drugs.<sup>3,4</sup> In particular, encapsulation of chemotherapeutic agents in Liposomes formulations may result in an improvement of the biological activity both *in-vitro* and *in-vivo*, leading to a reduction in side effects, an increase in the cellular and bacterial penetration and may ensure a more biodistribution of the drug.<sup>5-8</sup>

It is well known that Liposomes, like other colloidal systems, have a great potential in ocular drug delivery with the advantage of being completely biodegradable, relatively non toxic as compound with traditional ophthalmic preparations.<sup>9-12</sup>

Due to the low viscosity of colloidal suspensions that does not allow sufficient retention time of the dosage form, this approach needs to be improved. One other strategy used to delay the precorneal drainage rate of liposomal formulations is coating the vesicles with polymers. Bochot *et al.*<sup>13</sup> improved the corneal bioavailability of a model drug via dispersion of liposomes in thermosensitive gel. The study of Pleyer *et al.*<sup>14</sup> have investigated the significant improvement of cyclosporine A distribution in cornea and anterior sclera when liposome-encapsulated drug incorporated into collagen shields. Durrani and Davies<sup>15</sup> have demonstrated that carbapol 1342-coated liposomal formulations increased the bioavailability of pilocarpine in rabbit eye compared with uncoated preparation.

The drug of choice in this study was flurbiprofen, which is well tolerated when used in treatment of ophthalmic disorders. It is one of the NSAIDs with valuable effect in prevention of postoperative ocular inflammation with insignificant side effect when instilled as eye drops in 0.03% concentration. The drug is also administered topically to the eye prior to some ocular surgeries (e.g. cataract extraction) in

order to prevent intraoperative miosis.<sup>16</sup>

The aims of this study were: 1) Preparation of liposomal formulation of non-steroidal anti-inflammatory drugs "Flurbiprofen" dispersed in thermosensitive gel. The gel was prepared from pluronic F 127; 2) investigation of the *in-vitro* release profile of the entrapped and free drug from the same gel concentration; 3) Quantitatively evaluate the time course of ocular distribution and disposition of topically applied flurbiprofen in different eye tissues.

## EXPERIMENTAL

### Materials

The following materials were used as received. Flurbiprofen ([±]-2-fluoro- $\alpha$ -methyl-4-biphenyl acetic acid) was purchased from Sigma Chemical Co., St. Louis, MO. Egg phosphatidylcholine (EPC) from Egg Yolk was purchased from Wako Pure Chem., Industries Ltd., Japan. Cholesterol (CHOL) was purchased from Nacali Tesque Inc. Kyoto, Japan. Poloxamer (POLOX) 407 (pluronic F 127, BASF) was a gift from Asahia Denka Co., Kyoto Japan. Nembutal was purchased from Dainippon pharmaceutical Co. Ltd. Japan.

### Methods

#### Preparation and characterization of liposomes

EPC of 0.25 mmol, 0.11 mmol CHOL and 0.102 mmol Flurbiprofen were dissolved in chloroform ( $\approx$  50 ml) in round bottom flask (100 ml volume) which is fitted into the rotatory evaporator (Rotavapor Model RE 111 SIBATA, Japan). A vacuum pump was used during drying process, and the condenser should be cooled with liquid nitrogen until complete evaporation of chloroform. The flask was kept in vacuum desiccator overnight for full dryness.<sup>17</sup> The thin dry film coating the flask was rehydrated by addition of 5 ml of phosphate buffer saline (PBS) at pH 7.4 previously filtered through 0.22  $\mu$ m syringe filter attached. The suspension was sonicated for 10 min in a bath sonicator and sonicated for additional 3 min with ultrasonic prob. (Nissei ultrasonic generator Model US.

300). The resultant vesicles were extruded five times through 0.2  $\mu\text{m}$  polycarbonate membrane under nitrogen gas purge (nitrogen gas pressure should be kept between 15-20 kg f/cm<sup>2</sup>). The liposomal suspension was collected and kept at 4° until be used. The volume was adjusted by PBS until the drug concentration became 0.5% w/v (stock).

The liposomes were separated from non-entrapped flurbiprofen by centrifugation at 2500 rpm in ultrafiltration membrane cones for 1/2 hr, the supernatant removed and the amount of drug entrapped was determined as the difference between the initial drug concentration in liposome suspension and in supernatant by HPLC system (LC-6A shimadzu, CO. Ltd., Japan).<sup>20</sup>

The particle size of the liposomes was measured in a dynamic light scattering spectrophotometer (LS-900, Otsuka Electronics, Osaka, Japan). The zeta potential of the liposomes was determined with a laser electrophoresis zeta-potential analyzer (LEZA-500T, Otsuka Electronics, Osaka, Japan).

#### Preparation of ophthalmic gels

An appropriate amount of POLOX powder (20% w/w) was dissolved in cold PBS (pH 7.4), previously filtered through 0.45  $\mu\text{m}$  millipore filter, and the appropriate amount of flurbiprofen was also dissolved to give drug concentration of 0.05% w/w. The mixture was left overnight in refrigerator to affect complete desolvation of the polymer. Warming the mixture to room temperature a clear viscous gel was formed. The gel was sterilized by keeping in boiling water bath for one hour. For preparation of ophthalmic liposome dispersed in the gel, an appropriate weight of liposomal suspension which give flurbiprofen concentration of 0.05% w/w was dispersed in cold sterilized solution of poloxamer (20% w/w) under laminar flow. The two ophthalmic gels were packed in tightly closed neutral glass container at 4° until ready for use.<sup>13</sup>

#### In-vitro release of flurbiprofen

The release of flurbiprofen, from gel containing either free or liposomal entrapped drug, were carried out under sink condition. The

membrane (25 mm diameter and 0.2  $\mu\text{m}$  pore size, FR 20 type, Fuji Photo Film Co. LTD Tokyo-Japan) was mounted on the flow-through-type diffusion cell as shown in Fig. 1 (effective diffusion area, 3.14 cm<sup>2</sup>). One g from each formula was placed in the donor compartment over the mounted membrane; the apparatus was thermostated at 37° in water bath throughout the experiment. The donor compartment was tightly covered to prevent the test gel from evaporation during the experiment. The other side of the membrane was continuously washed with PBS (pH 7.4) at a rate of 6 ml/hr (by the aid of Roller Pump, Furue Scientific Co. Ltd. Japan). Magnetic stirrer gently stirred the receptor fluid and three cells were adopted for each formula.<sup>18</sup> The receptor fluid was collected automatically at 0.5, 1.5, 3, 4.5, 6, 7.5, 9, 10.5 and 12 hr (Fraction collection FC 204 Gilson, Germany).

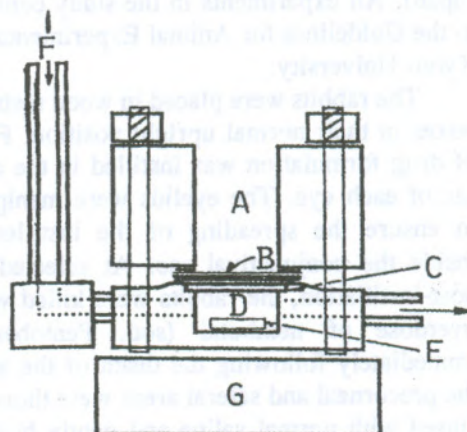


Fig. 1: Schematic view of the flow-through type diffusion cell used for the measurement of drug released *in-vitro*. A. donor compartment, B. membrane, C. screen support, D. receptor compartment, E. stirring bar, F. flow of the receptor fluid, G. magnetic stirrer.

#### Flurbiprofen assay

An HPLC system was used. The collected samples at timed intervals were mixed with 0.5 ml (0.002% w/v n-Butyl-paraben (TCI, Kasei-Tokyo-Japan) dissolved in Acetonitrile; AC, HPLC grade as internal standard IS). The standard solution prepared of 1 ml 0.005% w/v flurbiprofen in 10% AC aqueous solution, 0.5 ml (0.002% w/v IS in AC) and 8.5 ml PBS. The

solutions before injection in HPLC should be mixed well and filtered through 0.45  $\mu\text{m}$  HPLC filter. Analysis was performed on a Cosmosil 5C-18 AR packed column (size 4.6x150 mm, Nacalia, Tesque, Japan). Mixture of water, acetonitrile and glacial acetic acid (550:400:50) was used as the mobile phase with flow rate of 1.5 ml/min. Retention of the drug was monitored with UV spectrophotometric detector (SPD-6AV, Shimadzu, Japan) at 254 nm.<sup>19</sup>

#### ***In-vivo* performance of free and entrapped flurbiprofen**

Male Nippon albino rabbits, 2.0-3.0 kg were individually housed in cages in an air conditioned room and maintained on standard laboratory diet (ORC 4, Oriental Yeast, Tokyo, Japan). All experiments in the study conformed to the Guidelines for Animal Experimentation in Kyoto University.

The rabbits were placed in wood restraining boxes in their normal upright position. Fifty  $\mu\text{l}$  of drug formulation was instilled in the cul-de-sac of each eye. The eyelids were manipulated to ensure the spreading of the instilled dose inside the conjunctival sac. At selected times post-instillation, the rabbits were killed with an overdose of nembutal (sod. Pentobarbital). Immediately following the death of the animal, the precorneal and scleral areas were thoroughly rinsed with normal saline and gently blotted to remove excess fluid. The whole eye was enucleated from its socket and re-washed with saline and gently blotted. The aqueous humor samples were collected by a 26-gauge needle, attached to a syringe, introduced into the anterior chamber through the cornea. Vitreous body samples were withdrawn with 1 ml disposable syringe without needle after the junction between cornea and sclera had been cut with surgical knife.<sup>20</sup> The cornea and sclera were removed and washed with normal saline and gently blotted to remove residual fluid.<sup>21</sup> Each segment of the eye was put in suitable stoppered plastic tubes that had been immersed in broken ice after weighing the tube before and after packaging.

#### **Quantitative determinations of deposited flurbiprofen in different rabbits eye tissues**

The drug was extracted from the different tissues by adding PBS (pH 7.4) into the tissue in ratio of 5:1 (PBS:tissue, by weight) in tube, the tissue was homogenized by utilizing a homogenizer with metallic prop for sclera and cornea (Bio-miner, Nesei, Japan) and with plastic prop for soft segments as aqueous humor and vitreous body (OMNI TH, Yamato, Scient. Co. Ltd. Tokyo, Japan). Seven ml of acetone have been added to one gram (or the entire amount if it is less than one gram) of the homogenized tissue in stoppered glass test tube. The test tubes were shaken mechanically for 10 min, centrifuged for 10 min at 4° and 1500 rpm (TOMY, Refrigerator centrifuge RL-101, Japan). The clear acetonic layer was separated from the homogenized tissue and weighed accurately. The acetone was evaporated at 65-80°. The residue was dissolved in 200  $\mu\text{l}$  AC containing IS and 300 ml PBS and mixed in vortex for 1 min, the solution was filtered through 0.45  $\mu\text{m}$  HPLC filter and injected in HPLC apparatus.

A preliminary experiment, for drug protein binding, was carried out with the same protocol but the PBS had been previously saturated with flurbiprofen and added to non-drug treated rabbit eye tissues<sup>22</sup> to elucidate the amount of bound drug with protein, if the percent of the extracted drug is less than 70%, the experiment is not valid.

## **RESULTS AND DISCUSSION**

#### **Zeta potential and size measurements**

The measurement of zeta potential showed no important modification after incubation of Liposomes with poloxamer gel because this type of Liposomes was very close to neutrality. The measured zeta potential were  $(-3.27 \pm 0.48$  and  $3.98 \pm 0.35$  mV; before and after incubation with poloxamer gel, Table 1. The size of Liposomes particles was increased from  $124.6 \pm 31.35$  before dispersion to  $158.4 \pm 20.99$  nm,  $n = 3$ , Table 1. This confirmed that poloxamer had been adsorbed on the surface of liposomes and

**Table 1:** Particle size and Zeta potential of liposomes suspended in PB (pH 7.4) and in poloxamer gel.

	Liposomes in PBS	Liposomes in poloxamer gel
Particle size (nm)	124.6±31.35	158.4±20.99
Zeta potential (mv)	-3.27±0.48	-3.98±0.35

Values are means ± SD (n= 4)

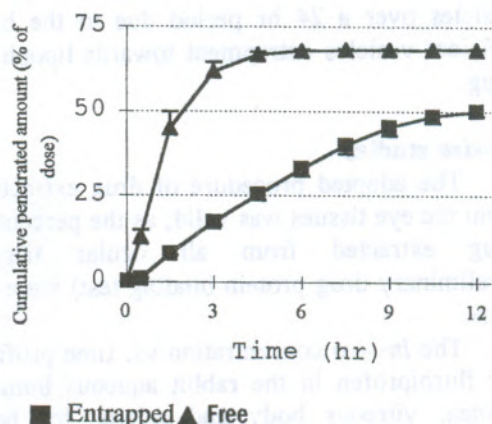
protected it from destabilization. The study of Bochot *et al.*<sup>13</sup> Suggested clearly that poloxamer can be adsorbed on the surface of Liposomes via its penetration of the lipid bilayer of EPC liposomes and project their polyoxyethylene groups from the vesicle surface.

#### *In-vitro* release of flurbiprofen

To evaluate the carrier capacity of liposomes as a drug delivery device, the drug release profile should be investigated. As shown in Fig. (2), the release of flurbiprofen from liposome suspended in poloxamer gel (entrapped drug) was characterized by gradual drug release that was extended for 12 hr. The cumulative percent of the drug released throughout this period was 50% which was significantly lower than that released from drug poloxamer gel (free drug) with the same drug concentration (0.05% w/w) as the cumulative percent was ≈69%. Comparing the cumulative percent throughout the first four hours of *in-vitro* release data (the same period for *in-vivo* study). It was illustrated that the difference was highly significance (P= 0.01). In all cases these differences are due to the good entrapment of the drug within liposomes (≈52.6% ±0.73 S.D. n= 3) as the drug is lipophilic and have an affinity to the lipid nature of liposomes layers. The kinetic of flurbiprofen released from the two formulations are illustrated in Table 2. It was revealed that the mechanism of drug release followed first order kinetic and the halfives for the drug released from entrapped drug was 26 hr which is so much higher than that of free drug (6.27 hr).

In the present study the leakage of entrapped lipophilic flurbiprofen drug from the EPC liposomes incubated with poloxamer gel

was reduced when compared to that of the gel without liposomes (free drug). This may be due to the formation of a high-density cloud from poloxamer around the vesicles that reduce dramatically the permeability of liposomes to flurbiprofen. As suggested by Torchilin *et al.*,<sup>23</sup> the flexible polyethylene glycol on the liposomes surface forms a conformational zone with high density in its central part. Therefore a relatively number of water-soluble, very flexible polymer molecules, as poloxamer in the present study, can create a sufficient number of high density conformation cloud over the vesicle surface, forming a protective umbrella, so dispersion of sterically stabilized liposomes into a thermosensitive gel was developed.



**Fig. 2:** Release profiles of flurbiprofen from entrapped and free drug formulations into PBS (pH 7.4) at 37°C, each point represents the mean ± S.E. (n= 3).

**Table 2:** First-order release rate constant ( $Kh^{-1}$ ) and half lives time ( $t_{1/2}$ , h) of flurbiprofen released from entrapped and free drug formulations.

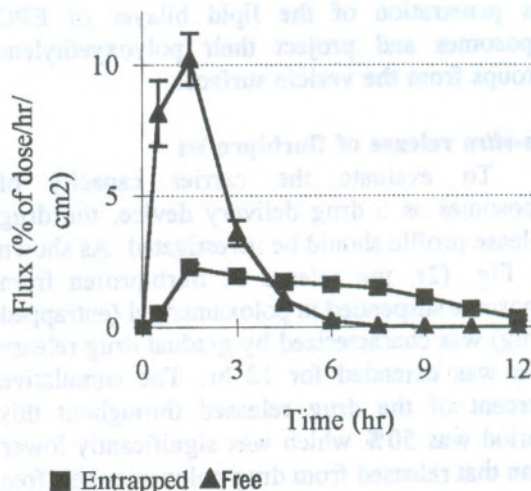
	Entrapped drug	Free drug
r= correlation coefficient	0.992	0.968
$Kh^{-1}$	0.267	0.1110
$t_{1/2}$ (h)	26.00	6.27

The efflux of entrapped and free flurbiprofen was illustrated in Fig (3). It was depicted that the percent of drug/h/cm<sup>2</sup> exhibits prolonged efflux rate for entrapped drug, which seems to be localized around the range from 2.27-1.12% and extended over a period of 9 hr. On the other hand a high and fast efflux rate was noticed for free drug which was ranged from 8-3.6 over a period of 3 hr. The drastically difference in efflux rate owing to the entrapment effect of vesicles towards drug, particularly our drug here, was lipophilic. Literatures suggested that ocular delivery via liposomes might benefit lipophilic compounds to a greater extent than hydrophilic compounds.<sup>24</sup> Taniguchi *et al.*<sup>25</sup> studied the release of dexamethasone valerate from phospholipid vesicles and showed that less than 5% of the steroid was released from the vesicles over a 24 hr period due to the high efficient vesicles entrapment towards lipophilic drug.

#### *In-vivo* studies

The adopted procedure of drug extraction from the eye tissues was valid, as the percent of drug extracted from all ocular tissues (preliminary drug protein binding test) were > 70%.

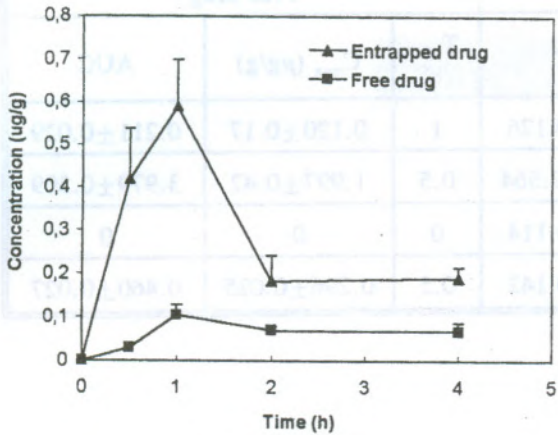
The *in-vivo* concentration vs. time profiles for flurbiprofen in the rabbit aqueous humor, cornea, vitreous body and sclera, for both formulation (entrapped and free drug) are illustrated, in Figures 4,5,6 and 7 respectively. These concentrations were measured after topical instillation of 50  $\mu$ l of 0.05% w/w for both formulations.



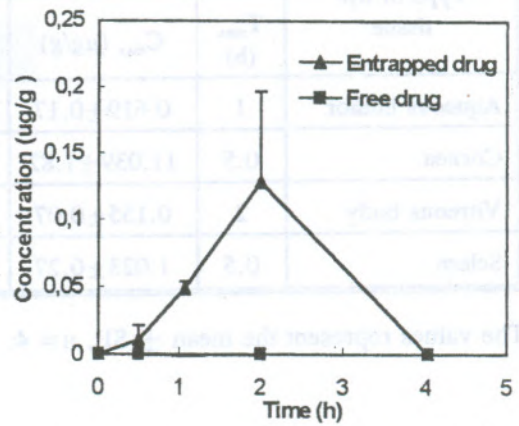
**Fig. 3:** Efflux of flurbiprofen from entrapped and free drug formulations into PBS (pH 7.4) at 37°C, each point represents the mean  $\pm$  S.E. (n= 3).

In all ocular tissues the levels of entrapped flurbiprofen were higher than those for free drug in gel. The highest flurbiprofen levels were observed in the cornea and sclera followed by aqueous humor and vitreous body, respectively. Table 3 depicts the values of pharmacokinetic parameters for entrapped and free drug in various ocular tissues.

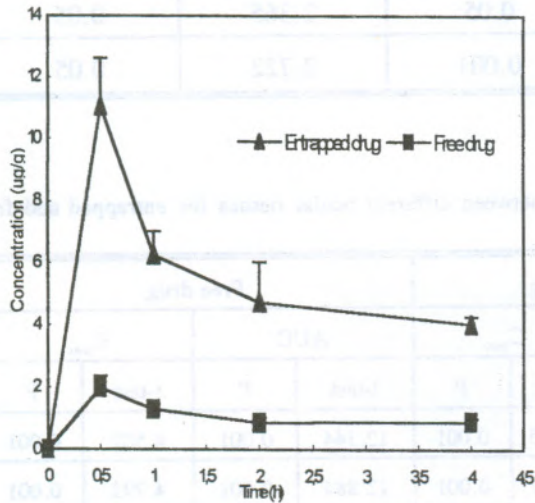
The time of maximum concentration ( $T_{max}$ ) for cornea and sclera (for both drug formulations) are the same, 0.5 hr indicating that the similarity in the pathway and the route of drug absorption to the eye.



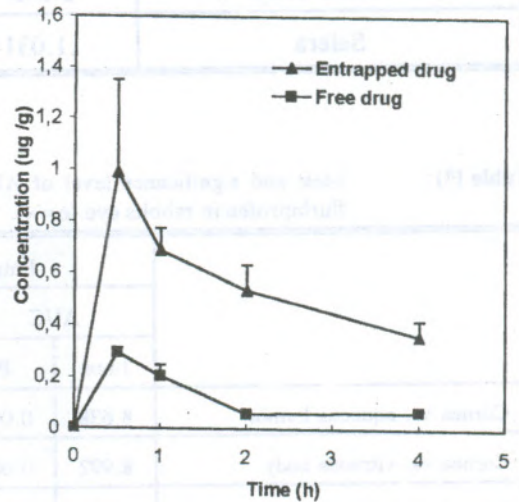
**Fig. 4:** Concentration vs. time profiles in the aqueous humor of rabbits eyes post instillation of 50 ul (0.05% w/v) flurbiprofen (entrapped and free drug formulations); the bars represent the standard error of the mean of four eyes.



**Fig. 6:** Concentration vs. time profiles in the vitreous body of rabbits eyes post installation of 50 ul (0.05% w/v) flurbiprofen (entrapped and free drug formulations); the bars represent the standard error of the mean of four eyes.



**Fig. 5:** Concentration vs. time profiles in the cornea of rabbits eyes post instillation of 50 ul (0.05% w/v) flurbiprofen (entrapped and free drug formulations); the bars represent the standard error of the mean of four eyes.



**Fig. 7:** Concentration vs. time profiles in the sclera of rabbits eyes post installation of 50 ul (0.05% w/v) flurbiprofen (entrapped and free drug formulations); the bars represent the standard error of the mean of four eyes.

**Table 3:** Values of pharmacokinetic parameters for flurbiprofen in various ocular tissues after topical instillation of entrapped and free drug formulations.

Type of eye tissue	Entrapped drug			Free drug		
	T <sub>max</sub> (h)	C <sub>max</sub> (μg/g)	AUC	T <sub>max</sub> (h)	C <sub>max</sub> (μg/g)	AUC
Aqueous humor	1	0.619±0.17	1.065±0.126	1	0.120±0.17	0.211±0.029
Cornea	0.5	11.039±1.82	21.514±2.364	0.5	1.997±0.42	3.979±0.309
Vitreous body	2	0.155±0.07	0.234±0.114	0	0	0
Sclera	0.5	1.023±0.27	2.050±0.142	0.5	0.296±0.025	0.460±0.027

The values represent the mean ± SE, n = 4.

**Table (4)** t-test and significance level (P values) of AUC, C<sub>max</sub> between entrapped and free flurbiprofen for different eye rabbit tissue (n=4).

Pairs of comparison between entrapped and free drug	AUC		C <sub>max</sub>	
	t-test	P	t-test	P
Aqueous humor	6.580	0.001	2.852	0.01
Cornea	7.355	0.001	4.839	0.001
Vitreous body	2.045	0.05	2.365	0.05
Sclera	11.031	0.001	2.722	0.05

**Table (5)** t-test and significance level of AUC, C<sub>max</sub> between different ocular tissues for entrapped and free flurbiprofen in rabbits eye (n=4).

	Entrapped drug				Free drug			
	AUC		C <sub>max</sub>		AUC		C <sub>max</sub>	
	t-test	P	t-test	P	t-test	P	t-test	P
Cornea vs. aqueous humor	8.638	0.001	5.695	0.001	12.144	0.001	4.502	0.001
Cornea vs. vitreous body	8.992	0.001	5.972	0.001	12.884	0.001	4.793	0.001
Cornea vs. sclera	8.219	0.001	5.442	0.001	11.349	0.001	4.076	0.001
Aqueous humor vs. vitreous body	4.875	0.001	2.497	0.01	7.058	0.001	16.884	0.001
Aqueous humor vs. sclera	5.197	0.001	1.271	0.1	6.159	0.001	5.839	0.001
Vitreous body vs. sclera	9.979	0.001	3.170	0.01	16.857	0.001	12.039	0.001



The area under time concentration curve, AUC, was taken as criterion for measuring the extent of drug absorption in different ocular tissues, while maximum concentration,  $C_{max}$ , was considered as criterion for the extent of drug intensity. The two parameters are illustrated in Table 3 which revealed that the cornea exhibits the higher values when compared to the other tissues with very highly significance differences ( $P= 0.001$ ) for both drug formulations, followed by sclera which also have a higher values with highly to very highly significance differences, except that of the  $C_{max}$ , the difference between sclera and aqueous humor of entrapped drug showed insignificance differences ( $P= 0.1$ ,  $n= 4$ ), Table 5.

It was reported that sclera is a tough and thick fibrous tissues composed of collagen and mucopolysaccharides and the thin conjunctival layer lining the interior sclera has relatively insignificant barrier function against drug.<sup>26</sup> The epithelial cells of the cornea are an extension to those of sclera and the stratified epithelial cells with tight junction of cornea are considered to comprise a lipophilic barrier to corneal drug penetration.<sup>27</sup>

Inspection and comparing the  $C_{max}$  and AUC for both formulations, Table 3 showed that the entrapment of the drug in vesicles resulting in 5.3 and 5 and 5 fold increase in AUC and 5.5, 3.5 and 5.2 fold increase in  $C_{max}$  for cornea, sclera and aqueous humor respectively when compared to these of the free drug.

The statistical analysis of the differences between both formulations with regard to the AUC and  $C_{max}$  for ocular tissues (Table 4), revealed that cornea, sclera and aqueous humor exhibit very highly significant differences ( $P= 0.001$ ) for AUC, while for  $C_{max}$ , the differences were very highly, highly and significant for cornea, aqueous humor, sclera and vitreous body respectively.

Table 3, and Figure 7 revealed that the disposition of the entrapped drug in vitreous body, which seems to be very low when compared to the other ocular tissues, and no drug was detected after instillation of free drug. As previously mentioned cornea and sclera are the major routes of drug absorption to ocular tissues. The results obtained in this study,

particularly for vitreous body, confirming these finding. Anatomical examination of the eye revealed that aqueous humor is the nearest part to cornea and interior sclera, while vitreous body is the farrest one, so a highly significance differences (Table 5) in the amount of drug disposed between vitreous body and aqueous humor or all other ocular tissues ( $P= 0.001$ ,  $n= 4$ ). On the other hand the absence of drug in vitreous body after application of free drug formulation indicating that entrapment of the drug within the phospholipid vesicles prolonged the precorneal residence of the drug and delaying the drug efflux into the cornea.

The results of this study postulated that entrapment of the drug in neutral liposomal vesicles improved ocular absorption and enhanced to a great extent, the bioavailability of drug in the ocular tissues. Several mechanisms have been proposed<sup>28</sup> to elucidate the ocular effect of liposomes, but adsorption or lipid exchanges seem to be most probably involved. It has been proposed that liposomes can insert themselves into the corneal cell membrane or simply remain adsorbed on the cell surface.<sup>28</sup>

Some studies revealed that positively charged vesicles can enhance the bioavailability of entrapped drug over neutral or negatively charged vesicles,<sup>12</sup> because at physiological pH, the corneal epithelium is negatively charged and thus electrostatic attraction may enhances absorption.<sup>24</sup> Singh *et al.*<sup>29</sup> and Schaeffer *et al.*<sup>30</sup> found that topical administration of triamcinolone acetonide and indoxole entrapped in MLV (multilamellar vesicles) neutral liposomes led to higher drug level in ocular tissues than an equal dose prepared as suspension or solubilized with polysorbate 80 in normal saline, respectively. While dihydrostreptomycin entrapped in positively charged vesicles was delivered less effectively to the cornea<sup>31</sup> Lee *et al.*<sup>32</sup> found that the amount of insulin delivered to the cornea and to the iris/ciliary body increased when encapsulated in neutral liposomes formed from EPC with 25 mol % CHOL added.

## Conclusion

Flurbiprofen is a potent non-steroidal anti-inflammatory agent, it was entrapped in EPC

liposomal vesicles by dispersion in thermosensitive gel. The size and zeta-potential, for liposomes before and after incubation in poloxamer gel, were measured. The release rates constant of the first order kinetic and the half lives were calculated and it was found that the entrapped drug was higher than the free drug by 4.1 folds. The *in-vivo* performance of the drug from both formulations was studied. It was revealed that entrapment of the drug enhances, to a greater extent, the precorneal retention, penetration and drug disposition in ocular rabbit tissues when compared to non-entrapped (free) drug. These findings depicted that medicated ophthalmic liposomes, particularly when dispersed in an ocular tolerated gel, may be the promising formulations for ocular drug targeting.

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