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Design development and evaluation of novel ophthalmic nano lipid *in situ* gel-forming solution using timolol hydrochloride

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ABSTRACT

The present research work was aimed to design, develop and evaluate the nano lipid-based drug delivery system by incorporating timolol hydrochloride drug for ocular therapy and improve the release of the drug through the ocular route. Nanolipids in situ gels were prepared by film hydration method involving two steps. First nano lipids were formulated with the help of organic solvents, and then they were incorporated into a gel by using gelling agents. FTIR spectrum studies were carried out for drug and the formulations which reveal that there was no interaction between the drug and excipients used. The various formulations prepared were subjected for the different evaluation parameters, which showed good and effective results for visual appearance, pH, gelation study, viscosity and ocular irritation studies. It was further observed from this research work that formulation TF2 (HPMC K-15M 0.2%w/v and Carbopol 940 0.4%w/v) had a maximum entrapment efficiency of 97.30%, drug content of about 97.67% and drug release of about 84.29% for 10 hrs. Stability studies were carried out for TF2 formulation, and they found that they were stable throughout the study period. It was finally concluded from the present work that formulations prepared were more suitable and had good patient compliance compared to the eye drops.

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INTRODUCTION

Ocular dosage forms are being designed to be instilled onto the external surface of the eye and can be administered inside or adjacent of the eye. There has been significant attention to the development of controlled drug delivery systems for a few decades. The structure of the eye is unique, so it restricts the entry of drug molecules at the site of action (Gaudana *et al.*, 2009).

Various stimuli can form gels theses includes

1. Physical stimuli: Change in temperature, electric fields, light, pressure, sound, and magnetic fields.
2. Chemical stimuli: Change in pH and ion activation from biological fluids.
3. Biological or biochemical stimuli: Change in glucose level.

From the above various stimuli only pH, ion activated, and temperature stimuli can be used for designing of ophthalmic drug delivery system.

Types of *in situ* ophthalmic gel are

1. *In situ* gelling based on change in pH
2. *In situ* gelling based on Temperature Modulation
3. Ion-activated in situ Gellation (Nanjawade *et al.*, 2007).

In the present research work, timolol hydrochloride nano lipid *in situ* gel was formulated by using the desired quantity of Carbopol 940 and HPMC K-15M (gelling agents), and other ingredients like benzalkonium chloride (preservative) and sodium chloride (isotonic with tear fluid and adjust pH) were added to the gel batches in sufficient quantity.

MATERIALS AND METHODS

Pure drug of Timolol hydrochloride was purchase from Yarrow chem product, Mumbai. Carbopol 940 and HPMC K 15M were also purchased from CDH laboratory reagent, India.

Study of interaction of the drug with excipients used in the formulation

Infrared spectra of timolol hydrochloride with used lipids were recorded on FTIR spectra photometer. The absorption maxima of timolol hydrochloride in the spectrum obtained being examined in position and relative intensity to those in the IR spectra of prepared *in situ* gel (Eaga *et al.*, 2009).

Manufacture of nano lipids *in situ* gel

Formulation of nano lipids

Film hydration technique was used for the preparation of nano lipids. In round bottom flask mixture of vesicles like lecithin and cholesterol were dissolved in a volatile organic solvent (methanol). The rotary evaporator was rotated at 60ºC for 45 min. The organic solvent is removed with gentle agitation, leaving a thin film of lipid on the walls of a rotary evaporator.

The aqueous phase containing timolol hydrochloride drug was slowly added with intermittent, constant shaking of the flask at room temperature and followed by sonication for 30 min. Nanolipid solution was cooled by kept in 4-8ºC in the freezer.

Formulation of nano lipid in-situ gel

Nanolipid *in situ* gels were prepared purely based on drug entrapment efficiency and morphology. The batch which gave maximum entrapment and good surface morphology were selected for preparation of *in-situ* gel. The appropriate weight of Carbopol 940 and HPMC K-15M were sprinkled over prepared nano lipid dispersion under constant stirring with the help of glass rod which avoids the formation of lumps. Benzalkonium chloride as preservative and sodium chloride to make gel formulations isotonic with tear fluid were added to the gel batches in sufficient quantity to adjust the pH (Table 1) (Lavanya *et al.*, 2014).

Drug entrapment efficiency

Ultracentrifugation method was used to separate the unentrapped drug from the nano lipid formulation where the nano lipid dispersion was centrifuged at 14000 rpm for about 90 min. The clear supernatant solution which formed was collected. Then it's further diluted with pH 7.2 stimulated tear fluid and was analyzed by UV spectrophotometric method.

Drug entrapment efficiency was calculated by using the equation. The amount of timolol hydrochloride encapsulated in the nano lipid was determined by using EE = (Total drug concentration – free drug concentration) / total drug concentration $\times$ 100 where, EE is entrapment efficiency (%) (Padma *et al.*, 2010).

Estimation of drug content

50 mg of equivalent nano lipid suspension was taken into a standard volumetric flask. Then it was lysed with 100 ml of propane-1-ol by continuous shaking. Then about 0.1 ml of solution was diluted to 10 ml with stimulated tear fluid 7.2. The absorbance was measured at 295 nm for timolol hydrochloride, and drug content was calculated with the help of calibration curve (Ramachandra *et al.*, 2012; Nagesh *et al.*, 2012).

Visual appearance and pH

Presence of particular matter can be observed by visual appearance and clarity. A digital glass electrode pH meter was used to check the pH of the formulations and pH was determined by bringing the electrode near the surface of the formulations and allowing it to equilibrate for about 1 min (Nagalakshmi *et al.*, 2014).

In vitro gellation study

The gelling capacity of the formulations was determined by placing a drop of the polymer solution in a vial which contains about 2 ml of freshly prepared simulated tear fluid (STF) pH 7.2 and equilibrated at 37ºC. Than gel formation was observed and time required for gelation and dissolution of the gel formed was noted (Moorthi *et al.*, 2012).

Viscosity study

Brookfield viscometer was used to determine the viscosity of the formulations by using CPE - 42 spindle at 10, 20, 50 &100 revolutions per min (Organisation for Economic Co-operation and Development, 2012; Sampath *et al.*, 2012).

In vitro drug release of nano lipid *in situ* gel

In vitro release studies of timolol hydrochloride nano lipid, the *in-situ* gel was performed at 37ºC using stimulated tear fluid (pH 7.2) as the release medium.

Nanolipid *in-situ* gel (5 ml) containing timolol hydrochloride was accurately weighed and transferred to the dialysis membrane. The gel was gently pushed down to the surface of the dialysis membrane. To wet the gel stimulated tear fluid (1 ml, pH 7.2) was added in the reservoir compartment.

The receiving compartment was stirred magnetically (100 rpm) at 37ºC. At regular interval, 1 ml of samples were withdrawn from the receiving compartment, and the amount of timolol hydrochloride released from *in situ* gel was determined spectrophotometer at 295nm (Shimadzu1800).

After the withdrawal of the sample at a particular interval, an equal quantity of stimulated was replaced (Shashank *et al.*, 2012).

Ocular irritation studies

Albino rabbit's six numbers were used as test species for the study. Rabbits were housed and well maintained in the animal house at room temperature (27ºC)—each weighing 2-3kg. The eyes of animals were marked as test and control.

Control animal group had not received any sample were as test group received the formulation (0.5 ml), and the animal eyes were observed for 1, 24, 48, 72 hrs. A scoring system graded ocular changes. Animals eyes were observed for redness, swelling, and watering (Nayak and Srinivasa, 2017a).

Accelerated stability studies

The optimized nano lipid dispersion, which had higher entrapment efficiency subjected to short term accelerated stability study at 4ºC ± 2ºC and 27ºC ± 2ºC as per modified ICH guidelines. Samples were analyzed for drug content (Nayak and Srinivasa, 2017b).

RESULTS AND DISCUSSION

Study of interaction of the drug with excipients used in the formulation

The FTIR spectrum studies of Timolol hydrochloride drug and *in-situ* gel were analyzed. Primary functional groups peaks {3315.52 (NH_{str}), 3049.90 (Ar-CH_{str}), 1615.26 (C=N) and 808.42 (Br)} of timolol hydrochloride were present in loaded *in-situ* gel prepared (Table 2, Table 3, Figure 1 and Figure 2).

This proves the fact that there was no potential interaction of the drug with the excipients used in the formulation. This indicates the stable nature of drugs in all formulations prepared.

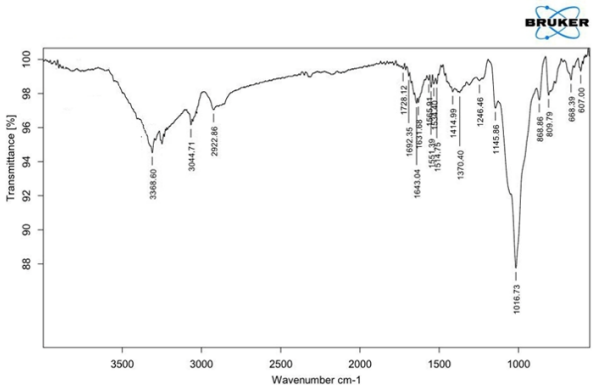

Figure 1: FTIR spectra of Timolol hydrochloride

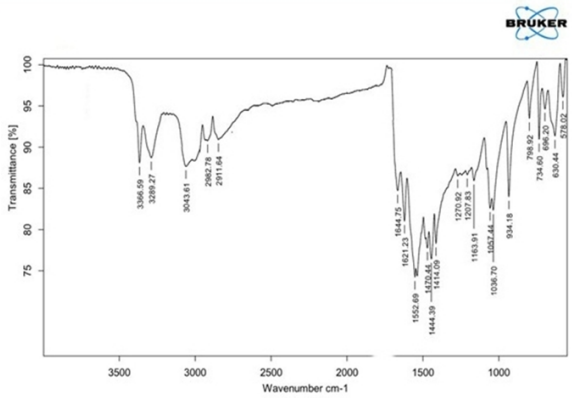

Figure 2: FTIR spectra of formulated Timolol hydrochloride *in-situ* gel

Drug entrapment efficiency

The entrapment efficiency was calculated, and it is expressed in term of percentage. Entrapment efficiency was within the range of 73.40% to 97.30% for timolol hydrochloride loaded *in-situ* gel formulations. TF2 formulation showed maximum entrapment efficiency of 97.30% when compared with other formulations (Table 4 and Figure 3).

Estimation of drug content

Timolol hydrochloride loaded *in situ* gel formulations were determined spectrophotometrically at 295nm. Drug content of all formulations was in a range of 66.69% to 97.67%, as shown in (Table 5). The TF2 formulation showed maximum drug content of about 97.67%.

Table 1: Formulation of Timolol hydrochloride loaded nanolipid *in-situ* gels

Ingredients	TF1	TF2	TF3	TF4	TF5	TF6
Timolol hydrochloride % w/v	0.05	0.05	0.05	0.05	0.05	0.05
Lecithin % w/v	0.05	0.05	0.1	0.05	0.15	0.2
Cholesterol % v/v	0.05	0.1	0.05	0.15	0.1	0.05
Methanol % v/v	7.5	7.5	7.5	7.5	7.5	7.5
Water % v/v	10	10	10	10	10	10
HPMC K 15M % w/v	0.2	0.2	0.4	0.4	0.3	0.2
Carbopol 940 % w/v	0.2	0.4	0.2	0.4	0.2	0.3
EDTA % w/v	0.1	0.1	0.1	0.1	0.1	0.1
Benzolkonium Chloride % v/v	0.01	0.01	0.01	0.01	0.01	0.01
Sodium chloride % w/v	0.9	0.9	0.9	0.9	0.9	0.9
Stimulated Tear Fluid % v/v	100	100	100	100	100	100

Table 2: FTIR spectra data of Timolol hydrochloride

IR (KBr)	Peaks cm-1
NHstr	3315.52
Ar-CHstr	3049.90
C=N	1615.26
Br	808.42

Table 3: FTIR spectra data of formulated Timolol hydrochloride *in-situ* gel

IR (KBr)	Peaks cm-1
NHstr	3366.59
Ar-CHstr	3043.61
OHstr	2982.78
C=N	1621.23
Br	834.18

Table 4: Entrapment Efficiency of Timolol hydrochloride loaded nano lipid *in-situ* gels

Formulation	Entrapment Efficiency %
TF1	95.27 ± 1.707
TF2	97.30 ± 1.326
TF3	73.40 ± 0.604
TF4	79.96 ± 0.356
TF5	66.28 ± 1.896
TF6	93.43 ± 1.506

Table 5: Drug Content Estimation of Timolol hydrochloride loaded nano lipid *in-situ* gels

S.No	Formulations	Drug content%
1	TF1	81.24±0.724
2	TF2	97.67±0.637
3	TF3	66.69±1.367
4	TF4	88.09±0.545
5	TF5	76.65±0.756
6	TF6	88.43±1.009

Table 6: Visual Appearance and pH of Timolol hydrochloride nano lipid *in-situ* gel formulations

S.No	Formulations	Visual appearance	pH
1	TF1	Clear	4.6 ± 0.123
2	TF2	Clear	4.2 ± 0.354
3	TF3	Cloudy	4.6 ± 0.345
4	TF4	Clear	4.1 ± 0.032
5	TF5	Cloudy	4.8 ± 0.867
6	TF6	Clear	4.3 ± 0.323

Table 7: Gelling Capacity of Timolol hydrochloride nano lipid *in-situ* gel formulations

S.No	Formulations	Gellation capacity
1	TF1	+++
2	TF2	++++
3	TF3	+++
4	TF4	++++
5	TF5	+++
6	TF6	++++

Table 8: Viscosity Study of Timolol hydrochloride nano lipid *in-situ* gel formulations

Angular velocity	Viscosity(CPS)					
(rpm)	TF1	TF2	TF3	TF4	TF5	TF6
10	527±1.456	905±1.845	713±1.234	768±0.389	702±0.456	645±1.346
20	485±1.678	807±0.456	612±0.345	678±1.450	605±0.950	421±1.945
50	323±1.543	708±1.567	305±0.456	467±0.345	382±1.423	389±1.586
100	256±1.789	596±0.213	189±0.478	285±0.386	199±0.765	256±0.945

Table 9: Cumulative percentage drug release profile of Timolol hydrochloride nano lipid *in situ* gel formulations

Time(hrs)	TF1 (%)	TF2 (%)	TF3 (%)	TF4 (%)	TF5 (%)	TF 6 (%)
0.5	7.42±0.041	6.25±0.458	7.98±0.895	18.07±0.256	16.05±0.546	13.07±0.405
1	37.04±0.506	18.42±0.356	22.13±1.207	41.01±0.645	38.45±0.305	30.04±0.549
2	51.16±3.061	41.32±3.457	39.30±1.264	53.0±0.856	43.07±1.805	35.8 ±1.234
4	65.18±1.987	51.67±0.87	54.62±1.089	67.46±0.309	57.98±0.895	52.05±1.867
6	82.30±1.640	69.57±1.23	68.45±0.956	85.59±1.006	80.03±0.856	70.90±1.867
10	98.50±1.45	84.29±1.760	86.98±1.079	85.82±1.176	84.05±1.265	80.03±0.466

Visual appearance and pH

The presence of any particular matter was observed visually. Timolol hydrochloride nano lipid *in situ* gel pH range lies between 4.1- 4.8 pH (Table 6). Nano-lipid *in-situ* gel shows a maximum pH of 4.8 for TF5 formulation and minimum for TF4 with pH 4.1. The pH of the reported formulations was non-irritable to the eye.

In-vitro gellation study

Freshly prepared simulated tear fluid pH 7.2 was used to determine the galling capacity. Gellation study revealed that the formulations TF1, TF3 &TF5 had a suitable gelation property and was found that gellation was maintained for about 8 hrs. Formulations TF2, TF4 & TF6 exhibited immediate gellation which remains for about 8-10 hrs as shown in (Table 7)

Viscosity study

The Highest viscosity for TF6 formulation leads to retarded drug release up to a considerable extent 84.29% in 10 hrs when compared with other formulations. Low viscosity formulations show the

Table 10: Grading of Ocular Lesions

	Cornea	
1	No ulceration or opacity	0
2	Slight dulling of opacity	1
3	Easily discernible translucent area	2
4	Nacrous area: no details of iris visible	3
5	Opaque cornea; iris not discernible through the opacity	4
	Iris	
1	Normal	0
2	Congestion, swelling , iris reactive to light (a sluggish reaction)	1
3	Hemorrhage or no reaction to light	2
	Conjuctivea	
1	Normal	0
2	Some blood vessels hyperaemic (injected)	1
3	Individual vessels not easily discernible	2
4	Diffuse beefy red	3

Table 11: Ocular Irritation Study

S.No	Parts of Eye	TF4
1	Cornea	normal
2	Iris	normal
3	Conjunctivae	normal
4	Total	normal

Table 12: Stability Data of OptimizedFormulation

		Drug content			
	Storage Conditions	Initial	1 month	2 months	3 months
TF2	4°C±2°C	97.23±1.517	96.04±1.567	95.37±1.231	94.34±1.491
	27°C±2°C	97.23±0.438	95.87±1.349	95.07±1.519	94.06±1.076

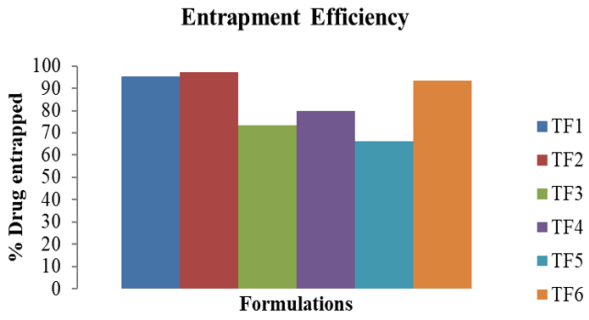

Figure 3: Comparative entrapment efficiencies of Timolol hydrochloride loaded nanolipid *in-situ* gels

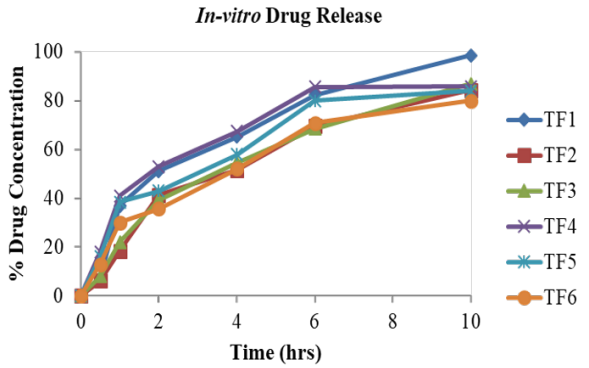

Figure 4: *In-vitro* drug releases of Timolol hydrocholride nanolipid *in-situ* gels formulations

highest drug release. Intermediate viscosity of formulations has shown maximum retardation of drug release due to the dense nature of the polymers. Carbopol 940 and HPMC K-15M as polymer system have contributed majorly towards building viscosity of the formulation. Prepared nano lipid *in-situ* gel for-

mulations viscosity was in the range between 256 - 905 cps, as shown in Table 8.

In-vitro release of nano lipid *in situ* gel

The timolol hydrochloride nano lipid *in-situ* gels showed a two-step release pattern initial burst release and followed by slow release phase. An initial burst release helps to achieve the therapeutic concentration in minimal time followed by the constant release of the drug.

Developed formulations showed 35.3% drug release in 2h and 98.50% in 10h. It was observed that early release phases occurred mainly due to diffusion in the polymer matrix. In contrast, during the later phases, the release has mediated both diffusions of the therapeutic agent and degradation of the polymer matrix itself. Based on the *in vitro* release profiles, TF2 was selected as optimized formulations (Table 9 and Figure 4).

Ocular irritation studies

It was carried on albino rabbits. Ocular irritation studies indicate that the optimized formulation TF2 was non-irritant. It had excellent ocular tolerance, and no ocular damage or abnormal clinical signs to the cornea, iris, or conjunctivae were visible, as shown in Table 10 and Table 11.

Accelerated stability studies

The stability studies of optimized formulation TF4 timolol hydrochloride nano lipid *in situ* gel formulations was determined at 4°C ± 2°C and 27°C ± 2°C for 3 months. The formulation was observed visually for precipitation. The drug content was determined for every 30 days for 3 months. The drug content was determined, and there was less difference between the formulations kept at different temperatures, as shown in Table 12.

CONCLUSION

It was concluded from the above work that formulation TF2 has a maximum entrapment efficiency of 97.30% and drug content of about 97.67%. TF2 formulation containing HPMC K-15M and Carbopol 940 about 0.2% w/v and 0.4% w/v respectively showed the drug release of about 84.29% for 10 hrs. The work concluded that the formulations prepared were more suitable and patient compliance.

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Conflict of Interest

The authors declare that they have no conflict of interest for this study.

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