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Original Article

# Design and Characterization of Timolol Loaded Gellan Gum Nanoparticles for Improved Ocular Drug Delivery

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## ABSTRACT

**Aim:** Glaucoma treatment often involves the topical application of Timolol maleate (TM) to the eye. However, achieving the desired bioavailability via the ocular route can be challenging due to poor retention and penetration of the drug. To address this issue, Gellan Gum (GG) polymer was employed in the current research to formulate nanoparticles of TM to enhance its release during ocular delivery. **Materials and Methods:** TM loaded GG Nanoparticles (TMNPs), were formulated by ionotropic gelation method. The study involved the fabrication of different experimental batches of TMNPs, with varying concentrations of gellan gum (0.06% and 0.12%) and aluminium chloride (0.01, 0.02, and 0.03%) and evaluating the impact of these concentrations on the particle size and Entrapment Efficiency (EE%), of TMNPs. The optimized batch of prepared TMNPs was further evaluated for different parameters such as DSC, SEM, FTIR, *in vitro* release, and *ex vivo* tolerance and permeation studies. **Results:** The particle size and EE% of different batches of TMNPs were found to be in the range of 135.2 to 1519 nm and 20.51 to 78.56% respectively. TMNPs showed a prolonged *in vitro* drug release (62.11%) profile for 12 hr. *Ex vivo* ocular tolerance studies confirmed the TMNPs to be non-irritant and safe for ocular drug delivery. Permeation studies revealed that a higher amount of TM from TMNPs (1.9 fold) was taken up by goat's cornea as compared to drug solution. **Conclusion:** In conclusion, TMNPs are found to depict sustained release and can be considered potential and safe carriers for the ocular drug delivery of TM.

**Keywords:** Nanoparticles, Glaucoma, Timolol maleate, Gellan gum, Biopolymer

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## INTRODUCTION

A World Health Organization (WHO) report estimates that by 2022, someone will lose their sight globally every minute and every 5 sec.[1] Worldwide, glaucoma is one of the major causes of irreversible visual loss, and cataracts as a cause of global blindness.[2] Glaucoma disease is represented by optic nerve damage, an increase in Intraocular Pressure (IOP), and reduced retinal sensitivity which leads to loss of vision. The standard healthy intra-ocular pressure range is 15-20 mm Hg and above 22 mm Hg intra-ocular pressure, glaucoma is observed. Aqueous humour circulation in the eye stimulates intraocular pressure. Aqueous humor circulation is the outflow of the eye through two major pathways one that passes out through the trabecular meshwork and schlemm's channel known as the conventional pathway and other is the uveoscleral pathway. Timolol maleate is classified under non-selective beta-blockers which are useful in the treatment of an ocular disease called glaucoma; it reduces the aqueous humor production which ultimately leads to the decreased IOP of the eye. There are many factors which affect the bioavailability of marketed ocular timolol maleate such as blinking reflex, nasolacrimal drainage, lacrimation and drainage by gravity, even the frequent instillation and naturally accompanying patient compliance are also there. Various drug delivery methods have been investigated during the past decades, which mainly included two strategies: to prolong the contact time and increase corneal permeability of drug.[3-5] Nanotechnology based ocular drug delivery systems are a promising platform owing to their small size and customizable surface properties that facilitate corneal penetration via ocular tissues and provide higher ocular bioavailability. Therefore, problems related to the conventional eye drops of TM and unintended side effects are overcome by nanoparticles. Nanoparticles (NPs) enhance drug permeation through corneal as well as non-corneal barriers and also increase the bioavailability of the drug.[6] Several polymers like albumin, chitosan, gelatin, gellan gum, HPMC, polyacrylamide, polymethylmethacrylate, polyalkylcyanoacrylate, polylactic-co-co-glycolic acid, polycaprolactone are used in the preparation of nanoparticles.[7] But natural polysaccharides are said to be the best polysaccharides because they are biocompatible, inexpensive and easily available.[8]

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A water-soluble, natural polysaccharide gellan gum is employed extensively for the development of ophthalmic drug delivery system. GG is naturally produced by the aerobic fermentation of sugar by a bacterium named *Sphingomonaselodea* (earlier *Pseudomonas elodea*) which is gram-negative. Gellan Gum has a linear structure formed of repetition in the molar ratio of 2:1:1 of tetra-saccharide glucose units, glucuronic acid and rhamnose. GG has a free COOH group which makes it an anionic polymer. In the presence of positive ions like $Na^{+}$, $Ca^{2+}$, $Mg^{2+}$, $Zn^{2+}$, $Al^{3+}$ it rapidly undergoes gelation even at a very low concentration of polymer. GG is employed in various dosage forms owing to its drastic distinctive and dynamic properties such as gelling, thickening, good tolerance, much-adhesiveness, non-toxicity, easy biodegradability, and good biocompatibility etc.,[9] Therefore, in this present research TM loaded GG Nanoparticles (TMNPs) were formulated using aluminum chloride as a cross-linker and the prepared TMNPs were further evaluated for various parameters.

## MATERIALS AND METHODS

### Materials

Timolol Maleate (TM) was gifted from FDC Ltd., Raigad. GG and remaining all the chemicals used for the study was purchased from the Research-Lab Fine Chem Industries, Mumbai.

### Preparation of Timolol maleate-loaded GG nanoparticles (TMNPs)

TMNPs were fabricated using the ionotropic gelation method.[10,11] Firstly, GG aqueous solution was prepared at 60-65°C in acidified water of pH-4 and drug solution was prepared via dissolving the TM in distilled water. The TM solution was then introduced dropwise into the gellan gum solution. Subsequently, the drug-polymer mixture was injected dropwise (using a 16-gauze syringe fitted with needles) into an equal volume of aqueous aluminium chloride solution that had been cooled to (4°C) under constant stirring at 1000 rpm. The pH of final mixture of drug-polymer-crosslinker solution was maintained at 7.4. Further, the mixture was continuously stirred for 2 hr at the temperature 25°C for the complete cross-linking reaction to occur.[12] The resultant nanodispersion was then centrifuged at the speed of 12,000 rpm for 15 min at 4°C temperature and the obtained nanoparticles in the form of pellet and supernatant was collected separated. The TMNPs so obtained were lyophilized and were kept in the refrigerator till the time of use. Also, supernatant of TMNPs was extracted separately in order to determine entrapment efficiency.[13] Six trial batches of TMNPs were prepared by varying the concentration of GG (0.06% and 0.12%) and aluminium chloride (0.01, 0.02, and 03%) and their effect on particle size and entrapment efficiency of TMNPs was evaluated (Table 1). The optimized batch (GG 0.06% and aluminium chloride 0.01%) with minimum size and maximum entrapment efficiency was selected for further characterization.

### Characterization of Timolol Maleate Loaded Gellan Gum Nanoparticles (TMNPs)

### Determination of particle size, polydispersity index and zeta potential

Particle size, Zeta Potential (ZP) and PDI (Polydispersity Index) of the developed TMNPs, were analyzed via Dynamic Light Scattering (DLS) technique on Malvern zetasizer (Nano ZS90, Malvern Instruments Ltd., U.K). A homogeneous dispersion was employed for determining the particle size, PDI and ZP which was obtained by diluting the samples with distilled water (10 times).[14]

### Determination of % Encapsulation Efficacy

The supernatant obtained during formulation of TMNPs was collected separately, diluted and analyzed at $\lambda_{max}$ 263 nm using a UV-spectrophotometer against blank NPs supernatant in triplicate (Shimadzu UV1800 Spectrophotometer; Shimadzu Corp., Kyoto, Japan) for determination of unentrapped drug.[15,25] EE% was determined by using the equation given below:

$$EE\% = (WT - WE / WT) * 100\%$$

Where, EE denotes the encapsulation efficiency; WT denotes the total drug amount and WE denote the amount of unentrapped drug.

### Fourier Transform Infrared Spectroscopy (FT-IR)

FT-IR spectrums of the drug Timolol maleate, GG and TMNPs was obtained on FTIR spectrophotometer (Alpha, Bruker, Germany) within 4000-400 $cm^{-1}$ range of scanning using KBr to determine drug loading and drug excipients interaction.[16]

### Differential Scanning Calorimetry (DSC)

DSC thermograms of TM, GG and TMNPs were obtained on DSC (Hitachi High-Tech Analytical Science, UK) in the 25-200°C range of temperature. DSC was conducted to identify the physical state of TM present inside the prepared nanoparticles. All the samples were weighed accurately and sealed in a pan made up of aluminium and the temperature was maintained at 10°C $min^{-1}$ from 40 to 250°C.[17]

### *In vitro* Release Studies

The *in vitro* release studies of the pure TM and TMNPs were performed (12 hr) with the help of USP dissolution apparatus II. TMNPs (drug equivalent to 3 mg/mL) and pure drug TM were dispersed in simulated tear fluid. Samples were placed into dialysis bags that were pre-treated and dipped into 50 mL volume beaker containing dissolution medium (STF, simulated tear fluid, 37±0.5°C, 50 rpm, pH 7.4). 1 mL fractions of sample were taken and replaced with equivalent volume of fresh STF at predefined intervals. A UV- spectrophotometer was used to monitor all

the samples at 263 nm. Then via calibration curve equation, the amount of Timolol maleate can be found which is released into the medium. The entire study was performed in triplicate and the mechanism of drug release was determined via different kinetic models.[18]

### Scanning Electron Microscopy (SEM)

SEM was done to assess the surface morphology of TMNPs (Hitach -8010, Japan). A drop of TMNPs dispersion was placed over the conductive paper which is mounted on the adhesive cuprum stud with a coating of platinum and investigated under SEM at the acceleration voltage of 25 KV and magnification of 5,000X.[19]

### *Ex vivo* Ocular Tolerance Study

Hen's Egg Chorioallantoic Membrane (HET-CAM) test was used to investigate the *ex vivo* ocular tolerance of TMNPs. In brief, hen's fertilized eggs were provided from a poultry farm and they were incubated for 10 days at temperature 37±0.5°C during this tenure, eggs were held upside and rotated gently every 12 hr to prevent chorioallantoic membrane from adhering to the shell. Eggs were candled with a light source to identify the air sac and eggs with viable embryos were used for further testing. This study was carried out in triplicate using TMNPs dispersion, negative control (0.9% NaCl), and positive control (0.1 N NaOH). All the samples were directly poured onto the CAM layer and then irritation effects for 300 sec were observed.[20,21] Finally, we observed the irritation onset time and the Potential Irritation (PI) index was determined through the below-mentioned equation:

$$PI = 5\,(301 - i)/300 + 7(301 - j)/300 + 9(301 - k)/300$$

Where, onset time in a sec of different factors are indicated, i=hemorrhage, j=vasoconstriction and k=coagulation. The eye irritation reaction was evaluated on the basis of PI score range given in Table 2.

### *Ex vivo* Permeation Study

Transcorneal permeation of the TMNPs and pure drug solution was studied using freshly obtained goat's eyes from nearby butchers, employing a Franz diffusion cell (active surface area = 0.9 $cm^2$ and volume capacity =10 mL). The cornea was cautiously extracted and rinsed multiple times with PBS to eliminate any proteinaceous substance. The extracted cornea was positioned between the lower and upper compartments of the Franz diffusion cell, with the epithelial surface facing the upper compartment. Samples (TMNPs or drug solution, 3 mg/mL) were added to the upper compartment, while the lower compartment was filled with freshly prepared STF (10 mL, 50 rpm). The medium was kept at 37°C±0.5°C, and at predetermined intervals, 2 mL of the sample was removed from the cell and replaced with an equal volume of fresh media. The extracted samples were then examined at 263 nm using a UV spectroscopy, and drug permeation was computed using the standard calibration curve equation, determining the percentage of drug permeated.[11]

## RESULTS AND DISCUSSION

### Determination of Particle Size, Polydispersity Index and Zeta Potential

Various concentrations of gellan gum and aluminium chloride were tested to achieve nanoparticles of the smallest size (Table 1). At a concentration of 0.06% w/v GG, the smallest nanodispersions of TMNPs were obtained with 0.01% w/v aluminium chloride and as the concentration of aluminium chloride increased, the size of the nanodispersion also increased. This is because a higher concentration of $Al^{3+}$ ions promotes the formation of a more complex network structure, which leads to larger particle sizes. Additionally, when the concentration of aluminium chloride was increased from 0.01% to 0.03% w/v at 0.12% w/v GG concentration, micro-dispersions were formed. This might be due to higher polymer concentration which caused the dispersion to become more viscous, resulting in the formation of larger droplets during the dripping process. This, in turn, led to the creation of particles with a larger diameter.[11] The particle size and PDI of optimized TMNPs is 135.2 nm and 0.294 respectively (Figure 1a). The formulated TMNPs were found to be in the nano range and a single peak was observed in the zeta scan which indicates monodisperse particles signifying that the method of formulation of nanoparticles was optimized. Also, the low value of polydispersity index depicts that the formulation was monodisperse. Zeta potential of TMNPs was found to be +20.9 mV (Figure 1b) which suggests higher electrostatic repulsion between particles in dispersion and thus preventing aggregation that makes it more stable.

**Table 1: Particle size and % EE of different batches of TMNPs.**

| Sl. No. | Gellan Gum (%w/v) | Aluminum chloride (%w/v) | Particle size (nm) | EE% |
|---|---|---|---|---|
| 1 | 0.06 | 0.01 | 135 | 78.56 |
| 2 | 0.06 | 0.02 | 339 | 63.14 |
| 3 | 0.06 | 0.03 | 587 | 59.23 |
| 4 | 0.12 | 0.01 | 1066 | 38.75 |
| 5 | 0.12 | 0.02 | 1147 | 25.37 |
| 6 | 0.12 | 0.03 | 1519 | 20.51 |

## Determination of % Entrapment Efficacy

The percentage was determined using the method described earlier. Entrapment efficacy of TMNPs was decreased, when the concentration of aluminium chloride was increased from 0.01% to 0.03% w/v at 0.06 and 0.12% w/v GG concentration (Table 1). The results suggest that a high concentration of crosslinker leads to excessive crosslinking, resulting in decreased entrapment efficiency due to reduced pore size, which impedes drug diffusion. Also, on increasing the polymer concentration, the viscosity of the mixture was enhanced which ultimately hindered the interaction between the cross-linker and polymer.[11,13] The % EE of an optimized batch of TMNPs was found to be 78.56±0.04%.

## Fourier Transform Infrared Spectroscopy (FT-IR)

FTIR spectra of TM, gellan gum polymer, physical mixture and TMNPs are depicted in Figure 2. FTIR spectrum of TM showed peaks at 3308 $cm^{-1}$ (O-H/N-H stretching vibrations), 2948 $cm^{-1}$and 2839 $cm^{-1}$ (C-H stretching vibrations), 1750 $cm^{-1}$ (COOH group of maleic acid) and 1477 $cm^{-1}$ ( N-H bending vibrations), 1584 $cm^{-1}$ (C=N stretching vibrations), 986 $cm^{-1}$ (C-O stretching vibrations) and 1,320 $cm^{-1}$ (S-N). FTIR spectra of gellan gum depicted the characteristic broadband at 3377 $cm^{-1}$ (aliphatic alcohol) and peaks at 2972 ($CH_2$ group), 1047 $cm^{-1}$ (CO stretching of hydroxyl group), 1670 and 1453 $cm^{-1}$ (asymmetric and symmetric stretching of -COO group) and 866 $cm^{-1}$ (C-H).[22] All characteristic peaks of timolol maleate drug and GG polymer were observed in IR spectra of PM demonstrating no interaction among polymer and the TM drug. The IR spectra of TMNPs depicted all the characteristic peaks of TM and GG signifying zero interaction between them while preparation of the nanoparticles. However, the carboxyl ions peaks (1670 $cm^{-1}$ and 1453 $cm^{-1}$) of gellan gum were vanished which might be because of the cross-linking of carboxylic group with $Al^{3+}$ ion.

**Table 2: Evaluation of eye irritation reaction.**

| PI Score | Irritation reaction | Scores |
|---|---|---|
| 0-0.9 | Non-irritant | 0 |
| 1-4.9 | Was slight-irritant | 1 |
| 5-8.9 | Moderate-irritant | 2 |
| 9-21 | Severe-irritant | 3 |

## Differential Scanning Calorimetry (DSC)

Thermograms from the DSC analysis of Timolol maleate, gellan gum polymer and TMNPs are represented in Figure 3. DSC curve of TM depicted a sharp endothermic peak at 204.4°C which represent the melting point and crystalline nature of drug. The DSC curve of the GG depicted a broad endothermic peak at 86.21°C which indicates the melting point and amorphous nature of GG. The TMNPs thermogram demonstrated that the endothermic peak of the TM drug had widened, indicating that the crystalline form of the TM had changed into amorphous form following encapsulation in TMNPs.

## *In vitro* Release Studies

The Percent Cumulative Drug Release (% CDR) of TM drug solution and TMNPs formulation has been illustrated in Figure 4. The pure TM solution showed quick release of 47.64% drug within 30 min and 98.11% in 3 hr. whereas in the case of prepared TMNPs a sustained drug profile with 3.87% release in the first 0.5 hr and 62.11% releases in 12 hr have been observed. Different *in vitro* kinetic models such as Korsmeyer-Peppas, zero order, higuchi and first order were used to examine the drug release data obtained from *in vitro* release investigation. With a value of $R^2$=0.993, the Korsmeyer-Peppas model was found to have the maximum regression coefficient. The n value was found to be 0.82 which indicates the drug release to be non fickian transport. Therefore, it can be concluded that the release of TM drug from gellan gum nanoparticles followed anomalous transport that included both diffusion and swelling-controlled drug release.

## Scanning Electron Microscopy

Surface morphology of the prepared TMNPs was analyzed by SEM technique. Figure 1c shows the SEM image of TMNPs which showed that the surface of TMNPs was irregular and cuboidal in shape.

## *Ex vivo* Ocular Tolerance Study

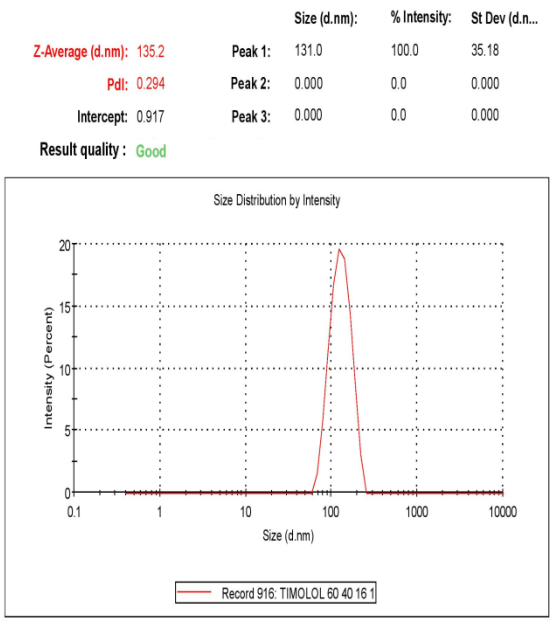
The result obtained from HET-CAM studies revealed that both TMNPs and negative control (0.9% NaCl) had 0.0 irritation score which is due to the absence of any damage signs (Figure 5 and Table 3) to the chorioallantois membranes whereas positive control (0.1 N NaOH) showing serious reaction with the mean irritation score of 3. Hence it can be concluded that TMNPS were found to be non-irritant and safe.

**Table 3: Shows the irritation score of *in vitro* HET-CAM assay.**

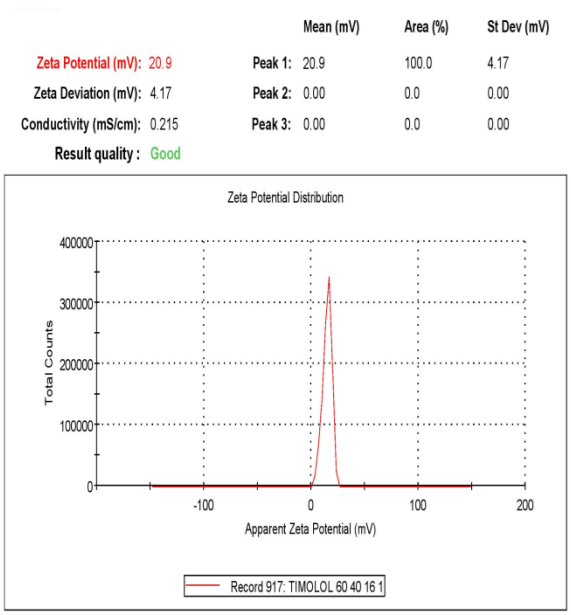
| Sample | Irritation potential (mean) | Irritation score (mean) | Observation |
|---|---|---|---|
| 0.9% NaCl (negative control) | 0.07 | 0 | No reaction |
| 0.1 N NaOH (positive control) | 14.07 | 3 | Severe reaction |
| TMNPs (test control) | 0.07 | 0 | No reaction |

## *Ex vivo* Permeation Study

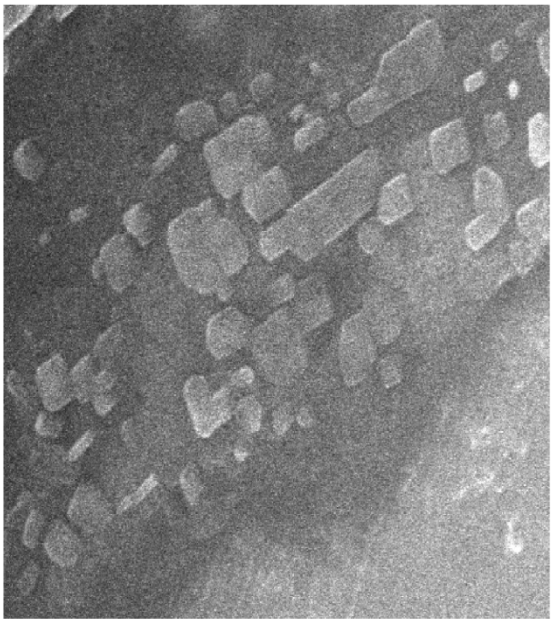
The permeability of a substance through the corneal barrier is influenced by several factors such as its chemical properties, lipid/water partition coefficient, size, etc., The corneal epithelium is primarily a lipid-based barrier, hindering the penetration of hydrophilic drugs, while the aqueous stroma of the cornea is the primary barrier for hydrophobic agents.[23] The TMNPs showed a significant increase in permeation (35.11±1.52%) compared to the drug solution (17.98±1.61%) within 4 hr, representing a nearly 1.9-fold increase. According to reports, submicron particles can penetrate the corneal epithelium cells through endocytosis.[24] The enhanced permeation of TMNPs in comparison to drug solution could be attributed to their nano size, and mucoadhesive properties of gellan gum which results in greater retention on the corneal surface and improved adhesion.




(a)




(b)



(c)

**Figure 1:** (a): Particle size and PDI analysis of TMNPs; (b): Zeta potential of TMNPs; (c): SEM image of TMNPs.

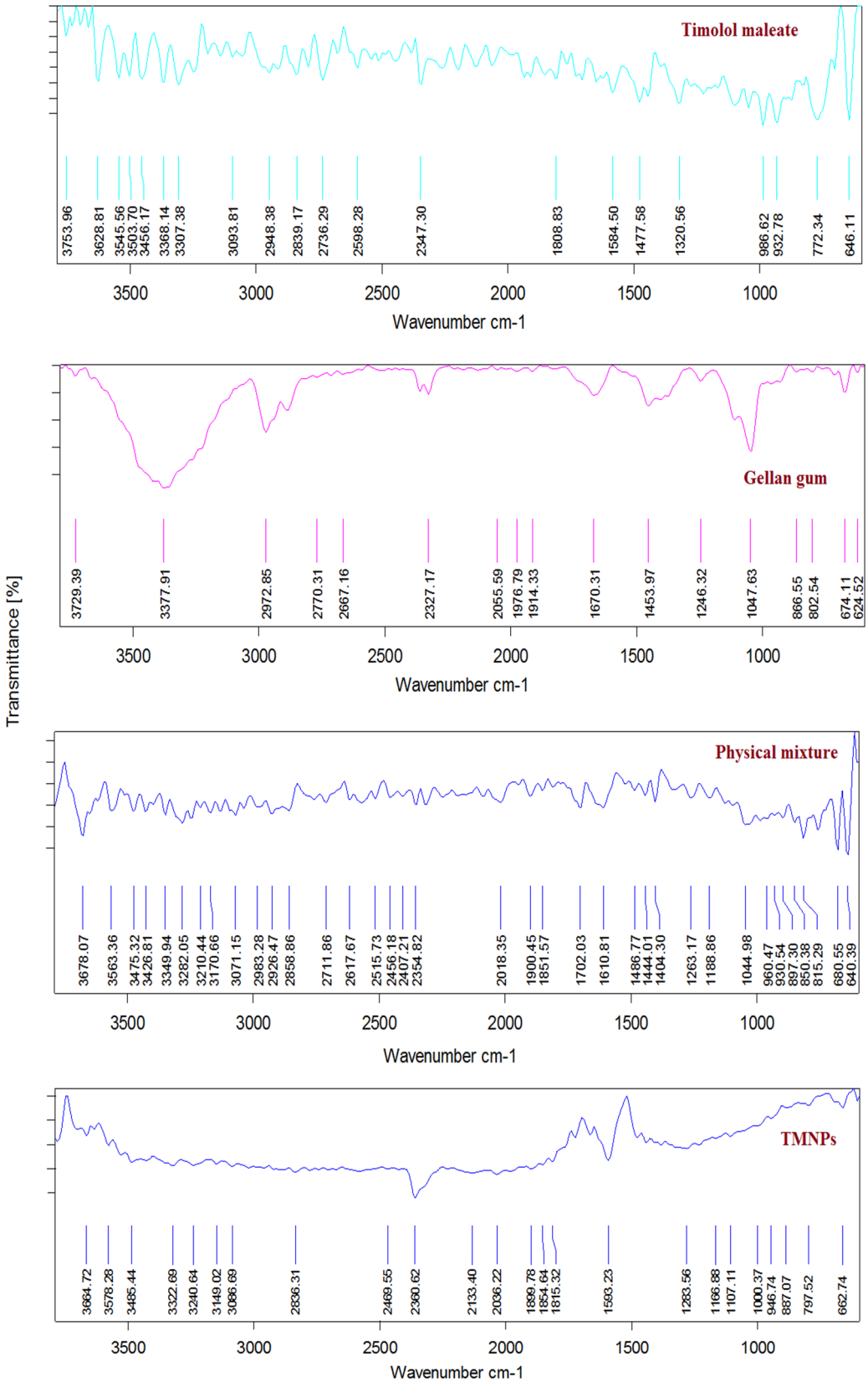



**Figure 2:** FT-IR spectra of TM, gellan gum, Physical mixture and TMNPs.

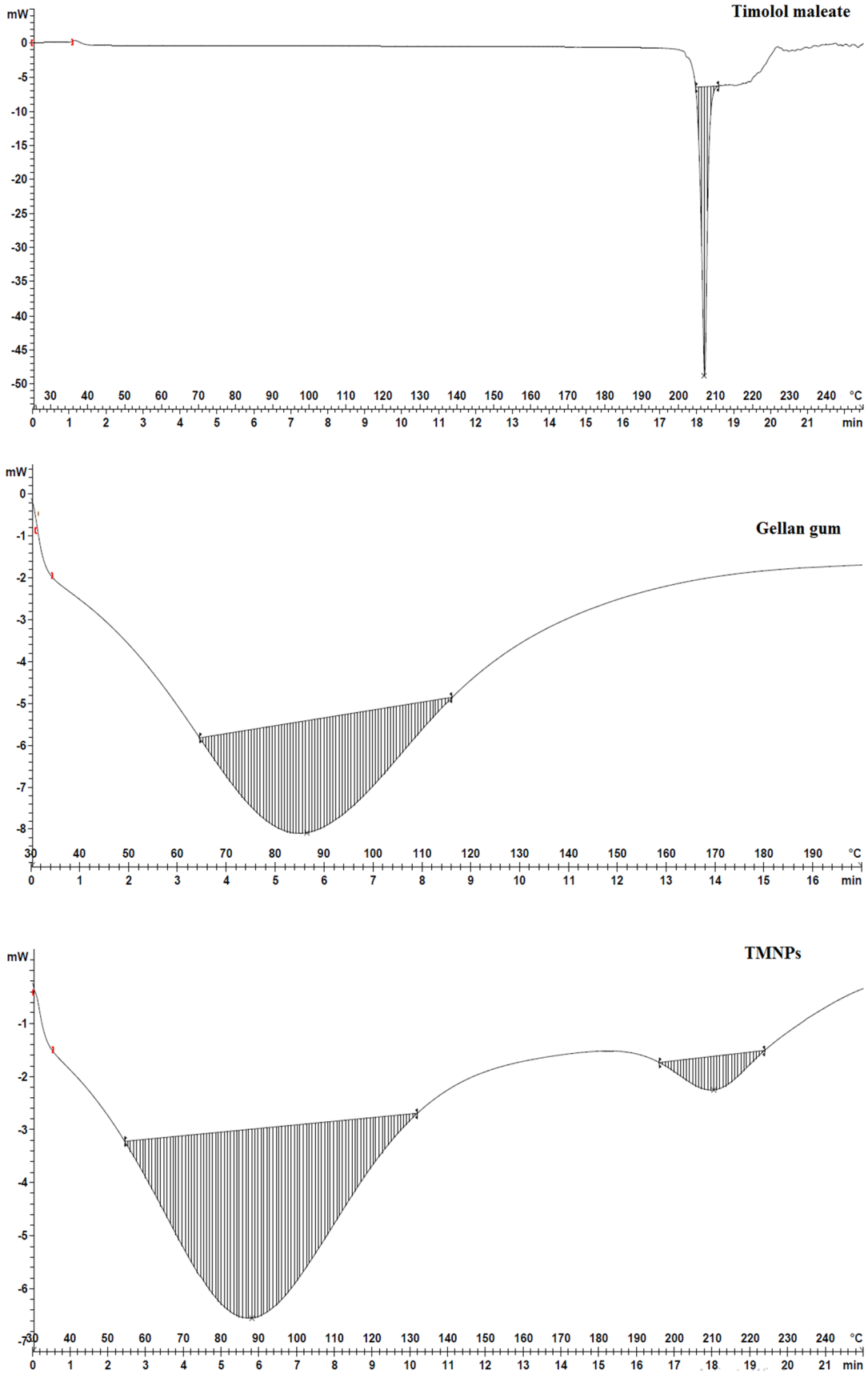



**Figure 3:** DSC Thermogram of TM, gellan gum and TMNPs.

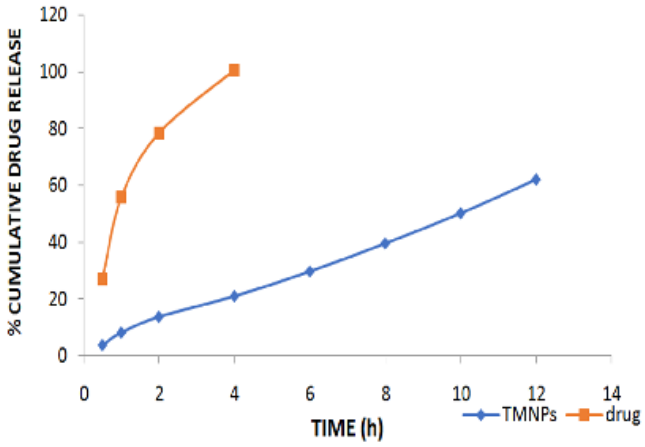



**Figure 4 :** *In vitro* drug release profile of drug solution and TMNPs.

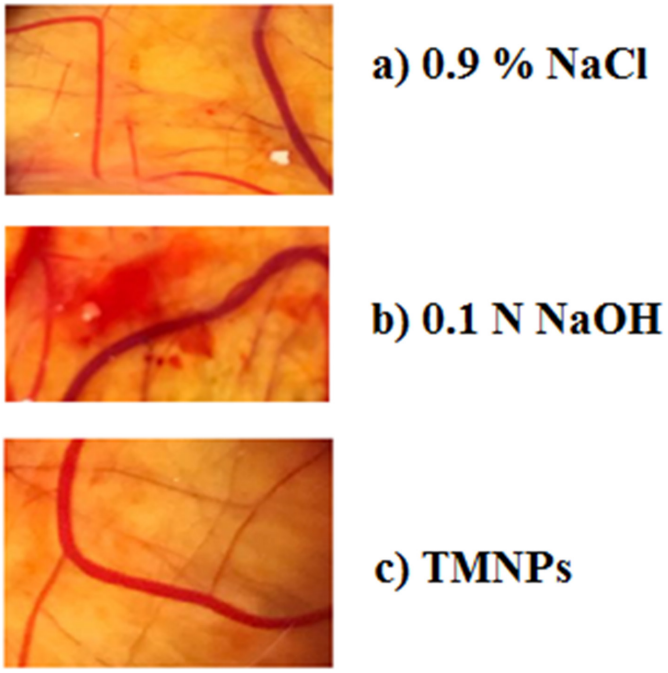



**Figure 5:** HET CAM assay images of 0.9% Nacl, 0.1 N NaOH and TMNPs.

## CONCLUSION

It is concluded that TMNPs are potential and safe carriers for the ocular drug delivery of TM and can be considered a good alternative to the conventional dosage form. *In vitro* drug release studies exhibited sustained release of TM from prepared TMNPs and permeation studies suggested that a significant amount (1.9 fold) of TM from TMNPs was permeated via corneal membrane as compared to drug solution. Also, the irritation potential of TMNPs was assessed via HET CAM assay which revealed that the TMNPs are safe and non-irritant. Further, this formulation can be evaluated for pharmacokinetic and pharmacodynamic studies in animal and human subjects.

## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

## ABBREVIATIONS

**EE:** Entrapment efficiency; **GG:** Gellan gum; **IOP:** Intraocular pressure; **NPs:** Nanoparticles; **TM:** Timolol maleate; **TMNPs:** TM loaded GG nanoparticles.

## REFERENCES

1. Pitkänen L, Ranta VP, Moilanen H, Urtti A. Permeability of retinal pigment epithelium: effects of permeant molecular weight and lipophilicity. Invest Ophthalmol Vis Sci. 2005;46(2):641-6. doi: 10.1167/iovs.04-1051, PMID 15671294.
2. You M, Rong R, Zeng Z, Xia X, Ji D. Transneuronal degeneration in the brain during glaucoma. Front Aging Neurosci. 2021;13:643685. doi: 10.3389/fnagi.2021.643685, PMID 33889083.
3. Kandav G, Bhatt DC, Singh SK. Effect of different molecular weights of chitosan on formulation and evaluation of allopurinol-loaded nanoparticles for kidney targeting and in management of hyperuricemic nephrolithiasis. AAPS PharmSciTech. 2022;23(5):144. doi: 10.1208/s12249-022-02297-7, PMID 35578122.
4. Tayal K, Kandav G, Girotra P, Singh SK. Formulation and evaluation of chitosan coated magnetic nanoparticles of amoxicillin trihydrate. Pharm Lett. 2015;7:241-25.
5. Yu S, Wang QM, Wang X, Liu D, Zhang W, Ye T, *et al*. Liposome incorporated ion sensitive *in situ* gels for opthalmic delivery of timolol maleate. Int J Pharm. 2015;480(1-2):128-36. doi: 10.1016/j.ijpharm.2015.01.032, PMID 25615987.
6. Pignatello R, Bucolo C, Ferrara P, Maltese A, Puleo A, Puglisi G. Eudragit RS100® nanosuspensions for the ophthalmic controlled delivery of ibuprofen. Eur J Pharm Sci. 2002;16(1-2):53-61. doi: 10.1016/s0928-0987(02)00057-x, PMID 12113891.
7. Ilka R, Mohseni M, Kianirad M, Naseripour M, Ashtari K, Mehravi B. Nanogel-based natural polymers as smart carriers for the controlled delivery of timolol maleate through the cornea for glaucoma. Int J Biol Macromol. 2018;109:955-62. doi: 10.1016/j.ijbiomac.2017.11.090, PMID 29154878.
8. Kandav G, Bhatt DC, Jindal DK, Singh SK. Formulation, optimization, and evaluation of allopurinol-loaded bovine serum albumin nanoparticles for targeting kidney in management of hyperuricemic nephrolithiasis: formulation, optimization, and evaluation of ABNPs for kidney targeting. AAPS PharmSciTech. 2020;21(5):164. doi: 10.1208/s12249-020-01695-z, PMID 32488630.
9. Milivojevic M, Pajic-Lijakovic I, Bugarski B, Nayak AK, Hasnain MS. Gellan gum in drug delivery applications. Nat Polysaccharides Drug Deliv Biomed Appl. 2019:145-86.
10. Kandav G, Bhatt DC, Jindal DK. Formulation and evaluation of allopurinol loaded chitosan nanoparticles. Int J Appl Pharm. 2019;11(3):49-52. doi: 10.22159/ijap.2019v11i3.31932.
11. Modi D, Nirmal J, Warsi MH, Bhatia M, Hasan N, Kesharwani P, *et al*. Formulation and development of tacrolimus-gellan gum nanoformulation for treatment of dry eye disease. Colloids Surf B Biointerfaces. 2022;211:112255. doi: 10.1016/j.colsurfb.2021.112255, PMID 34942465.
12. Maiti S, Ranjit S, Mondol R, Ray S, Sa B. Al+3 ion cross-linked and acetalated gellan hydrogel network beads for prolonged release of glipizide. Carbohydr Polym. 2011;85(1):164-72. doi: 10.1016/j.carbpol.2011.02.010.
13. Boni FI, Prezotti FG, Cury BSF. Gellan gum microspheres crosslinked with trivalent ion: effect of polymer and crosslinker concentrations on drug release and mucoadhesive properties. Drug Dev Ind Pharm. 2016;42(8):1283-90. doi: 10.3109/03639045.2015.1125915, PMID 26616390.
14. Bhagav P, Upadhyay H, Chandran S. Brimonidine tartrate-eudragit long-acting nanoparticles: formulation, optimization, *in vitro* and *in vivo* evaluation. AAPS PharmSciTech. 2011;12(4):1087-101. doi: 10.1208/s12249-011-9675-1, PMID 21879393.
15. Li X, Nie SF, Kong J, Li N, Ju CY, Pan WS. A controlled-release ocular delivery system for ibuprofen based on nanostructured lipid carriers. Int J Pharm. 2008;363(1-2):177-82. doi: 10.1016/j.ijpharm.2008.07.017, PMID 18706987.
16. Kandav G, Singh SK. Review of nanoemulsion formulation and characterization techniques. Indian J Pharm Sci. 2018;80(5):781-9.
17. Tighsazzadeh M, Mitchell JC, Boateng JS. Development and evaluation of performance characteristics of timolol-loaded composite ocular films as potential delivery platforms for treatment of glaucoma. Int J Pharm. 2019;566:111-25. doi: 10.1016/j.ijpharm.2019.05.059, PMID 31129346.
18. Zhao R, Li J, Wang J, Yin Z, Zhu Y, Liu W. Development of timolol-loaded galactosylated chitosan nanoparticles and evaluation of their potential for ocular drug delivery. AAPS PharmSciTech. 2017;18(4):997-1008. doi: 10.1208/s12249-016-0669-x, PMID 28101726.

19. Agnihotri SA, Aminabhavi TM. Chitosan nanoparticles for prolonged delivery of timolol maleate. Drug Dev Ind Pharm. 2007;33(11):1254-62. doi: 10.1080/03639040701384942, PMID 18058322.
20. Rajpal Deshmukh G, Hema Kumar K, Suresh Reddy PV, Srinivasa Rao B. Venkata Satish Kumar C. Evaluation of eye irritation potential of aqueous leaf extract of *Achyranthes aspera* by *in vitro* and *in vivo* method. Int Sch Res Not.
21. Ahuja M, Singh S, Kumar A. Evaluation of carboxymethyl gellan gum as a mucoadhesive polymer. Int J Biol Macromol. 2013;53:114-21. doi: 10.1016/j.ijbiomac.2012.10.033, PMID 23178342.
22. Dixit R, Verma A, Singh UP, Soni S, Mishra AK, Bansal AK, *et al*. Preparation and characterization of gellan-chitosan polyelectrolyte complex beads. Lat Am J Pharm. 2011;30(6):1186-95.
23. Gupta H, Aqil M, Khar RK, Ali A, Bhatnagar A, Mittal G. Biodegradable levofloxacin nanoparticles for sustained ocular drug delivery. J Drug Target. 2011;19(6):409-17. doi: 10.3109/1061186X.2010.504268, PMID 20678034.
24. Calvo P, Alonso MJ, Vila-Jato JL, Robinson JR. Improved ocular bioavailability of indomethacin by novel ocular drug carriers. J Pharm Pharmacol. 1996;48(11):1147-52. doi: 10.1111/j.2042-7158.1996.tb03911.x, PMID 8961163.
25. Kandav G, Bhatt DC, Jindal DK. Validation of HPLC method for the quantification of allopurinol in serum and kidney homogenates of mice. Res J Pharm Technol. 2020;13(1):373-6. doi: 10.5958/0974-360X.2020.00074.8.

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