

Timolol Maleate loaded Chitosan Mucoadhesive Nanoparticles for Ocular drug delivery system

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ABSTRACT

Many effective anti-glaucoma drugs, available for the treatment of ocular hypertension and open angle glaucoma are associated with rapid and extensive precorneal loss caused by drainage and high tear fluid turnover. The objective of this study involved the design of mucoadhesive nanoparticulate carrier system containing Timolol Maleate for ocular drug delivery to improve its corneal absorption. Nanoparticles were prepared by template polymerisation method and the process optimisation using different combination ratios of mucoadhesive polymers was carried out. The developed nanoparticle was checked for its particle size and zeta potential.

The *invitro* release characteristics of the optimised nanoparticle were compared with the marketed formulation. The potential irritancy was evaluated using HET-CAM method. Transcorneal permeation data was performed using goat cornea. The study was carried out with an aim of giving a promising delivery system for management of glaucoma.

Keywords: mucoadhesive, nanoparticle, timolol maleate, chitosan, glaucoma.

1 MATERIALS USED

Timolol Maleate (TM), Chitosan (CS), Acrylic acid (AA), Potassium persulfate ($K_2S_2O_8$), Sodium Hydroxide pellets, Sodium Chloride, Sodium dicarbonate, Calcium chloride dihydrate, Potassium chloride.

2 METHODOLOGY

2.1 Preformulation studies

Preformulation studies which encompass the purity of drug candidate, its calibration curve in artificial tear fluid and the compatibility between the drug and the selected polymers were evaluated.

2.2 Formulation and optimisation of chitosan-polyacrylic acid (CS-PAA) nanoparticles

Different batches of CS-PAA nanoparticles were prepared by template polymerisation method. In order to induce polymerisation of acrylic monomers, an indicator potassium persulfate was added to the solution of chitosan dissolved in 50ml of acrylic acid. The pH value of the system was maintained at about 4. The mixture was placed at 70°C, under magnetic stirring and nitrogen atmosphere. The reaction was stopped once the solution became opaque. The solution was dialyzed against distilled water in a 12-14 KD cut off membrane for three days, to remove the indicator and acrylic molecules that did not react.

2.3 Evaluation of CS-PAA nanoparticles

2.3.1 Particle size, zeta potential and poly dispersity index

Out of the different batches prepared, the batch which showed good opalescence was dialyzed against water for 72 hours and evaluated for particle size, zeta potential and poly dispersity index.

2.3.2 Encapsulation efficiency (EE)

The Timolol Maleate loaded CS-PAA nanoparticles were tested for their encapsulation efficiency

$$EE = \frac{TD - FD}{FD} \times 100$$

Where TD- Total amount of drug
FD- Free amount of drug

2.3.3 *Invitro* drug release study

This was studied by using dialysis bag diffusion technique using the medium as simulated tear fluid of pH 7.4 at 37±0.5°C.

2.3.4 *In vitro* transcorneal permeation study

Goat corneas were used to study the permeation of Timolol Maleate across the corneal membrane. Whole eye-balls of goat were procured from a slaughter house and transported to laboratory in cold condition in normal saline maintained at 4°C. The corneas were carefully removed along with a 5–6 mm of surrounding scleral tissue and washed with cold saline. The washed corneas were kept in cold freshly prepared solution of tear buffer of pH 7.4. The study was carried out in Franz diffusion cells. The whole system was maintained at 37 ± 0.5°C. After a predetermined period, 5ml of the medium was removed and the amount of Timolol Maleate was analysed for 6hrs by UV-Visible spectrometer at wavelength of 294nm

2.3.5 Irritancy test (HET-CAM method)

Chorioallantoic Membrane (CAM) was treated with the test formulation and the development of endpoints like haemorrhage, hyperemia and coagulation were observed.

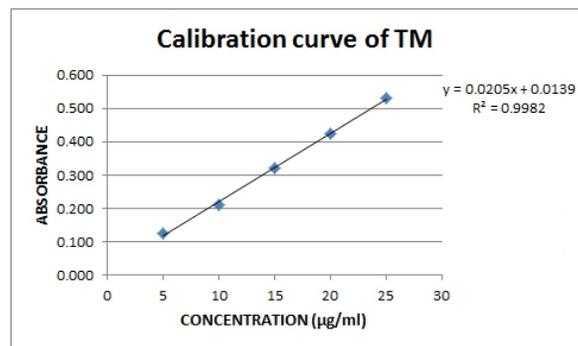
2.3.6 Release kinetics

In order to elucidate mode and mechanism of drug release, the *in vitro* release data obtained for the formulation was fitted into various kinetic models.

3 RESULTS AND DISCUSSIONS

3.1 Preformulation studies

The purity of the drug by non-aqueous titration method was found to be 99.02% which compiled with the official specification of 98.5-101%.



It was concluded that the perfect linearity between the concentration and absorbance was observed when the concentration range was from 5µg/mL to 25µg/mL.

3.2 FTIR

It was found that the selected excipients were found to be compatible in entrapping the selected drug Timolol Maleate.

3.3 Formulation and optimisation of CS-PAA nanoparticles

Synthesis of chitosan-poly(acrylic acid) nanoparticles was done by template polymerisation method. As the polymerization time extended, the amount of PAA in the solution increased, and the system changed initially from a clear solution to an opalescent emulsion indicating the formation of CS-PAA nanoparticles. The electrostatic interaction between PAA (negative charge) and CS (positive charge) promoted the self-assembly of nanoparticles

| UNDER ATMOSPHERIC CONDITIONS | | | | | |
|------------------------------|-------|-------|--|---------------|-------------|
| Batch | AA | CS | K ₂ S ₂ O ₈ | RESULT | Consistency |
| | | | | (opalescence) | |
| F1 | 3 mM | 3 mM | 0.1 mM | Negative | -- |
| F2 | 6 mM | 6 mM | 0.1 mM | Negative | -- |
| F3 | 12 mM | 12 mM | 0.1 mM | positive | Unstable |

| UNDER INERT CONDITIONS | | | | | |
|------------------------|-------|-------|--|---------------|-------------|
| Batch | AA | CS | K ₂ S ₂ O ₈ | RESULT | Consistency |
| | | | | (opalescence) | |
| F4 | 3 mM | 3 mM | 0.1 mM | Less | Stable |
| F5 | 6 mM | 3 mM | 0.1 mM | Moderate | Stable |
| F6 | 12 mM | 3 mM | 0.1 mM | high | Stable |
| F7 | 3 mM | 6 mM | 0.1 mM | less | Stable |
| F8 | 6 mM | 6 mM | 0.1 mM | high | Stable |
| F9 | 12 mM | 6 mM | 0.1 mM | moderate | Stable |
| F10 | 3 mM | 12 mM | 0.1 mM | less | Stable |
| F11 | 6 mM | 12 mM | 0.1 mM | less | Stable |
| F12 | 12 mM | 12 mM | 0.1 mM | Moderate | Stable |

3.4 Evaluation of CS-PAA nanoparticles

Since the optimised formulation F6 and F8 showed appreciable values for the below parameters, they were found ideal for ocular drug delivery

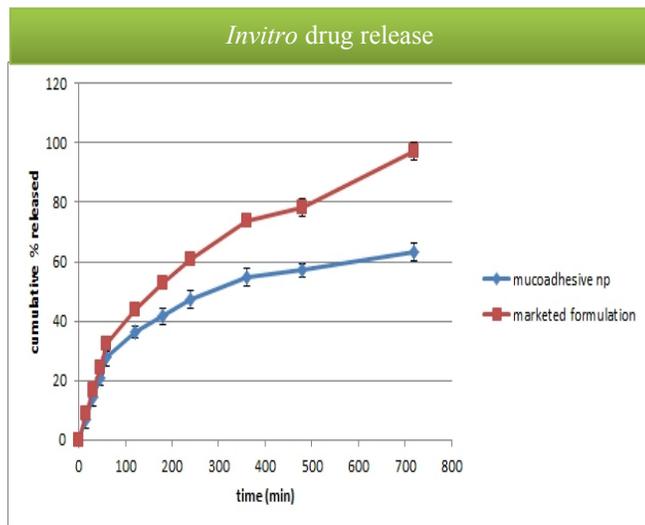
| BATCH | AA | CS | K ₂ S ₂ O ₈ | Z-Average | Zeta potential | PDI |
|-------|-------|------|--|-----------|----------------|-------|
| F6 | 12 mM | 3 mM | 0.1 mM | 378.1nm | -7.42mV | 0.432 |
| F8 | 6 mM | 6 mM | 0.1 mM | 141.29nm | 23.30mV | 0.339 |

3.5 Encapsulation efficiency

Among all the batches, F8 showed the maximum entrapment efficiency of 69.75 %

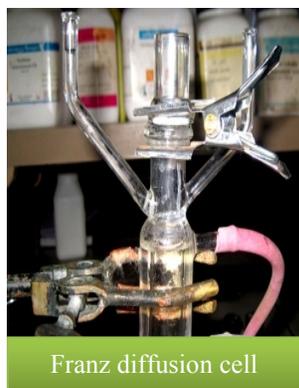
3.6 *In vitro* drug release study

The *in vitro* release of the drug from the polymeric nanoparticles was studied by the dialysis bag diffusion technique. The medium used was simulated tear fluid of pH 7.4 at 37°C. The release profiles of mucoadhesive nanoparticle formulation of Timolol maleate and of commercial eye drop were compared.

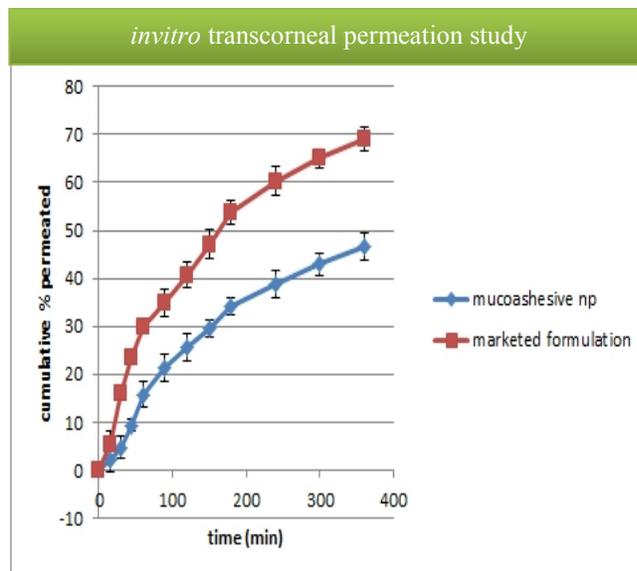


The release profile of Timolol Maleate loaded nanoparticles showed an initial burst release followed by a slow release. Only about 30% drug was released within an hour and then 63% of drug was released within 12hr indicating the sustained release of drug from the nanoparticle core shell. This demonstrated that the mucoadhesive nanoparticle formulation were better able to retain Timolol Maleate.

2.3.4 *In vitro* transcorneal permeation study

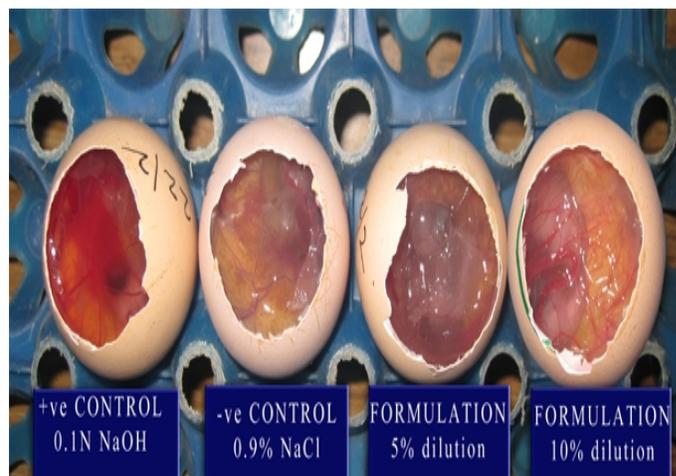


The below graph shows that the permeation rate of the nanoparticle formulation is slower as compared to same concentration of marketed formulation for Timolol Maleate. It indicates that the mucoadhesive nanoparticle formulation gives a sustained effect in ocular milieu.



3.8 Irritancy Test (HET-CAM method)

Since the end points were not observed, the developed formulation is non-irritant

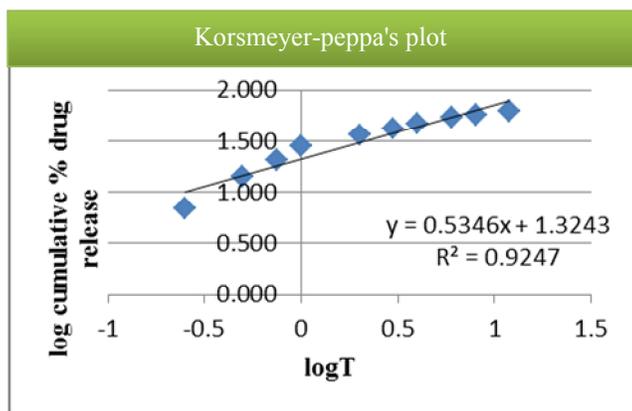


3.9 Release kinetics

The *in vitro* release data obtained for mucoadhesive nanoparticle formulation, in simulated tear fluid pH 7.4, was fitted into various kinetic models. The results are shown in the below table.

| Release kinetics | | | | |
|------------------|----------------|-------------|-----------|--------------|
| FORMULATION | R ² | | | Peppa's plot |
| | Zero order | First order | Higuchi's | |
| Ideal batch | 0.7791 | 0.875 | 0.9502 | N=0.534 |

Korsmeyer-Peppas's plot showed that the developed formulation undergo anomalous diffusion.



4 CONCLUSION

From all of the above studies, it is to be highlighted that the developed formulation is a viable alternative to the conventional eye drops, and due to its sustained effect in ocular milieu, it helps in reducing the dose and dosing frequency which in turn reduces toxicity and also increases patient compliance.

Therefore, this formulation is expected to be promising for management of glaucoma

5 REFERENCE

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