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Research Article

Formulation and Evaluation of Tropicamide loaded Niosomes

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ABSTRACT

Tropicamide is an antimuscarinic drug used in eye disease. The niosomal vesicular drug delivery system facilitate the permeation of drug through the cornea because of the micron/nano size of vesicles containing drugs, which will increase the corneal penetration of drug, and increase the residence time of formulation in ocular cavity that result to increase the bioavailability of drug. Tropicamide loaded Niosomes by investigating the relationship between drug/Nonionic surfactant ratio were successfully prepared by thin film hydration method and compare the result of different grade of span used (20,40,60) with different ratio of cholesterol. niosomes were evaluated for particle size ,drug entrapment efficiency, drug content ,corneal permeation study and in–vitro drug release. Respectively as a result the niosomes designed showed nearly spherical particles with a mean particle size 156.3nm. Niosomes prepared using cholesterol and span 60 in the ratio (1:1) f9 shoed higher entrapment efficiency (84.35%) in-vitro drug release (94.02%) was optimized.

Keywords: Niosomes, *in-situ* gel, vesicles, ocular cul-de-sac, viscoelastic gel.

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INTRODUCTION

The drug delivery to the eyes is a difficult task for the formulation because the eye is protected by a series of complex defense mechanisms, which make it difficult to achieve an effective concentration of the drug at the target site of the eye. Traditional ophthalmic dosage forms include solutions, suspensions; ointments are still acceptable, such dosage forms are no longer sufficient to overcome the various ocular diseases like glaucoma due to poor bioavailability[1].

Marketed conventional preparations have many demerits, like poor absorption, permeation, retention time of drug in the ocular. The main objective of designing a ocular delivery is to achieve an maximum concentration of a drug at the target site for the longer time. Ocular retention time and loss of drug is based upon its physical-chemical properties as well as their ocular anatomy and physiology[2].

The many approaches have been used to enhance the retention time of drug in ocular cavity these are 2 categories:

The first one is depend on the use of controlled drug delivery systems, which provide the prolonged and continuous delivery of ophthalmic formulation.

The second one involves enhance corneal drug absorption and reduced precorneal drug loss [3, 4].

Most commonly available conventional ophthalmic preparations are eye drops and ointments in market. But these formulations when administration into the eye are rapidly loss from corneal layer due to tear flow and nasolachrymal drainage. Only a small amount (10%) is available for its therapeutic effect resulting in frequent dosing is required. So attempt was take to overcome to these problems noval pharmaceutical ophthalmic formulation have been developed to release the drug in sustained and controlled manner to increase the ocular bioavailability[5].

Niosomes

Niosomes is a non-ionic surfactant-based liposome. Niosomes are formed with or without incorporation of cholesterol or lipids as an excipient. Niosomes carry both water soluble & water insoluble drug its more penetrating capacity than other formulation like; suspension, emulsions. They are structurally similar to liposomes but it is more stable then liposome, however, the nonionic surfactants used to prepare niosomes make them more stable.

Niosomes are microscopic-lamellar, spherical, unilamellar and multilamellar structures which are formed on the

admixture of non-ionic surfactant (span 20, span 40, span 60) and cholesterol with subsequent hydration in aqueous media (distilled water).

Drug delivery through novel formulation (niosomes) is one of the best approaches to achieve effective drug concentration.[5,6,7]

Advantages:[8,9]

- Excellent reproducibility and feasible large scale production.
- Niosomes are chemically stable, high entrapment efficiency and have long storage time instead of liposome.
- Niosomes carry both hydrophilic and hydrophobic drugs.
- They have high biocompatibility with biological systems & low toxicity because of their non-ionic nature.
- Increased product stability of about 1 year in compare to liposome.
- Niosomes improved (bioavailability & permiatiom) therapeutic performance.

MATERIALS AND METHODS

Materials

Tropicamide was a gift sample from Bal pharma pvt. ltd. Span 20, Span 40 and span 60 were obtained from yerrow chem. Products All other solvents and reagents used for study were of analytical grade.

Thin film hydration method

Niosomes of ocular delivery are prepared by non-ionic surfactants because the irritation power of surfactants decreases in the following order: **cationic >anionic > ampholytic > non-ionic.** Niosomes of Tropicamide were prepared by thin film hydration method using non-ionic surfactants span 20, span40, span60 in different ratio with cholesterol as shown in Table.1. Accurately weighed quantity of surfactant and cholesterol in different molar ratios were weigh and dissolved in Chloroform: Methanol (2:1 v/v) solvent mixture and stirred until it gets completely dissolved. Then it was vortexed in a round bottomed flask at temperature 55 C to remove the solvent under reduced pressure in the rotary flask evaporator at 100 rpm for 30-40 min. After evaporation, the surfactant and cholesterol formed a thin film on the inner sides of round bottom flask. Then the film was hydrated with aqueous phase containing the drug in 30 ml of phosphate buffer saline pH 7.4 for 30 min at temperature 55ºC to obtain white dispersion of niosomes. The above white dispersion (Niosomes) was cooled in an ice bath and then using bath type sonicator sonicate for 20 minute. The resulted vesicles of niosomes were stored at 40C in a refrigerator for further studies.[11]

Evaluation of Tropicamide Loaded Niosomes:

Size analysis

Fourier Transform Infrared Spectroscopy:

It was important to check any kind of interaction between drug and excipient. It was done using Fourier Transformed Infrared Spectroscopy.IR spectra of pure Tropicamide and polymers were taken separately. Then to know if there is any interaction between drug and polymer, IR spectra of physical mixture of Tropicamide and polymers were taken in combination.[10]

Particle size:[9,10]

The average diameter and PDI of prepared batches of Tropicamide loaded niosomes were determined by photon correlation spectroscopy (PCS) using a zeta sizer (Malvern, Ver. 6.01) at a fixed angle at 25°C.Sample was diluted 10 times with distilled water and then it was analyzed for particle size. The readings were recorded in triplicate and reported.

Zeta potential

The zeta potential can be measured by determination of the movement velocity of the particles in an electric field and the particle charge. The niosomes was diluted 10 times with distilled water and analyzed by zetasizer (Malvern, Ver. 6.01).

Drug content analysis:[10,11]

The drug content was determined by dissolving the 1 ml of niosomes containing dispersed niosomes in methanol and continues stirring in mechanical stirrer for 30 minute to break the niosomal structure and release the entrapped drug. After suitable dilution with phosphate buffer pH 7.4, samples were analyzed spectrophotometrically using UV-visible spectrophotometer (Shimadzu, UV-1800, Thane) at wavelength of 257 nm and drug content was determined.

Drug entrapment efficiency:

The drug entrapment efficiency (EE), which corresponds to the percentage of Tropicamide encapsulated within and absorbed on to the niosomes .was determined by measuring the concentration of free Tropicamide in the dispersion medium. 1.0 ml of Tropicamide loaded niosomes was diluted up to 10 ml with methanol and centrifuged at 14000 r.p.m for 45 minutes. Supernatant was then filtered by 0.2µ membrane filter and analyzed by UV-VIS spectroscopy at 256 nm.

% Entrapment efficiency= Entrapped amount of drug/Total amount of drug added ×100

In-vitro release studies:

In-vitro drug release from the formulation was studied by the diffusion cell. *In-vitro* release studies was done using donor receiver compartment model using dialysis membrane soaked overnight in the receptor medium(simulated tear fluid, pH7.4).The diffusion medium was 100 ml of simulated tear fluid stirred at 100 rpm at (37ºC±0.5.ºC) One end of the diffusion tube was covered by a dialysis membrane. The 5 ml Niosomes formulations were filled in test tubes covered with a dialysis membrane and was placed such that it just touches the diffusion medium (STF) present in receptor compartment. The drug sample were withdrawn at the interval of0 .30 hour for the period of 12 hrs from diffusion medium and analyzed by a UV spectrophotometer at 257 nm using simulated tear fluid as a blank.

Accelerated stability studies for the optimized Formulation:

Stability of a pharmaceutical preparation can be defined as, "In a specific container/ closure system the capability of a particular formulation to remain within its physiochemical, microbilogical, therapeutic specification throughout the shelf life" Accelerated stability studies of the optimized formulation was carried out as per ICH guidelines , at refrigeration temperature by using stability chamber for

period of three month. Formulation was observed for viscosity, drug content and gel formulation ability.

Vitro Drug Release Kinetics:

In order to investigate the mechanism of release, the release data were analyzed with the following mathematical models: zero-order kinetic (Eq. 3), first-order kinetic (Eq. 4) and Higuchi kinetic (Eq. 5):

Zero-order model:

Drug dissolution from the dosage forms that do not disaggregate and release out the drug slowly can be represented. by this

$$Q_t = Q_0 + K_0 t \qquad (3)$$

Where

Q_t – The Q_t is content of drug dissolved in time t,

Q_o-The Q_o is initial content of drug in the solution (most times, $Q_o = 0$)

K_0- The K_0 is the zero order release constant. Its unit is {concentration/time}.

The *in- vitro* drug release studies were plotted between cumulative amount of drug released and time.

First order model:

This first order model has also been used to determine absorption or elimination process of drugs, although it is difficult to understand this mechanism absorption and elimination on the behalf of theoretical basis. The first order kinetics can be expressed by the equation:

$$\text{Log } Q_t = \text{Log } Q_0 - \frac{K_1 t}{2.303} \qquad (4)$$

Wher e:

Q_0 = initial concentration of drug,

K_1 = first order rate constant, and

T = time.

The graph was plotted between log cumulative percentages of drug remaining or time which would yield a straight line with a slope of [-K/2.303]

Higuchi model

The Higuchi model is based on the hypotheses accordingly this model expression is given by the equation: it is used to measure release rate of formulation

$$Q = K_H t^{1/2} \qquad (5)$$

Where,

K_H: is the Higuchi dissolution constant.

$t^{1/2}$= square root of time

The data obtained were plotted as cumulative percentage drug release vr. Square root of time.

Korsmeyer-Peppas model

The first 60% drug release data were fitted in Korsmeyern Peppas model.

$$\{M_t / M_\infty = Kt^n\} \qquad (6)$$

Where:

M_t / M_∞ = fraction of drug released at time t,

K = release rate constant.

n = release exponent.

The n value is used to characterize different release for cylindrical shaped matrices.

In this model, the value of n characterizes the release mechanism of drug as described in Table. To study the release kinetics, data obtained from in vitro drug release studies were plotted as log cumulative percentage drug release versus log time.

RESULTS AND DISCUSSION

Compatibility studies of Tropicamide and non-ionic surfactants:

Infra red spectra of pure drug Tropicamide as well as drug with non ionic surfactant shows characteristic absorption peaks shown in Figure No. 1 All the characteristic peaks of Tropicamide were present in spectra thus indicating compatibility between drug and non- ionic surfactants. It shows that there was no significant change in the drug.

Solubility:

Solubility result was found to be Tropicamide freely soluble in acetone, ethanol, methanol and phosphate buffer (pH 7.4).

Appearance:

Appearance of formulation is important parameter for ophthalmic drug delivery. All formulations were found to be turbid shown in Figure No.9

pH:

pH values for all the formulations was found to be satisfactory in the range of 7.0-7.4 as tabulated in Table No. 9 The pH was within acceptable range and hence would not cause any irritation upon administration of the formulations.

Particle size and Zeta potential analysis:

Particle size analysis:

The particle size analysis was performed for optimized formulation by using Malvern zeta sizer.

Zeta size of particle F9 was found 156.

Zeta potential:

Zeta potential are performed optimized formulation of Tropicamide loaded niosomes batch no F9 were shown in table no. Zeta potential of particles of F9 span 60 batch was found in the range of +16m.

Drug Entrappment:

Drug entrapment efficiency of niosomes formulations are shown in table no. 8 among the niosomes formulations highest drug entrapment efficiency 84.66% was observed with formulation F9 and lowest drug entrapment efficiency 78.7% was observed with formulation F4. As increased concentration of span and decreased concentration of cholesterol there is a increased entrapment efficiency of the niosomal formulation. span 60 shows more entrapment efficiency.

Drug content:

Drug content formulations are shown in table no.8 Among the niosomal formulations highest drug content was observed 96.34% of F11 formulation and the lowest drug content was observed 50.97% of F6 formulation. As the increased non-ionic surfactant concentration there is increased drug content of niosomal formulations.

***In-vitro* drug release study:**

In-vitro drug release from the formulation was studied for all formulations. The medium consist 900 ml of Phosphate buffer pH 7.4 stirred at 50 rpm at 37˚C±5˚C. The drug release of a formulation shows in table no.12.

***In-vitro* drug release kinetics:**

The drug release kinetics of that formulation best fitted in the Peppas - Korsmeyer model and the R^2 value was found to be 0.98. The drug release kinetic of a formulation shows in table no.13.

Accelerated stability studies for the optimized formulation:

Optimized formulation (F9) was evaluated for the stability studies at refrigeration temperature. Result revealed that no change were observed in visual appearance, clarity, pH, drug content, drug entrapment was determined at periodical interval for each formulation which was found satisfactory. The figure show in table no.14.

Formulation of Tropicamide Niosomes composition of Niosome:

Table-1 Niosomes with varying Cholesterol: Surfactant Molar Ratio

S.NO.	Drug(mg)	Cholesterol(mg)	Span 20(mg)	Solvent(chloroform:methanol) (2:1)
1	50	100	100	15
2	50	95	105	15
3	50	90	110	15
4	50	85	115	15

Table-2 Niosomes with varying Cholesterol : Surfactant Molar Ratio(span 40)

S.NO.	Drug(mg)	Cholesterol(mg)	Span 40(mg)	Solvent(chloroform:methanol) (2:1)
1	50	100	100	15
2	50	95	105	15
3	50	90	110	15
4	50	85	115	15

Table-3 Niosomes with varying Cholesterol: Surfactant Molar Ratio(span 60)

S.NO.	Drug(mg)	Cholesterol(mg)	Span 60(mg)	Solvent(chloroform:methanol) (2:1)
1	50	100	100	15
2	50	95	105	15
3	50	90	110	15
4	50	85	115	15

Table 4: Determination of drug entrapment and Drug content

Formulation	% Drug Entrapment	% Drug Content
F1(span20)	84	78
F2(span20)	82.88	68.53
F3(span20)	80.71	66.34
F4(span20)	78.75	78.77
F5(span40)	84.66	90.24
F6(span40)	82.95	50.97
F7(span40)	83.22	72.19
F8(span40)	83.68	95.85
F9(span60)	84.35	95.36
F10(span60)	83.22	86.3
F11(span60)	82.24	96.34
F12(span60)	82.25	91.70

Table 5: Determination of Clarity and pH

Formulation	visual appearance	pH
F1	Clear	7.233
F2	Clear	7.2
F3	Clear	7.066
F4	Clear	7.166
F5	Clear	7.266
F6	Clear	7.233
F7	Clear	7.133
F8	Clear	7.166
F9	Clear	7.233
F10	Clear	7.366
F11	Clear	7.2
F12	Clear	7.0333

Table 6: Particle size and zeta potential analysis

S.no.	Optimized formulation	Mean particle size(nm)	Zeta potential(mV)	Pdi
1.	F9	156.82	16	0.277

Table 7: Pure Drug Dissolution

Time	%cdr
0.5	0.391899
1	6.14987
2	11.388
3	17.14923
4	23.19031
5	27.40523
6	37.81308
7	41.74769
8	50.90769
9	54.55846
10	58.17846
11	62.90615
12	74.28615

Table 8: In-vitro Drug release study F9 (SPAN60) Formulation

Time	% CDR
0.5	0.159494
1	3.04704
2	5.167692
3	8.336308
4	12.31385
5	18.34508
6	27.68308
7	35.04554
8	51.97846
9	64.81077
10	80.11231
11	88.78154
12	96.30615

Table9: F14 Accelerated stability study of optimized formulation F9

Parameter	15 days	30 days	45 days
pH	7.1	7.1	7.2
Drug entrapment	84	83.48	83.20
Viscosity	5.34	5.28	5.11
Dug content	94.21	94.033	93.98

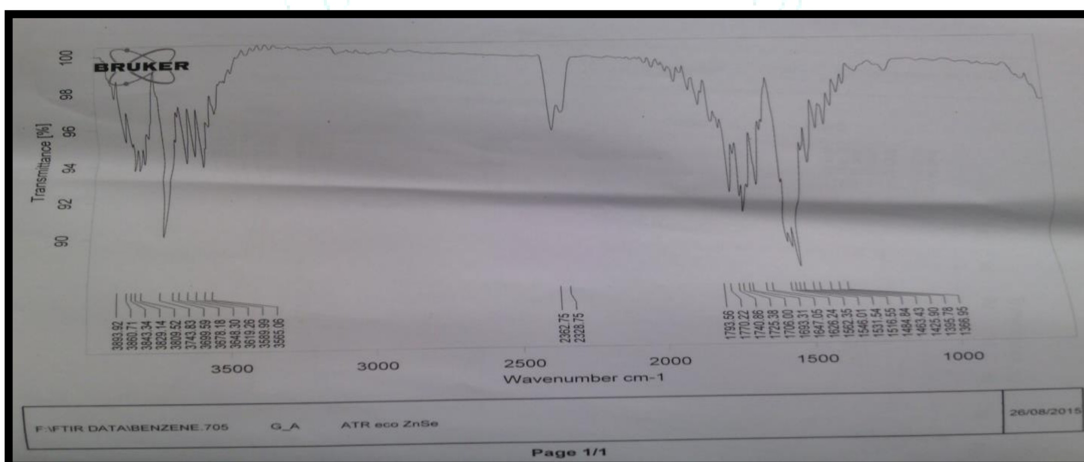

Figure 1: IR spectra of pure drug

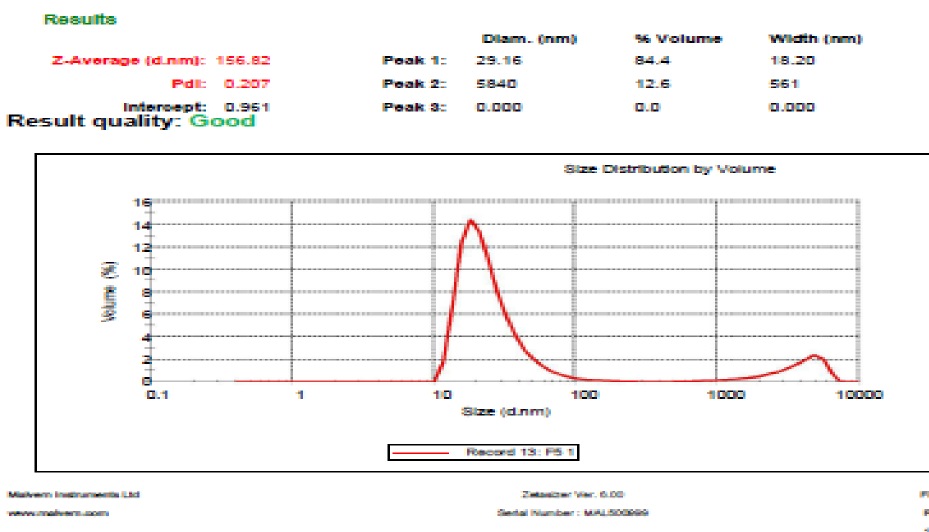

Figure 2: size distribution report

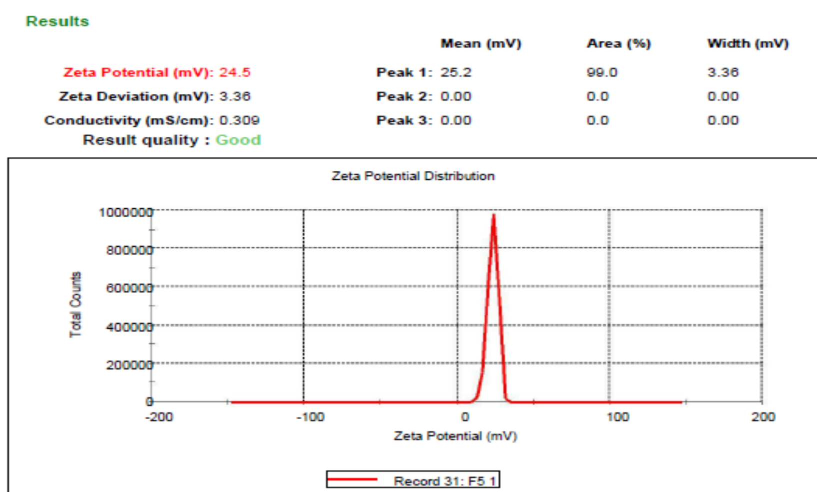

Figure 3: Zeta potential report

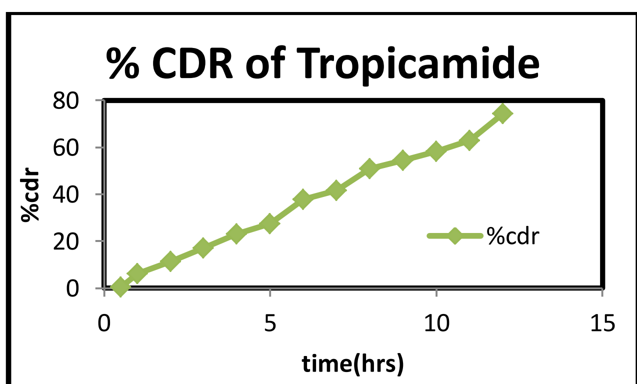

Figure 4: % CDR of Tropicamide

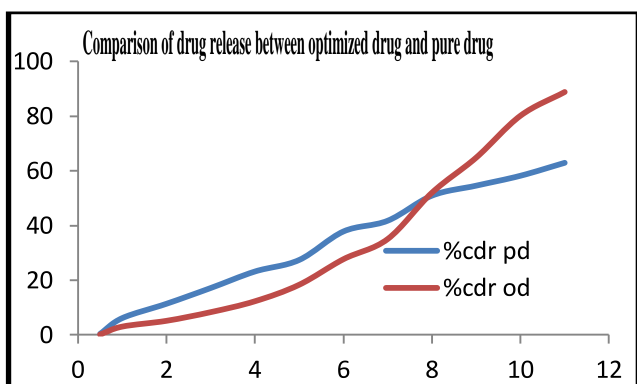

Figure 5: % CDR of pure drug and optimized formulation
{Comparison of drug release between optimized drug and pure drug (pd-pure drug)(od-optimized drug)}

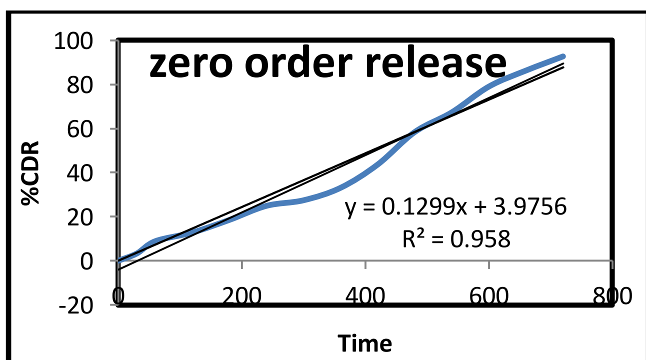

Fig: 6 Zero order release kinetics of optimized batch F9

CONCLUSION

In the last couple of years, continuous research has been going on for better delivery for ocular with the aim of more localized drug delivery and minimization of dosing frequency. An ophthalmic delivery system should preferably release the drug at a controlled rate to prolong the effect in reducing IOP and should be non toxic and comfortable for patient use. Tropicamide loaded Niosomes can be prepared by hand shaking method with Span 20,40,60 and cholesterol in the different ratio. In vitro release of Tropicamide from niosomes was very slow when compared to the release from Tropicamide solution. Our findings have shown that higher EE% of (84.22%) was obtained from niosomes prepared using Span 60/CH at F9 molar ratio with particle size diameter of 156.82 nm. In conclusion, niosomes could be a promising delivery system for Tropicamide with improved ocular bioavailability and prolonged drug release profiles.

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