ORIGINAL ARTICLE

The Development and Characterization of Eye Drops Containing Acyclovir Microspheres against Herpes Simplex-induced Keratitis

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ABSTRACT

Objective: Acyclovir (ACV), a highly specific inhibitor of herpes virus replication, is applied topically into the eye to treat ocular keratitis. The main aim of this study was to formulate and characterize eye drops containing ACV microspheres against herpes simplex-induced keratitis. The microspheres are small spherical particles with diameters ranging from 10 to 1000 μm. It increases bioavailability while decreasing side effects. **Materials and Methods:** The solvent evaporation technique was used to develop the microspheres for the sustained release drug delivery system. Crystalline methylcellulose, sodium alginate, calcium chloride, and chitosan were the polymers used. The drug content, encapsulation efficiency, *in vitro* drug release studies, stability studies, viscosity, pH measurement, and clarity test were all performed on the microspheres. **Results:** Particle size and shape, percent entrapment, *in vitro* drug release, compatibility studies, and other characteristics of microsomes were assessed. These drug delivery systems demonstrated good increased solubility and sustained release, which is necessary for bioavailability and therapeutic action, due to their matrix nature. The formulation has an acceptable shape and particle size, no chemical interactions, and is stable under refrigeration, according to the results of the characterization parameters and stability research. **Conclusion:** Eye drops containing microspheres of ACV could be considered a promising sustained drug delivery system for ocular keratitis treatment.

Key words: Acyclovir, eye drops, microsome, *in vitro* drug release

INTRODUCTION

Herpes simplex virus (HSV) can affect the eyes and cause infection. Herpes simplex keratitis is caused by recurrent infection of the cornea by the HSV.[1,2] The virus commonly transmits by droplet transmission or less frequently by direct inoculation. Herpes Keratitis remains the leading infection which is generally caused by corneal ulcers and also causes blindness. worldwide.[3,4]

The microsphere is small spherical particles, having diameters in the range between 10 and 1000 μm. It improves the bioavailability and reduces the side effects.[5,6] The main role of the microsphere is to enhance the permeability and the controlled delivery of the system of drug content.[7] Microsphere prepared by solvent evaporation technique for sustained release drug delivery system. There are two types of the microsphere.[8]

- Microcapsules
- Micrometrics

Microcapsules are those substances that entrapped the drug substance distinctively and are surrounded by capsules wall and micromeritics where the drug substance is dispersed throughout the matrix.[9,10] The microsphere is made up of

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polymer, waxes, and many the protective materials which are biodegradable synthetic polymers[11]

MATERIALS AND METHODS

Materials

Acyclovir (ACV) was a gift sample from Mylan laboratories limited, Nashik. Chitosan, sodium alginate and calcium chloride are available in the college laboratories. All other reagents used in this experiment are belongs to laboratory grade.

Methods

Preparation of microsomes

The ACV microsome was prepared according to the variable formulation in the given table. Briefly, sodium alginate solutions were prepared by dissolving them in the appropriate amount of distilled water. In the above solution, 1.5% w/v of ACV was added under homogenization for 5 min to achieve smooth dispersion of the drug in solutions. Further, the chitosan solution was prepared in 5% v/v aqueous acetic acid. In that solution, an accurate amount of calcium chloride was added to form an ACV alginate dispersion solution.

Then, this solution was filled in a 0.45 mm needle fitted in a syringe having dropped rate of 1 mL/min. Slowly drop-wise this dispersion solution was placed into the calcium chloride solution and stirred at 100 rpm at room temperature to yield opalescent beads. Prepared beads were allowed to harden for 2 h, the assize of beads was reduced, and were kept in a labeled self-sealing bag the composition of formulation is given in Table 1.

Method of formulation of microsomal eye drop suspension of ACV

An accurately weighed amount of crystalline methylcellulose (thickening agent) was dissolved in sterile water which was mixed together using a magnetic stirrer, at 25 rpm. Added prepared acyclovir microsomes equivalent to 1% of ACV.

Table 1: Composition of microsomes of ACV

Code	ACV (%)	Sodium alginate conc (% w/v)	Calcium chloride conc (%w/v)	Chitosan conc (%w/v)
F-1	1	1	5	1
F-2	1	1.5	5	1
F-3	1	2	5	1
F-4	1	2.5	5	1
F-5	1	3	5	1

ACV: Acyclovir

Then, benzalkonium chloride as a preservative, sodium chloride as a tonicity enhancer, and boric acid as a visualizer was added until a homogenous suspension was prepared. After the preparation of the suspension, the pH was adjusted and the formulated suspension was taken for further analysis. The composition of eye drop formulation was mention in Table 2.

Sterilization

After the preparation of the eye drop, suspension of ACV was sterilized by vacuum filtration using a 0.45 µm membrane filter and then UV light.

EVALUATION PARAMETERS

Characterization of the ACV microsomes

Calculation of % yield calculation

The percentage yield is calculated by:-

$$\%\ Yield = \frac{Practical\ yield}{Theoretical\ yield} * 100$$

Swelling index

For the swelling index, 50 mg of microsomes were placed in water and set aside to swell overnight, filtered, and weight, the degree of swelling is calculated by[18,19]

$$Swelling\ index\,(a) = \frac{\{Weight\ of\ swelled\ microsomes\,(Wg) - Initial\ weight\ of\ microsomes\,(Wo)\}}{The\ initial\ weight\ of\ microsomes\,(Wo)}$$

Particle size determination by optical microscopy

Four batches prepared (F1-F5) were analyzed for particle size by optical microscope.

First, the eyepiece micrometer was calibrated with the help of a stage micrometer. Then, microsphere was placed in the slide to cover it and observe under ×10 magnification. A hundred particles were counted and the average particle diameter was calculated using the formula[20,21]. The SEM analysis can be done of acyclovir microsomes and mentioned on Figure 1.

Where,

$Average\ particle\ diameter = \sum n * d/N$

n = Total no. of particles in that size range
d = Diameter of the particles of that size range
N = Total no. of particles.

Determination of % drug content and encapsulation efficiency

From each batch, take 20 mg of microsphere and mix in 100 mL of 0.1N HCl in a100 ml volumetric flask and kept aside for 24 h. Then filtered it with using Whatman filter

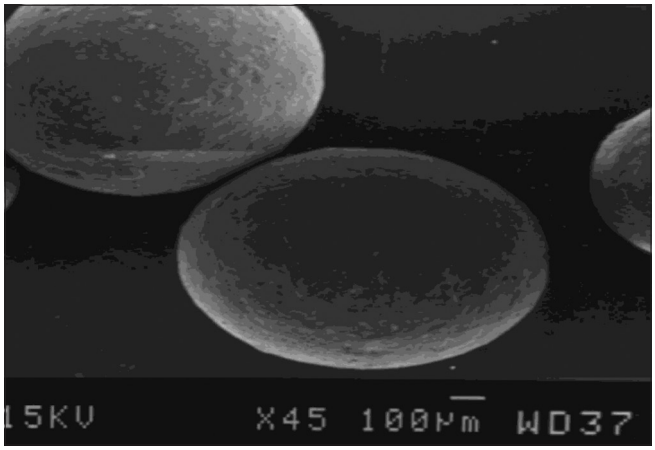

Figure 1: Surface morphology of optimized formulation

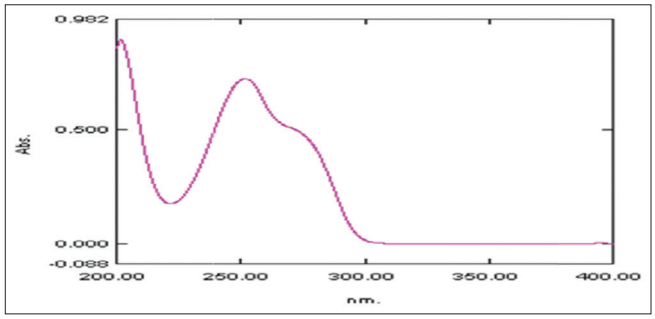

Figure 2: UV spectrum of acyclovir in phosphate buffer of pH7.4

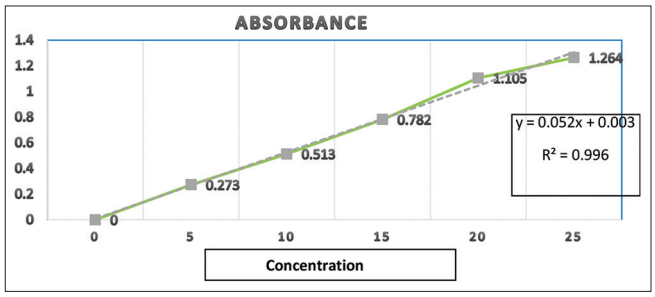

Figure 3: Calibration curve of Acyclovir in phosphate buffer (pH 7.4)

Table 2: Formulation of eye drop solution

S. No.	Name of ingredients	Quantities
1.	Microsome equivalent to acyclovir	1%
2.	Boric acid	0.5%
3.	Sodium chloride	5%
4.	Sodium metabisulphite	0.1%
5.	Benzalkonium chloride	0.01%
6.	Sterile water	q.s
7.	Crystalline methylcellulose	0.5%

paper no.1.after that 1 mL of filtrate was diluted with 50 mL of dimethyl sulfoxide in a volumetric flask, and sonicated for 15 min. Again, filter it further withdrawing 1 mL of solution

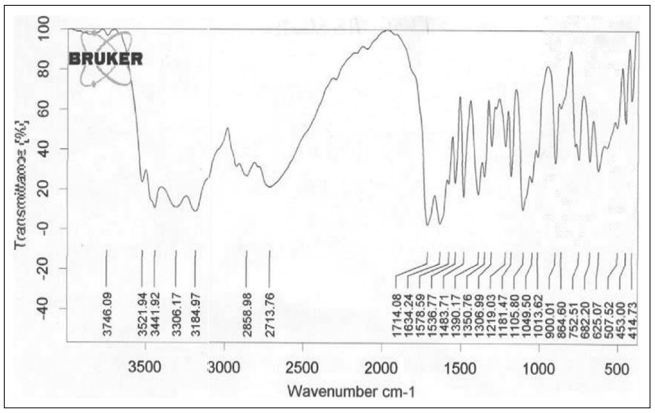

Figure 4: IR spectra of acyclovir

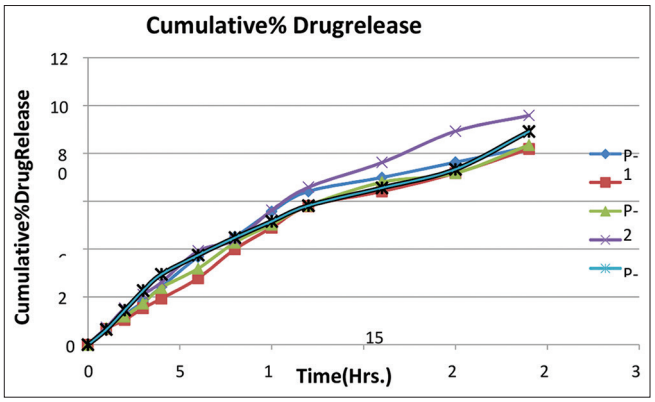

Figure 5: Cumulative % drug release of prepared microsomes

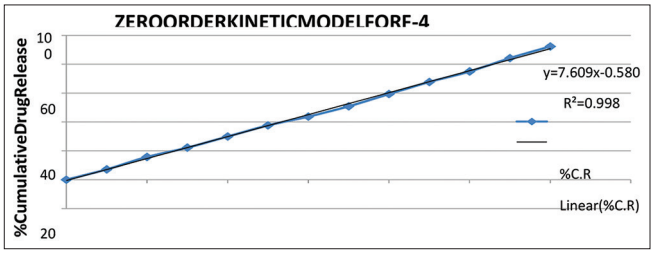

Figure 6: Zero-order kinetics for F-4

and diluting it with 10 mL of methanol, and the absorbance was measured at 252 nm using methanol as blank. After recording the absorbance, the drug content and encapsulation efficiency were calculated. The readings were taken thrice and the average reading was taken for further calculation.[11,12,22] The graph and calibration curve mention on Figures 2 and 3.

Amount of drug = Abs−Intercept/Slope (*10*100)/1000
% Drug content = Calculated amount of drug total/Amount of microspheres × 100
Encapsulation efficiency = Calculated drug content/ Theoretical drug content × 100

In vitro drug release studies

The *in vitro* dissolution studies were carried out using USP-34 paddle-type dissolution apparatus. 50 mg ACV-loaded

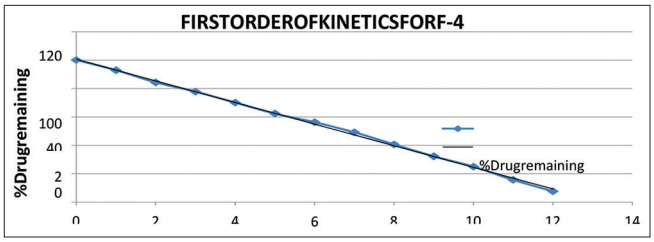

Figure 7: First-order kinetics for F-4

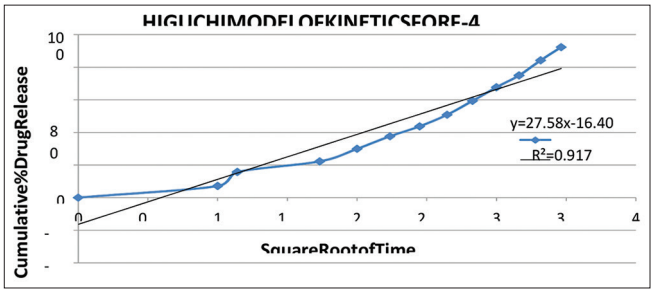

Figure 8: Higuchi model of kinetics for F-4

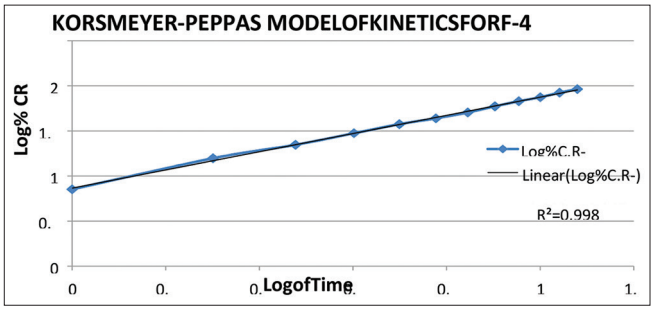

Figure 9: Korsmeyer–Peppas model of kinetics for F-4

microspheres were placed in a dialysis bag and introduced into 100 mL a dissolution medium of buffer solution pH 7.4 maintained at 37°C ± 0.5°C at a rotation speed of 50 RPM. 1 mL of aliquots was withdrawn at predetermined time intervals and an equivalent volume of fresh medium was replaced to maintain sink condition. The aliquots were diluted and analyzed spectrophotometrically at 252.0 nm to determine the concentration of the drug present. The readings were taken thrice and the average reading was taken for further calculation.[11,23,24,25] The Cumulative % drug release of prepared microsomes was mentioned in Figure 5.

Accelerated stability studies

The above-prepared samples were kept in sealed vials for 7 days at 40°C and 75% RH. Method of evaluation of microsomal eye drop solution of ACV.[11,26,31,32]

Measurement of pH

Accurately 2.5 mL of suspension formulation batches were measured using a digital pH meter. Before measurement, the pH meter should calibrate then readings were taken by dipping the glass rod into the formulations.[27,33,34]

Viscosity measurement

The viscosity of the eye drop suspension was determined using brook field viscometer, Spindle No.2 (Brookfield Engineering Labs., USA). All the formulated gels were sheared at 1.6–7.4 torque for 5 min. The shear stress was recorded for each formulation.[11,28]

Clarity testing (IP2007)

A clarity test was done against dark and white background board apparatus, for the presence of foreign particles.[11,12,29]

Stability studies

The stability of a drug is a great important factor that must be considered for its efficacy. Stability studies were done according to ICH guidelines for drug and formulation stability. The optimized formulation was kept in the stability chamber at a specified temperature and humidity (40°C ± 5°C and 75% RH), ambient condition, and (4°C ± 2°C and 15% RH) for 1 month. The chemical stability was assessed by the estimation of % of the drug remaining in the formulation, pH, and physical stability, which was evaluated by monitoring any change in pH, viscosity, and appearance.[13,30,31]

Data analysis and statistics

Data are expressed as mean ± SD. Statistical analysis was performed by student's test using MS Excel significance was defined at $P < 0.05$.

RESULTS AND DISCUSSION

To administer the precise dose and prolong the residence period of the drug in contact with the eyes which are not delivered in semisolid dosage form innovative, drug delivery is employed to achieve and promote ease of administration.

In pre-formulation studies, the organoleptic properties of ACV were found to be white crystalline, tasteless and odorless, and sparingly soluble in water. The melting point of the drug was found to be 256–258°C. λ_{max} was determined in phosphate buffer (pH 7.4) solvent at 252.0 nm. A standard calibration curve was prepared using a concentration range of 05–25 µg/mL and linearity equation as y = 0.052x + 0.003 with R^2 = 0.997. Drug ACV was also compatible with used excipients. It is physically stable and chemically stable as observed by Fourier transform infrared (FT-IR) spectra.

Pre-formulation studies

Organoleptic properties

Identification tests of ACV were done and it was observed that is crystalline in nature and white in color and has no odor.

The organoleptic properties of the received sample of ACV were found to be similar as mentioned in the literature. It is shown in Table 3.

Melting point

The melting point of ACV is observed to be in the range of 256–258°C with decomposition, that is, the substance chars as it starts to melt. It is shown in Table 4.

Solubility

The solubility of the drug ACV was observed in different solvents and it was mentioned in Table 5.

Determination of λ_{max} in 7.4pH buffer

Determination of λ_{max} was observed in a UV-Visible spectrophotometer and it was found to be 252 nm in phosphate buffer solvent. The result was mentioned in Table s 6-8 and Figures 1 and 2.

Calibration curve of ACV in phosphate buffer (pH7.4)

The calibration curve of ACV was performed in a phosphate buffer of pH 7.4. The calibration curve [Figure 4] was found to be linear in the concentration range of 4–20 µg/mL having a coefficient of regression value $R^2 = 0.999$ and line equation, $y = 0.053x + 0.012$.

FT-IR analysis

The Drug Acyclovir was compatible with used all the excipients. It is physically and chemically stable which was

Table 3: Results of identification tests of acyclovir

S. No.	Identification test	Observed result
1.	Appearance	Crystalline powder
2.	Color	White in color
3.	Odor	Odorless

Table 4: Melting point of acyclovir

Identification test	Observed result
Melting point	256–258°C

Table 5: Solubility of acyclovir

S. No.	Solvent	Solubility (mg/mL)
1.	Water	Sparingly soluble
2.	Phosphate buffer (pH 7.4)	Soluble
3.	Ethanol	Freely soluble
4.	0.1NHCl	Soluble
5.	Methanol	Freely soluble
6.	0.1NNaOH	Soluble

Table 6: Wave length of maximum absorbance (λ_{max}) of acyclovir

S. No.	Solvent	λ_{max} (nm)
1.	Phosphate buffer (pH 7.4)	252.0 nm

Table 7: Concentration and absorbance values for acyclovir in pH 7.4 buffer at 251.3 nm

Concentration (µg/mL)	0	5	10	15	20	25
Absorbance	0	0.273	0.513	0.782	1.105	1.264

Table 8: Interpretation of IR

Standard Peaks (cm^{-1})	Peak assigned	Observed peaks (cm^{-1}) acyclovir	Observed peaks (cm^{-1}) formulation
3700–3000	N-Hstr	3521	3446
3400–3000	O-H	3306	3311
3040–3000	C-Hstr.methyl group	2858	2974, 2876
1900–1600	C=Nstr.	1714	1712
1650–1600	C=Ostr.	1634	1631
1500–1300	C–Hb and methylene group	1483	1481

Principal peaks for IR of ACV are 3446, 3311, and 1712 cm^{-1}. The above spectrum matches the standard spectrum for ACV

Table 9: Results of characterization of acyclovir microsomes

Formulation	% Yield	Microsome Size (µm)	(%) Encapsulation	Swelling index
F1	87.21	819.41±1.931	79.15±1.21	4.14 (burst)
F2	83.6	817.26±1.152	83.26±1.45	4.04 (brurst)
F3	88.52	711.86±1.621	83.73±2.88	3.55
F4	91.15	631.13±0.638	91.36±1.73	4.18
F5	93.02	58174±0.932	81.24±0.46	3.88

Statistically significant difference among the values ($P<0.0001$)

observed by FT-IR spectra The result was shown in Figure 4 and Table 8.

Characterization of the ACV microsomes

Surface morphology by SEM analysis

The microsphere was formed in spherical with slightly elongated tips, and smooth surface were observed. The image shown in Figure 3.

In vitro drug release studies

In vitro release rate studies The Kinetics modelling of the drug release profile for formulation F4 (ACV Microsomes) followed zero order. First-order, Higuchi's model and Korsmeyer–Peppas model are shown in the graph. On the basis of the highest R2 value, F-4 is the best formulation having a maximum % of drug release (96.72%) also selected for further studies.

Table 10: R^2 values of all microsome formulations

Model	Equation	R^2
F1	y=3.449x+9.617	R^2=0.934
F2	y=3.571x+6.322	R^2=0.954
F3	y=3.474x+8.227	R^2=0.951
F4	y=4.061x+8.849	R^2=0.967
F5	R-value	R^2=0.948

Based on the highest R value, *P*-4 is the best formulation having a maximum % of drug release (95.82%) also selected for further studies

Evaluation of microsomal eye drop solution of ACV

Further, the Prepared microsomal eye drop solution of acyclovir was evaluated by different parameters like clarity irritation test viscosity, pH stability, kinetic modeling /Invitro release rate study etc.

The clarity test was found to clear with no irritation at pH7.4 having a viscosity of 5.6±0.8 cps .result was mentioned on Table 11.

Stability studies

All formulations were kept in the stability chamber at a specified temperature and humidity for 1 month. The chemical stability was assessed by the estimation of % drug remaining in the formulation, pH and physical stability was evaluated by monitoring any change. The result was mentioned in Table 12.

Kinetics modeling

The Kinetics modeling of drug release profile for formulation F4 (ACV Microsomes) are followed zero order. First-order Higuchi's model and Korsmeyer–Peppas model are shown in graph. Formulation F-4 is the best formulation having a maximum % of drug release (95.82%) also selected for further studies The result was mentioned in the Figures 6-9 and Table 13.

Table 11: Evaluation of acyclovir microsomal eye drop solution

Formulation code	pH	Clarity	Irritation test	Viscosity (cps)
F-1	7.2	Clear	Negative	5.2±1.8
F-2	7.1	Clear	Negative	5.3±2.0
F-3	7.4	Clear	Negative	5.5±1.2
F-4	7.4	Clear	Negative	5.6±0.8
F-5	7.8	Clear	Positive	5.2±1.2

Table 12: Stability of acyclovir microsomal eye drop solutions at different conditions

Conditions	Evaluation parameters	F1	F2	F3	F4	F5
4±2°C and 15% RH	% Drug remaining	90.13%	91.20%	89.13%	96.81%	83.61%
	pH	7.2	7.2	7.5	7.3	7.7
	Physical change	No	No	No	No	No
Ambient temperature	% Drug remaining	91.32%	98.13%	87.61%	96.53%	93.83
	pH	7.2	7.3	7.6	7.2	7.3
	Physical change	No	No	No	No	No
40±5°C and 75% RH	% Drug remaining	92.21%	98.99%	87.32%	93.53%	91.37%
	pH	7.2	7.3	7.3	7.2	7.3
	Physical change	No	No	No	No	No

Table 13: *In vitro* curve fits for various release systems for formulation F-4

Model	Equation	R2
Zero-order	y=7.609x–0.580	R^2=0.998
First-order	y=–7.609x+100.5	R^2=0.998
Higuchi	y=27.58–16.40	R^2=0.917
Korsmeyer–Peppas	y=1.009x+0.867	R^2=0.998

CONCLUSION

ACV microsomes were prepared using sodium alginate solutions containing 1% w/v ACV, and add 1% w/v or 2% w/v chitosan solution in a 5% v/v aq. acetic acid. Amounts of calcium chloride were added to the chitosan solutions. To make opalescent beads, the ACV-alginate dispersions were loaded into a syringe fitted with a 0.45 mm needle and slowly drop-wise added at a rate of 1 mL/min into chitosan-calcium chloride solutions stirred at 100 rpm at room temperature. A unique drug delivery strategy that can offer greater solubility, an extended period, an improved skin absorption is needed for ACV because it was only weakly soluble in water. As a permeability enhancer for ACV, nerolidol was employed. For a compatibility investigation, particle size and shape, percent entrapment, and *in vitro* drug release, microsomes were characterized. These drug delivery systems demonstrated good increased solubility and sustained release, which is necessary for bioavailability and therapeutic action, due to their matrix nature. The formulation has an acceptable shape and particle size, no chemical interactions, and is stable under refrigeration, according to the results of the characterization parameters and stability research. An extensive investigation is needed with reference to the depth of penetration into the eye, determination of zeta potential, and confirmation of the configuration of phospholipids in the lipid layer. There is a need to develop a suitable formulation for commercial exploitation.

AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

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