

## 1 ANIMAL STUDIES

### 1.1 INTRODUCTION

Generally, topical treatment of extra- or intraocular diseases with eyedrops is the most accepted by patients. Treatment with eyedrops, however, usually entails coping with a poor bioavailability because of the precorneal area, i.e., the site of drug action/absorption, is rapidly cleared of drugs by protective mechanisms of the eye, such as blinking, basal and reflex tearing, and nasolacrimal drainage. From here derives the need of frequent instillations, and hence the risk of side effects (Zambito et al., 2010). Using colloidal drug delivery systems that can increase the retention time of drugs in eyes, such as mucoadhesive nanoparticles and lipid carriers are interesting approach to increase the bioavailability of topically administered drugs. In order to evaluate the retention time or bioadhesion of these nanocarriers *in vivo*, precorneal studies are performed in rabbits which provide useful information for prediction of bioavailability in intraocular sections (Li et al., 2009).

Similarly, in order to study the behaviour of topically instilled fluorescent nanocarriers, ocular distribution studies are performed in mice by using confocal laser scanning microscopy. The combination of fluorescence technique with confocal microscopy provides a comprehensive tool for investigating drug delivery (White et al., 2005). Ophthalmology is one of the fields that take the most advantage of the potential for high-resolution imaging, non-invasive optical sectioning and three-dimensional reconstruction of confocal microscopy combined with sensitive, selective and versatile fluorescent probes (Furrer et al., 1997).

### 1.2 PERMISSION FOR ANIMAL EXPERIMENTS

All animal experiments were approved by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India, New Delhi, India. The protocol number of project was MSU/PHARM/IAEC/2011/23.

### 1.3 *IN VIVO* PRECORNEAL RETENTION STUDIES

#### 1.3.1 Materials and Reagents

TearFlo™ Schirmer filter paper strips (2mm×7mm) were purchased from Contacare Ophthalmics & Diagnostics (Gujarat, India). Syringe filters (0.45 μm) were purchased from Himedia Lab, Mumbai, India. Pipettes, Eppendorf tubes and collecting vials were purchased from Tarson's India. All other reagents were purchased from commercial sources and were of the highest available purity.

#### 1.3.2 Equipments

Ultrasonic Bath Sonicator (Ultrasonics Selec, Vetra, Italy)

High Pressure Liquid Chromatograph (Shimadzu, Kyoto, Japan)

#### 1.3.3 Animals

For precorneal retention studies of prepared formulations (GCV E, TA E, GCV CS HCl/HA NPs and TA lecithin/ CS HCl NPs), New Zealand albino rabbits (male, weighing 2.5–3.0 kg) were used.

#### 1.3.4 Method

The drug concentration in the precorneal area after instillation in rabbits was determined in order to evaluate the precorneal retention of formulations (GCV E, TA E, GCV CS HCl/HA NPs and TA lecithin/ CS HCl NPs) compared with GCV and TA aqueous solutions, by reported method of Li et al (Li et al., 2009).

### 1.4 OCULAR DISTRIBUTION STUDIES

#### 1.4.1 Materials

Optimal Cutting Temperature compound was purchased from Tissue-Tek® Sakura, USA. Sucrose and glycerol and all other analytical reagents were obtained from S.D. fine-chem limited, Baroda, India.

#### 1.4.2 Equipments

Cryostat (IES Minotome plus, International Equipment Company, Massachusetts, USA)

Ultrasonic Bath Sonicator (Ultrasonics Selec, Vetra, Italy)

Confocal Microscope (LSM 510, Carl Zeiss, Oberkochen, Germany)

#### 1.4.3 Animals

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Mouse experimental models are extremely useful in understanding human retinal conditions. Human and mouse genomes have high homology, with some estimates of up to 95% overlap. Moreover, mice have fast generation times and aging, thus reducing maintenance costs. Thus, for ocular distribution studies, un-anesthetized female adult CD1 mice weighting 30–35 g were used. The mice were fed with regular diet.

#### 1.4.4 Method

Ocular distribution studies on prepared formulations were performed as per reported method of Araujo et al. (Araujo et al., 2011). RESULTS AND DISCUSSION

#### 1.4.5 Ganciclovir Emulsomes

##### 1.4.5.1 In vivo precorneal retention time of GCV loaded emulsomes:

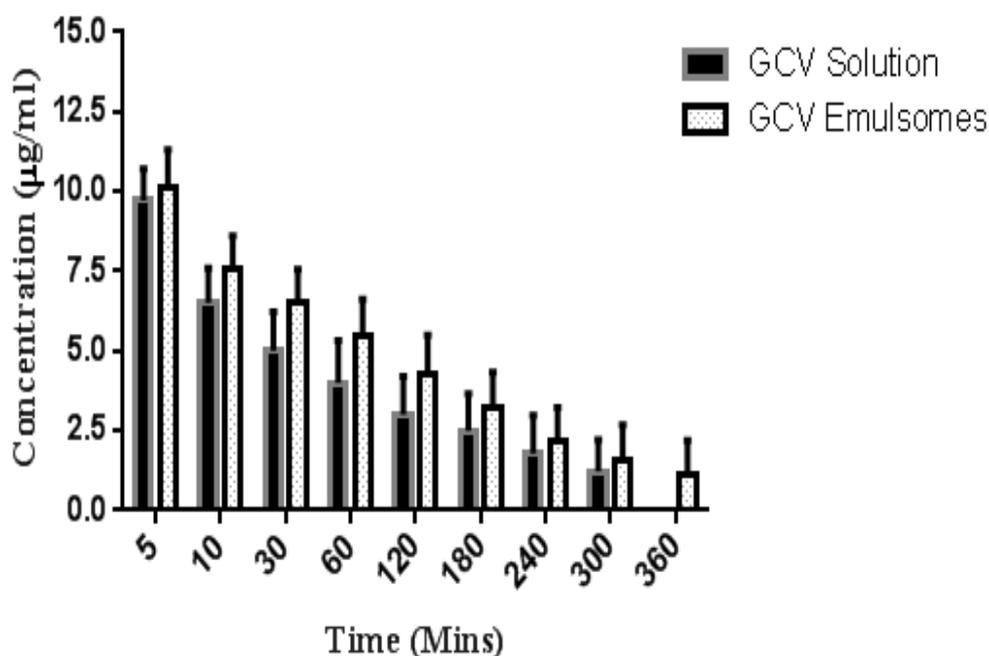


Fig 1.1 Ocular tear concentration of GCV from solution and emulsomes after topical administration in rabbit eye (n=3)

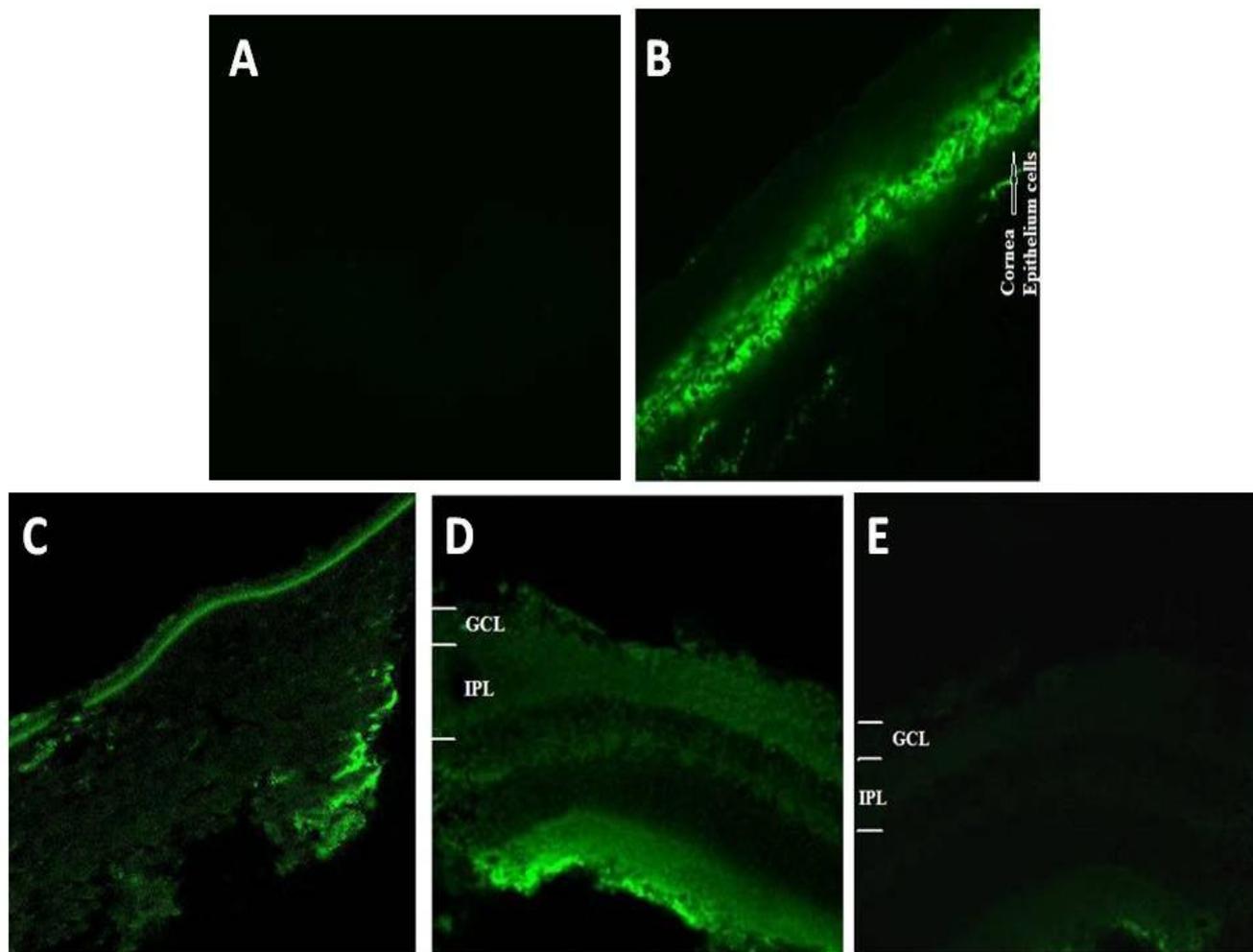
Fig 9.1 shows the plot of GCV concentration ( $\mu\text{g/ml}$ ) vs time profiles in the rabbit lachrymal fluid following the *in vivo* instillation of 150  $\mu\text{l}$  of formulation and GCV solution into the conjunctival sac. Ocular distribution studies of sodium fluorescein loaded emulsomes:

Confocal microscopy was used to study the intraocular distribution of sodium fluorescein loaded emulsomes after they were applied as eye drops in the mice eye.

Figure 9.2 A) shows the retinal images of sodium fluorescein solution after 30 mins of eye drop distribution. No fluorescence was detected in the case of eyes treated with sodium fluorescein solution.

Fig. 9.2 B) shows the image taken at 30 min after application of sodium fluorescein loaded emulsomes in cornea. Corneal epithelium cells are clearly evident in the figure. Strong fluorescence was observed in these cells suggesting the accumulation of emulsomes on corneal epithelium.

Fig. 9.2 C) shows the image of sclera, taken at 30 min after topical application of sodium fluorescein loaded emulsomes. Strong fluorescence observed in these cells suggests that SF might have reached sclera through either corneal or conjunctival route.



**Fig 1.2** Fluorescence microscopic images of Sodium fluorescein solution A) in retina after 60 min B) in cornea after 30 mins, C) in sclera after 30 mins, D) in retina after 60 mins, E) in retina after 120 mins

Fig.9.2 D) and E) shows the images taken at 60 and 120 min after application of sodium fluorescein loaded emulsomes in retina. Triamcinolone acetonide emulsomes:

#### 1.4.5.2 In vivo precorneal retention time of TA loaded emulsomes:

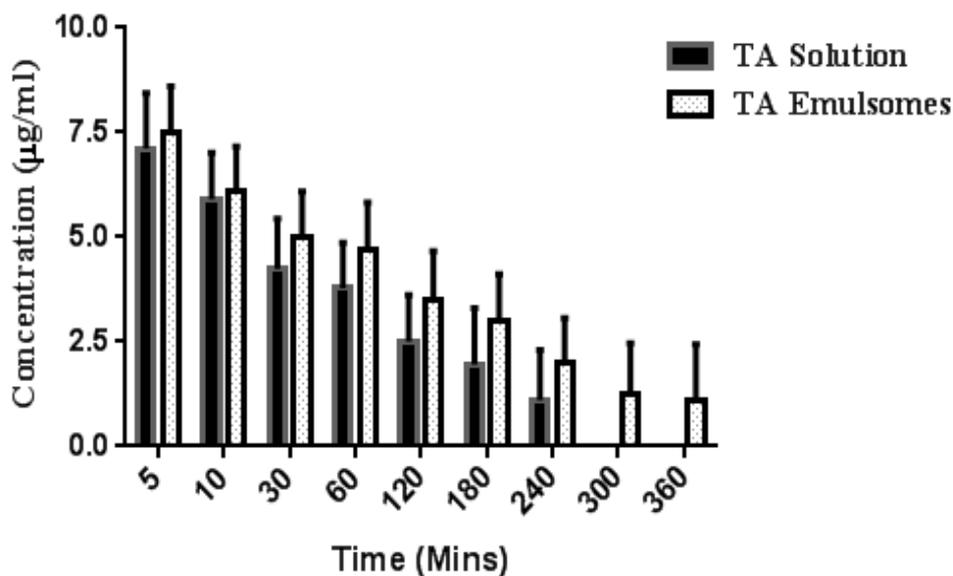
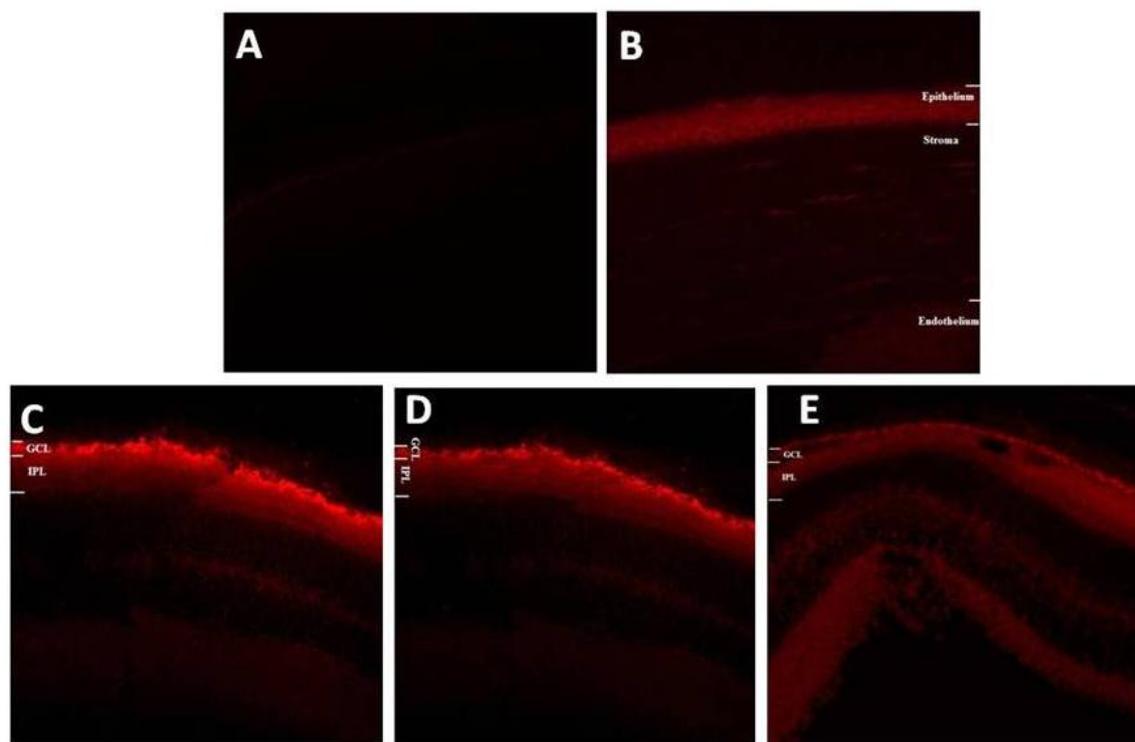


Fig 1.3 Ocular tear TA concentration of TA emulsomes and TA solution in rabbit eye (n=3)

Fig. 9.3 shows the plot of TA concentration ( $\mu\text{g/ml}$ ) vs time profiles in the rabbit lachrymal fluid following the *in vivo* instillation of 150  $\mu\text{l}$  of formulation and TA solution into the conjunctival sac.

#### 1.4.5.3 Ocular distribution studies of Nile Red loaded emulsomes:



**Fig 1.4 Fluorescence microscopic images of A) Nile red solution in retina after 30 mins, B) Nile red loaded emulsomes in cornea after 10 mins, C) in retina after 30 mins, D) in retina after 60 mins, E) in retina after 120 mins**

Figure 9.4 A) shows the retinal images of mice eye after 30 mins of Nile red solution administration. No fluorescence was detected in the case of eyes treated with Nile red solution.

Fig. 9.4 B) shows the corneal image of mice eye after 30 mins of topical administration of NR loaded emulsomes.

Further, Figs. 9.4 C), D) and E) shows the fluorescence microscopic images of Nile red loaded emulsomes in retina after 30, 60 and 120 mins of topical application, respectively

The strong fluorescence in the IPL layer suggests that these Nile red loaded emulsomes might have reached the posterior segment of eye by either corneal or conjunctival pathways, or both.

### 1.4.6 Ganciclovir loaded Nanoparticles

#### 1.4.6.1 In vivo precorneal retention time of GCV loaded nanoparticles

Fig.9.5 shows the plot of GCV concentration ( $\mu\text{g/ml}$ ) vs time profiles in the rabbit lachrymal fluid following the *in vivo* instillation of 150  $\mu\text{l}$  of formulation and GCV solution into the conjunctival sac.

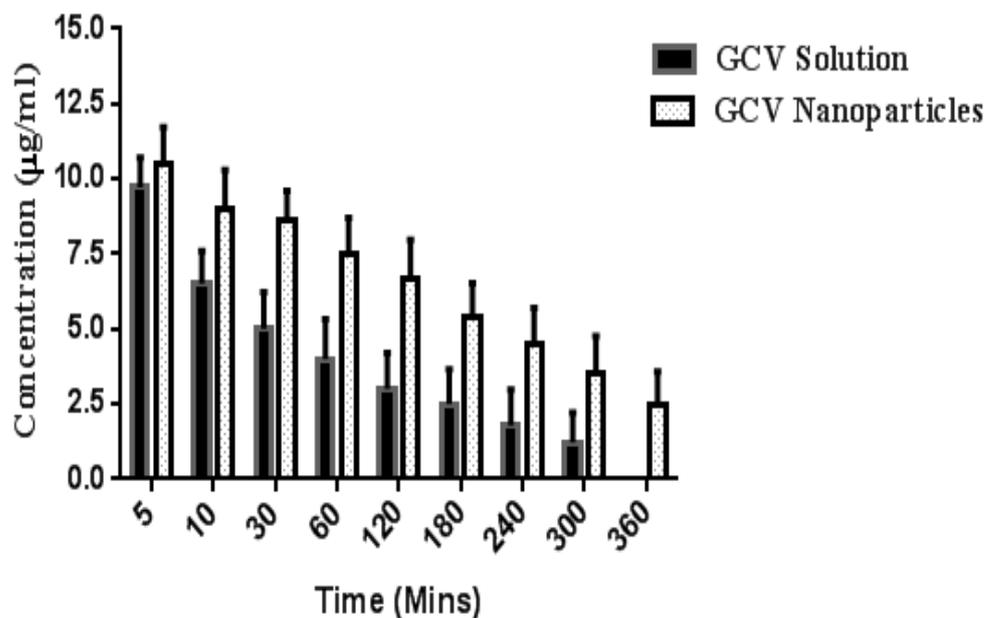
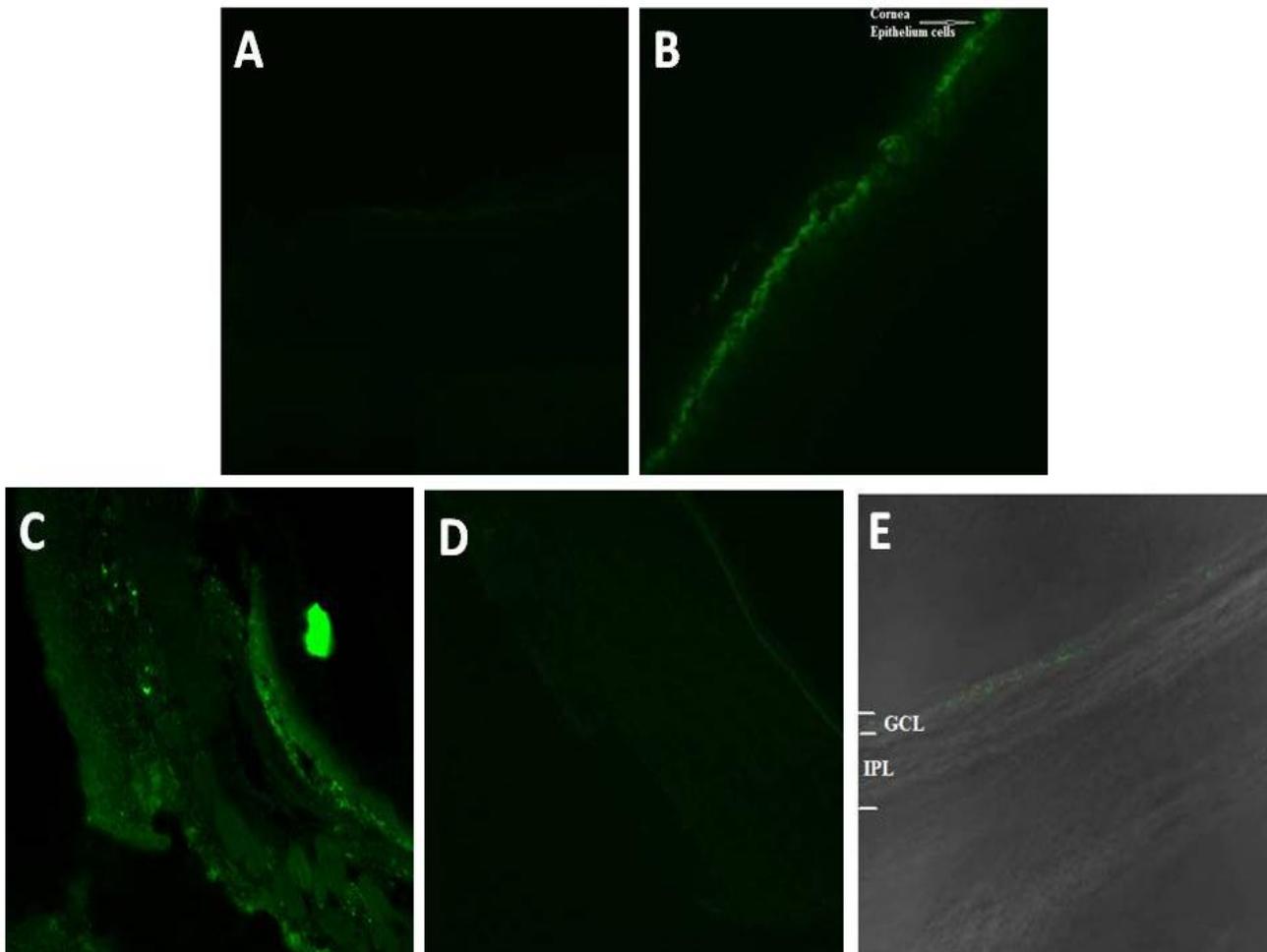


Fig 1.5 Ocular tear concentration of GCV in solution and CS HCl nanoparticles after topical administration of rabbit eye (n=3)

#### 1.4.6.2 Ocular distribution studies of sodium fluorescein loaded nanoparticles:



**Fig 1.6** Fluorescence microscopic images of sodium fluorescein solution in retina after 30 mins, B) sodium fluorescein loaded nanoparticles in cornea after 30 mins, C) in conjunctiva after 30 mins, D) in sclera after 30 mins, E) in retina after 30 mins

Figure 9.6 A) shows the retinal images of mice eye after 30 mins of sodium fluorescein solution administration. No fluorescence was detected in the case of eyes treated with sodium fluorescein solution.

Fig. 9.6 B) and C) shows the image of mice cornea and conjunctiva, respectively, after 30 mins of topical administration of sodium fluorescein loaded nanoparticles.

Fig.9.6 D) showed the image of sclera after 30 mins of eye drop administration.

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Fig 9.6 E) shows the image of retina after 30 mins of eye drop administration. Despite weak fluorescence in sclera section, no fluorescence was observed in the retinal section of mice eye indicating inability of chitosan/HA nanoparticles to reach into the posterior segment of eye either by corneal or non- corneal route through topical application.

#### 1.4.7 Triamcinolone acetonide loaded Nanoparticles

##### 1.4.7.1 In vivo precorneal retention time of TA loaded nanoparticles:

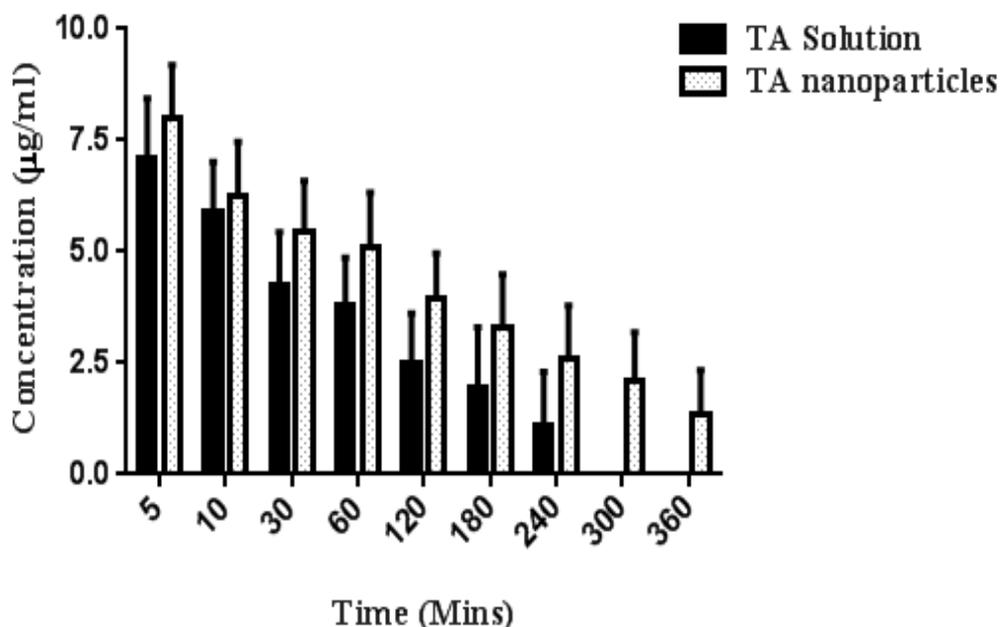
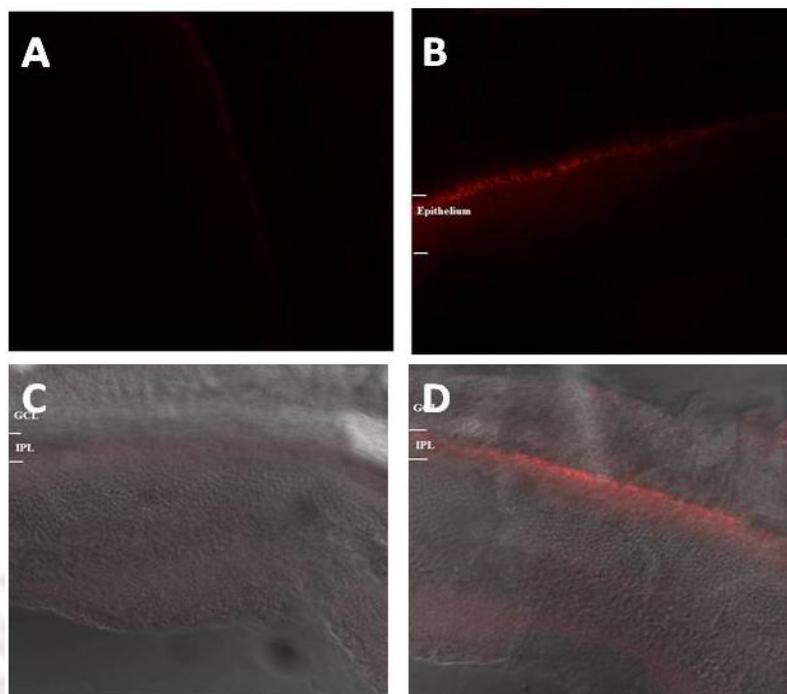


Fig 1.7 Ocular tear concentration of TA in solution and CS HCl nanoparticles after topical administration in rabbit eye (n=3)

Results showed that, in the first few minutes, the TA tear film concentration was high in both TA solution and TA nanoparticles. After 10<sup>th</sup> min, concentration of TA in precorneal area decreased in TA solution which could be because of the mechanical elimination of the excess of instilled volume from the cul-de-sac. These positively charged nanoparticles will have retained for a longer period of time on ocular surface and then gradually got transported inside the eye.

### 1.4.7.2 Ocular distribution studies of Nile Red loaded nanoparticles



**Fig 1.8** A) Fluorescence microscopic images of Nile red solution in retina after 30 mins, B) Nile red loaded nanoparticles in cornea after 30 mins, C) in retina after 30 mins, D) in retina after 60 mins

Figure 9.8 A) shows the retinal images of mice eye after 30 mins of Nile red solution administration. No fluorescence was detected in the case of eyes treated with Nile red solution.

Fig. 9.8 B) shows the image of mice cornea after 30 mins of topical administration of Nile red loaded nanoparticles. Fig 9.8 C) and D) show the fluorescence microscopic images of Nile red loaded nanoparticles in retina after 30 and 60 mins of topical administration of nanoparticles. No fluorescence was observed in the retina section of eye indicating, inability of these carriers to reach the posterior segment of eye by topical administration through either corneal or conjunctival route.

**1.5 REFERENCES**

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