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Proniosomal Gel: An Approach To Sustain And Improve The Ocular Delivery Of Brimondine Tartrate Formulation ,In Vitro And In - Vivo Characterization

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ABSTRACT-there are many challenges faced by ocular drug delivery system due to the anatomy and physiology of eye and we can say eye is the unique part of our body . Brimondine tartrate(BRT) is a highly selective α 2- adrenergic agonist drug. Brimondine tartrate has been used to treat glaucoma. Glaucoma is a complex disease, impact interior part of ocular system. Main problems encountered in the drug delivery to the eye is small epithelial surface so residence time is short and so bioavailability of drug decreases. To improve the ocular residence time and enhance the bioavailability proniosomal gel of Brimonidine Tartrate has been formulated. BRT loaded proniosomal gel has been prepared by co- ceravtion phase separation method by used different type non inonic surfactant like(Span 20,40,60,80) and combination of different non ionic surfactant(span20+40, span40+60, span 20+80). Total 12 formulation were prepared but divided in two Batch. Batch A and Batch B. Batch A was prepared by using single surfactant with another chemicals and in Batch B combination of surfactant were used and were evaluated for their vesicles size, drug content, Viscosity &pH, encapsulation Efficiency, and drug release and optimized the formulation and these formulation showed expected results. Batch A formulations F5 and F6, showed 92 and94 % drug release respectively in 24 h and encapsulation efficiency of drug was found to be 92 %, Viscosity and PH of gel was also suitable for ocular delivery. In batch B F3 formulation showed good results. Drug release studies showed 90% drug release over 24 hrs. In-vivo study Was performed on healthy male rabbits which showed no irritation caused by the prepared formulations which was assured by the draize test.

KEYWORDS- Proniosomal gel, glaucoma, surfactant

I. INTRODUCTION

Ocular drug delivery system have various convenient properties so I keep great interest . It is a non -invasive route. Today in the marketed various type ocular formulation present in the form of eye drop and eye gel. Main problems with these formulation short reside time specially with eye drops. Different type of disease occurred in the eye and by different reasons like dry eye, inflammation, allergy, glaucoma, conjunctivitis, dermatitis. Mostly surgery done in pain and inflammation disease like vitreoretinal, cataract and glaucoma(mostly in secondary glaucoma.(1) Glaucoma is a complicated disease that was occur in the eye and impact the inner portion of eye. when the normal fluid pressure in side eye increase, glaucoma was occurs. That situation occurs when the uveoscleral outflow increase result imbalance fluid over production o blockage of the drainage system accumulation occurs inside the eye and that lead to intra ocular pressure(IOP)rise, Increase in IOP to carry impairment in blood flow so optic nerve was degrade that was cause in blindness , vision loss. Loss of vision, headache blindness, these are the symptoms of glaucoma. (2) Different types of drugs used in the treatment of glaucoma, some are decreasing production of aqueous humor and some are increasing the uveosceral outflow. Brimonidine is a highly selective alpha 2-adrenergic agonist and it is a hydrophilic drug, It exerts its IOP- lowering effect via a dual mechanism: by inhibiting the enzyme adenylate, it reduces aqueous humor synthesis, while at the same time it moderately enhances outflow through the trabecular and uveoscleral pathways. In the eye, alpha-1 adrenoceptors play a role in vasoconstriction; mydriasis, eyelid retraction, and elevation of intraocular pressure (IOP) whereas alpha -2 adrenoceptors are responsible for IOP reduction via complex Gi-coupled signaling cascade pathway. Activation of alpha-2 receptors leads to inhibition of adenylyl cyclase and

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reduction of cyclic AMP levels. As a result, there is a decrease in nor epinephrine (NE) release at synaptic junction, NE - induced stimulation of beta - 2 adrenoceptors, and production of aqueous humor by the ciliary epithelium .An elevated IOP us the most significant risk factor for developing glaucomatous optic neuropathy, which is associated with progressive visual field loss and functional disability. (3) Brimonidine tartrate eye drop mostly used in the treatment of glaucoma in daily thee time in entire life, frequency of dose administration is too much because bioavailability of drug is very poor. Eye mostly prescribe dosage form but delivery in the ocular system challenging because 1-3 % drug enter the eye after instillation of drug in the eye due to small surface area, loss of drug via conjunctival absorption, naso lacrimal drainage, precorneal metabolism, reflex blinking, tear dilution, etc. present available dosage form like ointment, suspension also degrade drug by metabolic enzyme and cause irritation and blurring of vision Last two decades in the pharmaceutical science has led to refabrication of various types of drugs delivery system to increase the bioavailability of ocular dosage form and increase residence time in ocular surface area overcome the problem associated with patient compliance and ocular barriers also provide sustained release. (4) These result received when drug loaded in the vesicular drug carriers. vesicular carriers are such as niosomes , liposomes, proniosomes, proliposomes, phystosomes, aquasomes, etc. Liposomes have stability problems. Liposomes can undergo chemical degradation rick as hydrolysis and oxidation. Noisome sassociated with such type of problems like aggregation, fusion, leakage, physical stability, etc(.5) To overcome these type of problems formulate proniosomal gel. Proniosomes are versatile drug delivery system by this amphipathy nature and stability. In vesicles of proniosomes both type of drug encapsulated hydrophobic and hydrophilic. (6) Proniosomal gels are translucent gels and liquid lamellar crystal of vesicular bilayers which can formed by the addition of small quantities of gelling agent or water to dry prepared formulation (mixture of non -ionic surfactant, lecithin and cholesterol .The principle advantage of proniosomes is that the amount of carrier required for maintaining the surfactant ratio can easily adjusted (7).When we are used proniosomes on skin and eye hydrate by water it convert in niosomes because ease to use on transdermal route and ocular route . Proniosomal gel enhances skin penetration and enhances corneal permeation and increase bioavailability of drug and residence time of ocular dose. Texture of proniosomal gel make stable during the storage and transport (8)).Formation of vesicles depends on used of surfactant ,cholesterol are used stringency form of vesicles. Lecithin used to increase of the entrapment efficiency of the drug in the vesicles. and also increase penetration. main purpose this study is to explore the used of Brimonidine tartrate loaded proniosomal gel to improve the residence time in corneal surface increase ocular drug bioavailability and also safe the drug degradation by the metabolic enzyme.

II. MATERIALS AND METHOD

2.1 Materials

Brimonidine tartrate was recieved as a gift sample. Cholesterol was purchased from LobaChemie Pvt. Ltd, Span 20 Central Drug House New Delhi, Span 40 from Central Drug House (P) LTD. Bombay- New Delhi Span, 60 Central Drug House (P) LTD Bombay-New Delhi, Span 80 from Central Drug House New Delhi , Lecithin from Hi Media LaboratiiesPvt. Ltd. Ethyl alcohol from Central Drug House (P) Ltd (India).

2.2 Method

Coacervation phase separation method is a simple technique for the preparation of proniosomes. Nonionic surfactant is the most commonly used surfactant in the preparation of vesicles due to their compatibility, stability, and toxicity. Cholesterol and lecithin are generally regarded as a membrane stabilizers and penetration enhance. Ethanol was chosen as it forms large vesicles with higher entrapment when compared with other alcohols. In this method all the required ingredients like surfactants, carriers, cholesterol are taken in a clean and dry stopper glass vial and solvents should be added to it. These ingredients are all heated and after heating, all ingredients should be mixed with glass rod. Until all ingredients dissolved ,the stopper of glass vial, to prevent loss of solvents and then it is being needed add small amount of buffer and again heated on water bath at 60- 70°c for 5 minute, then the mixture must be cooled at room temperature till dispersion gets converted in to proniosomal gel.(9**)** Formulation table is shown in table no.1

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Table:1. Formulation table (Batch A) Brimonidine tartrrate loaded proniosomal gel

Formulation Code	Surfactants (1500)	Lecithin (900mg)	Cholesterol (200 m)	Alcohol (ml)	Drug (mg)	Observation
F1	Span 20	-	200	5	100	Gel not form
F2	Span40	-	200	5	100	Gel not form
F3	Sp60	-	200	5	100	Gel not form
F4.	Span 80	-	200	5	100	Gel form
F5	Span 40	900mg	200	5	100	Gel form
F6	Span 60	900mg	200	5	100	Gel form

Table:2. table(Batch B) Brimonidine tartrate loaded proniosomal gel

Formulation code	Surfactant com(mg)	Lecithin (mg)	Cholesterol (mg)	Ethyl alcohol (ml)	Drug (mg)	Observation
F1	Sp20+sp40	-	200	5	100	Oily yellowish Gel form
F2	Sp40+sp60	900	200	5	100	Yellowish Gel form
F3	Sp80+sp60	-	200	5	100	Oily yellowish gel form
F4	Sp60+sp20	-	200	5	100	Creamy Gel form
F5	Sp40+sp80	-	200	5	100	Gel form
F6	Sp80+sp20	-	200	5	100	Gel form

2.3Compatibility study of brimonidine tartrate with cholesterol using infrared spectroscopy

The infrared absorption spectra of brimonidine tartrate and other ingredient at were obtained at ambient temperature using FTIR -8400 (Shimadzu, Japan). kyoto Approximately, 1-3 mg of BRT in potassium bromide disk. The spectra were recorded 500-3500. (10)

2.4 X -ray diffractrometer

The phase formation of powder samples was analyzed by X- ray diffraction (XRD) technique using an X- ray powder Diffractometer (Rigaku corporation Japan, smart lab 3kw) with Cuka radiation (k=1.5405 .)min the sample run slow scan in the range of 10^{O}-70 .The peaks were appearing after the analysis with Brimonidine tartrate and recorded.(11)

2.5 In vitro characterization of Brimonidine tartrate loaded proniosomal ge1.

All result are shows in result table no. 3.4,5 and figure no 4.1,4.2(a, b, c) 4.3, 4.4,4.5,4.64.7

Vesicles morphology- vesicles morphology involves the measurements of size and shape of proniosomal gel Vesicles by the use of optical microscope . In a glass vial 100 mg of proniosomal gel were taken and hydrate it with buffer solution and after appropriate hydration, shake it for 10 minute (12)

2.5.2 Transmission electron microscopy- By using transmission electron microscopy (saif New Delhi) proniosomes can be visualized. The proniosomes are hydrated and drop proniosomes dispersion is diluted 10 fold using deionized water. A drop of diluted proniosomes dispersion is applied to a carbon coated mesh copper grid and is left for 1 min to allow some of the proniosomes to adhere to the carbon substrate. The remaining dispersion is removed by adsorbing the drop with corner of a piece of filter paper. After rinsing grid (deionized water for 3-5 s) a drop of 2% aqueous solution of uranyl acetate is applied for 1 s. The remaining solution is removed by absorbing the liquid with the tip of a piece of filter and the sample is air dried. The sample is observed at 80 kv.(13)

2.5.3 Determination of viscosity and pH – Accurately weighed gel was taken and then diluted with pH 7.4 phosphate buffers and checked the pH by using pH meter and brook field viscometer is used to determine the viscosity of gel. (14)

2.5.4 Drug content- Proniosomal formulation equivalent to 250 mg of drug was taken in a standard flask they were mixed with 50 ml, of propanol by shaking and 1 ml, of mixture was then diluted with phosphate buffer the absorbance at 244 nm by spectroscopically and calibrated calibration curve. (15)

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2.5.5 Encapsulation efficiency- To evaluate the loading capacity of proniosomal system for BRT loaded gel (100mg) was dispersed in distilled water and warmed a little for the formation of niosmoes. Then the dispersion was centrifuged at 1800m rpm for 40 min the clear fraction was used for the determination of free drug at 281 nm spectrophotomatriaclly. the percentage encapsulation efficiency was calculated from following equation (16)

$$\% \text{ Encapsulation Efficiency} = \frac{\text{Total drug - free drug}}{\text{Total drug}} \times 100$$

2.5.6 Drug release: The in-vitro studies can be performed by using cellophane tube membrane. Proniosomal gel placed in membrane. The proniosomal gel dialyzed against suitable dissolution medium(7.4 pH phosphate buffer) at room temperature; the samples are withdrawn from the medium at suitable intervals, sample were replaced with equal amount of dissolution medium and analyzed for % release of drug using a suitable intervals, and analyzed by suitable UV spectroscopy and also attend sink conditions it is mandatory. (7,)

2.6 IN-VIVO STUDY

Ocular irritation evaluation-

Draize test most reliable test for determination of ocular irritation of prepared formulation .3 New Zealand male albino rabbits were subjected to the administration of the proniosomal gel and the market eye drops in order to evaluate the irritancy of both. Draize test depends on scoring system ranging from o-3. Proniosomal gel was applied in the conjunctival sac of the right eye and the right eye was kept control by instillation of saline .The cornea, iris, and conjunctivae were examined for any signs of irritation or congestion caused by the formulation .Testing the ocular irritation after a interval of 1, 2, 3, 4, 6, 8,.24h after administration.

III. RESULT AND DISCUSSION

3.1 Compatibility study of brimonidine tartrate with cholesterol

Compatibility studies of BRT and cholesterol identified by IR spectroscopy no interaction found between BRT and cholesterol observed peaks found to be N-H bending 1630C=C stretching 1645N-H stretching 3320C-N 1358 these peaks shows no interaction between cholesterol and brimonidine tartrate no interaction between both chemical

3.2 X- ray diffractrometer

XRD analysis revealed that the characteristics intense peaks of pure drug appeared at scattering angles 2^{θ} of 2.2^{O}, 11.72^{O}, 12.36^{O}, 17.1^{O}, 18.78^{O}, 19.11^{O}, 21.2^{O}, 23.92, 24.42, 26.62, 28.5, 29.92, 35.5,. Thus the thermal behavior coupled with the crystallographic data suggested that crystallinity of pure BRT. Shown in fig no.4.1

3.3 Morphology

Optical microscopic photos shown in fig no.4.2 confirmed that prepared proniosomal gel were spherical with smooth surface. Which further ensures the safety for ocular administration. The perforation may be due to usage of surfactants span 60.

3.4 Transmission electron microscopy

The proniosomal gel appeared dark and with bright surrounding and a positive image. The vesicles were spherical.Shown in fig no.4.3.

3.5 Drug content

Presence of drug content in proniosomal gel formulation was found to be good range of drug content in different formulation showed 797.1 to 94□1.2 in group A and in group B 87□1.2 to 92□8.9.

3.6 Encapsulation efficiency

Encapsulation efficiency was found to be good (79□8.9 in group A and in group B found to be 87□5.8 to 95□1.3 % in proniosomal gel. In the group A Formulation F5 and F5 and group B Formulation F2 and F4 showed maximum encapsulation efficiency, this may be due to lecithin in the mean values of refractive index of appropriate amount. As shown in TEM image, amount of surfactants more no. Of vesicles formed by increased amount of surfactant. Also increase in hydrophobic domain; hence increase the encapsulation of drug. Cholesterol increase the rigidity of the bilayer as it acts as vesicular cement, it's promotes the hydophobicity of the bilayer, so enhance the incorporation of BRT inside the vesicles. Due to advantages of proniosomes compare to niosomes stop the leakage of drug .Good encapsulation efficiency of BRT in proniosomal gel increase bioavailability of drug and reduce dosing frequency. Results given in table no.3 and 4.

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3.7 Viscosity

In group A For Formulation F6 as the least viscosity compared to other formulations. This may be due to the little amount of solvent used. Viscosity of all proniosomal gel formulation was good as expected. There was no significant change in selected formulation and placebo formulation. Thus, it can be concluded that proniosomal gel formulation were not physically stable but also chemically stable and remained isotropic in nature, without interactions between proniosomal gel components and drug.results given in table no.3 and 4.

3.8 Ph

Ph is important parameter in ocular dosage form that was measured by ph meter. Prepared proniosomal gel formulations were found to be of suitable pH range ie. pH (6.9- 7.5) given in table no.3 and4.which showed, that these prepared proniosomal gel formulation can be used as ocular dosage form. Results given in table no.3 and4.

3.9 In vitro drug release studies

In vitro drug release studies from both batch different proniosomal gel formulation, all having the same quantity. This was done in order to maintain the sink condition. In vitro drug release was the highest in formulation F4 in batch A and F3 in batch B and the lower for F1 in batch A and F1 in batch B. The significant difference in proniosomal gel formulation was probably due to the mean size of vesicles, which was significantly smaller in proniosomal gel. The maximum release F4 in batch A and F3 in batch B. Prepared formulation showed drug release profile over 24 h, while compared Alphagan P showed % drug release within the first 2h. In vitro drug release gives an indication of how drug will behave in-vivo. Some formulation showed not acceptable result due to choice of surfactants and size of vesicles. Span 60 based proniosomal gel gives sustained release.results shown in fig no.4.4, to fig no.4.6

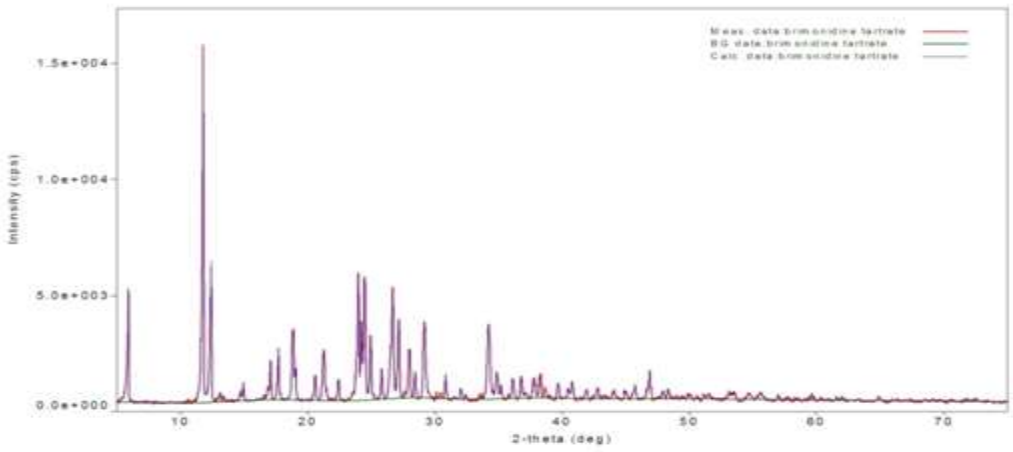
3.10 In - vivo characterization

Draiz test (ocular irritation test)

Draize test examine the ocular acceptability and shelter of topical applied proniosomal gel against Alphagan P. show so as to formulation No 6(batch A) cause no irritation on top of the Draiz scale of 0 to +3 throughout whole learning. The market BRT eye drop cause minor conjunctival irritation in first hour in single rabbit. The irritation cause is frequently due to the preservatives present in the marketplace eye drops, which lead to reaction blinking and irritation. Be deficient in of irritation cause by tested formulation indicate the protection of ocular management of it. And moreover observed that dwelling time of proniosomal gel that to be better than eye drops.results are shown in table no.5

IV. OBSERVATION

4.1XRD



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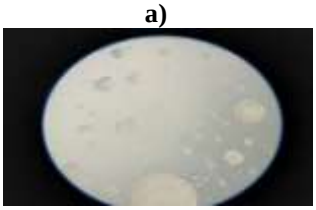
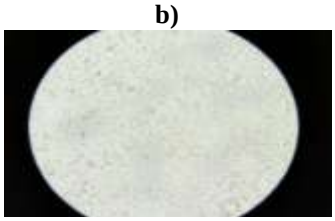
4.2 OPTICAL IMAGE

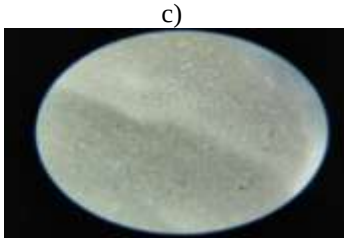
a)



b)

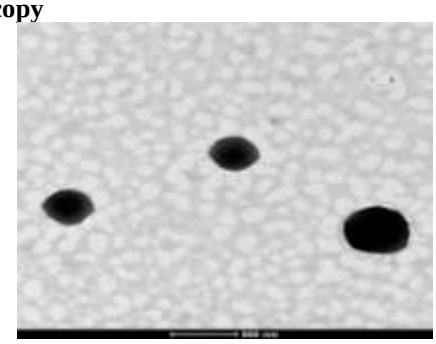


c)



I observed that use of different type surfactant altered the size of vesicles.

4.3Transmission electron microscopy



3.. Characterization table of brimonidine tartrate loaded proniosomal gel (Batch A)

Formulation code	% Drug content	% Encapsulation efficiency	PH	Viscosity (cp)	vesicles size (µm)
F1	85□2.9	80□4.6	6.9	8907	14.89□90
F2	-	-	-	-	-
F3	-	-	-	-	-
F4	89□7.1	79□8.9	7.1	8690	15.90□4.4
F5	95□3.9	92□3.9	7.4	9024	10□1.2
F6	94□1.2	93□8.9	7.1	9167	9.1□3.7

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4. Batch B

Formulation code	% Drug content	% Encapsulation efficiency	pH	Viscosity (cp)	vesicles size (µm)
F1	97□69	90□2.2	7.1	8912	13□8.8
F2	96□8.9	90□1.3	7.2	9889	12□1.9
F3	90□6.7	84□4.6	6.9	8765	13□9.9
F4	91□5.7	80□8.9	7.0	9543	11□9.5
F5	89□7.9	85□5.8	7.1	8921	14□1.2
F6	87□1.2	85□7.8	7.3	8289	15□3.6

Fig. no.4.4 Cumulative % release of graph batch A formulation

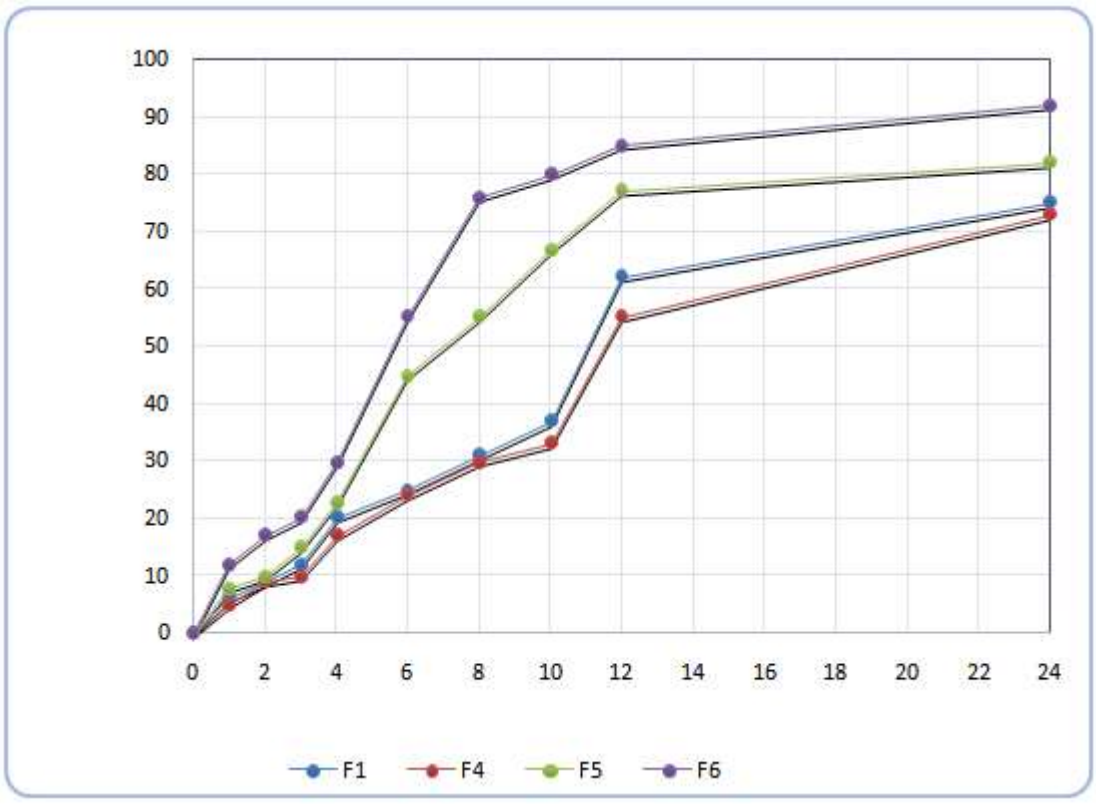

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Fig no. 4.5 : Cumulative %drug release graph (Batch B 1,2,3 formulation)

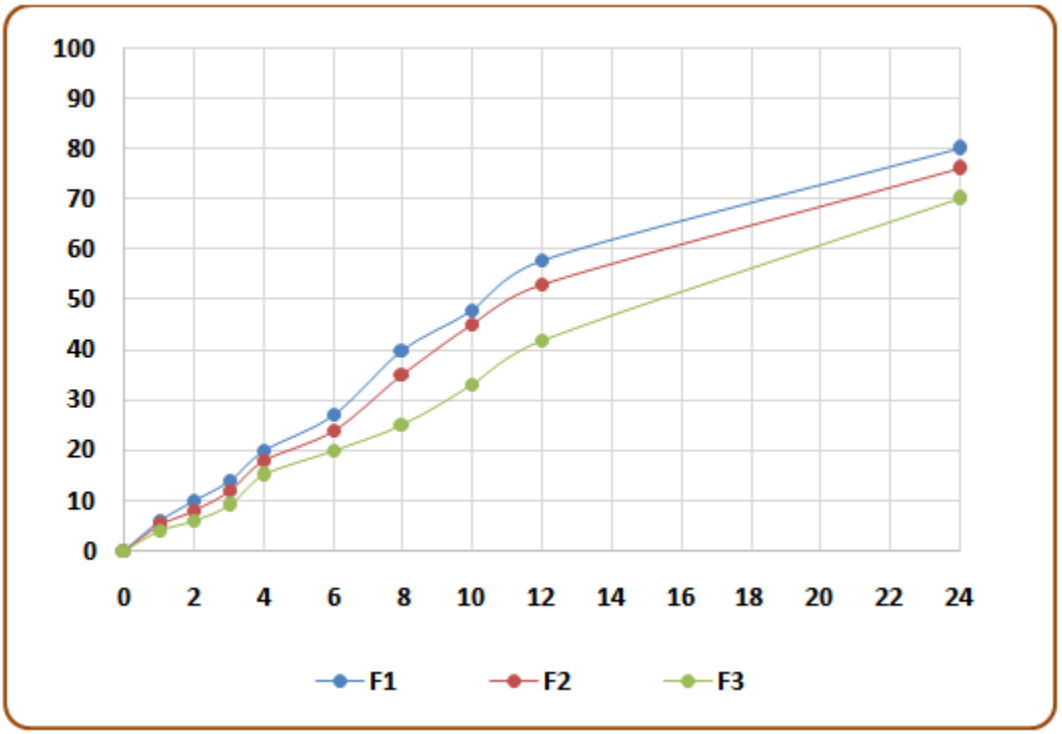

Fig no. 4.6: Cumulative % drug release graph (Batch B 4,5,6 formulation)

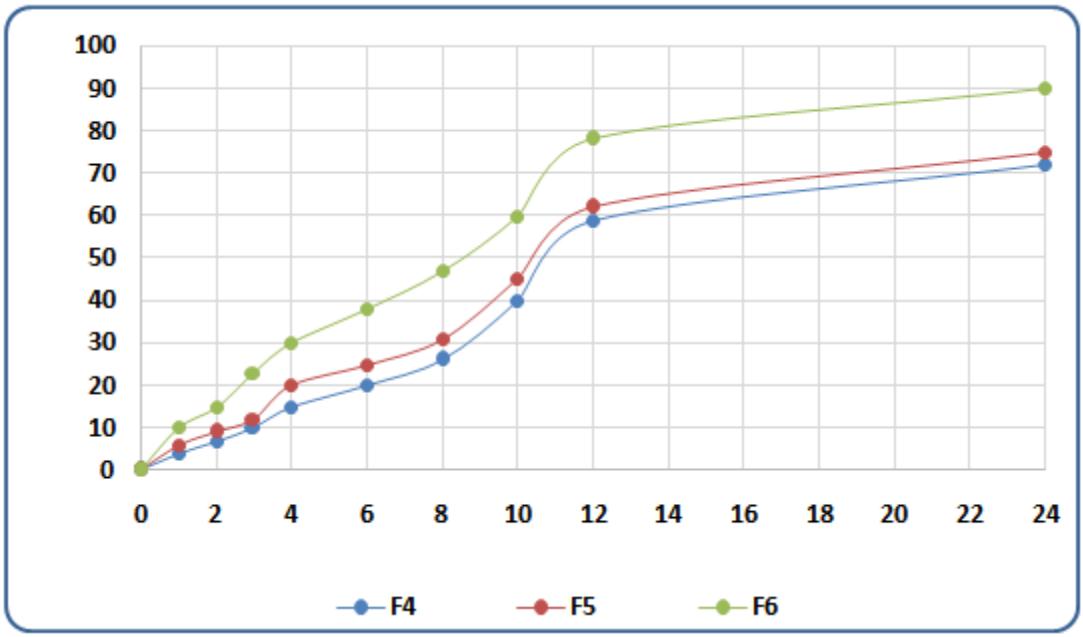

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Table no.5. Draiz test table

Group	Parameter	1h	2h	5h	8h	12h	24h
Span 60 based Proniosomal gel	Corneal	0	0	0	0	0	0
	Iris	0	0	0	0	0	0
	Conjunctiva	0	0	0	0	0	0
	Total score	0	0	0	0	0	0
Alphagan	Corneal	0	0	0	0	0	0
	Iris	0	0	0	0	0	0
	Conjunctiva	1	0	0	0	0	0
	Total Score	1	0	0	0	0	0

V. CONCLUSION

In ocular delivery system, proniosomal gel formulation could be considered as a very recent approach. In which both hydrophobic and hydrophilic drug can loaded and get desire effect. Our study highly emphasized on proper optimization, design, development, delivery approach of a short half life drug called BRT.

We have selected proper surfactant, cholesterol, and lecithin for prepare proniosomal gel formulation. It has been reviled that amount of ingredients could produce good viscosity, and penetrability. Moreover, particle size determination studies, and TEM concluded that prepared proniosomal gel retained within the nano range. Almost all the 10 formulation posse's good appearance and pH, good viscosity, encapsulation efficiency, drug content, and the cumulative percentage drug release. The overall plot we had given best out of best formulation named as F6 (batch A) and found it has good percentage cumulative drug release profile almost 94.56±23% drug release in 24 hr and posed handsome stability profile. Hence, it can be concluded that prepared BRT loaded proniosomal gel was a good candidate for ocular drug delivery.

BRT loaded proniosomal gel were successfully prepared by coacervation phase separation method. We could accept as a promising ocular drug delivery carrier for BRT and treatment of glaucoma with sustained release manner. In-vivo study on New Zealand albinio rabbit assured the accomplishment of the aim of our work. Formulate proniosomal gel showed that enhance ocular residence time and improve ocular bioavailability and sustained released from prepared vesicles

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