Formulation and charecterization of Acetazolamide loaded cubosomal gel for enhanced ocular drug delivery

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Abstract:

Aim: The present study is aimed to formulation and characterization of acetazolamide loaded cubosomal gel for enhanced ocular drug delivery. **Objective:** design and evaluate an acetazolamide-loaded cubosomal gel formulation aimed at improving the ocular bioavailability and therapeutic efficacy in the treatment of glaucoma. To analyze the role that lipid selection plays in and stabilizer concentration on the formation and performance of acetazolamide-loaded cubosomes. **Material and Method:** Top-down fabrication method was employed to produce loaded cubosomal dispersions composed of a GMO bulk phase and Poloxamer 407 were accurately weighed, melted at 60°C to form a homogeneous lipid phase, and acetazolamide was incorporated with continuous stirring. Preheated distilled water (70°C) was added dropwise under constant stirring to form a two-phase system, which was allowed to equilibrate for 24 hours to promote self-assembly. The dispersion was then homogenized at 8000–10,000 rpm and sonicated for 15 minutes to reduce particle size and stabilize the cubosomes, was maintained under ambient conditions protected from light, and used for subsequent gel formulation. **Results:** The first performed Preformulation study of drug and excipients Solubility, Melting point, UV Spectroscopy, FTIR, DSC. The formulated Acetazolamide cubosome gel were characterized by various tests like Entrapment efficacy, in vitro drug release, Particle size, Zeta potential, TEM, etc. F8 Batch of Cubosome out of nine formulations was found to be optimized. The average Particle size of the optimized F8 batch was measured at 124 nm, with a zeta potential of 20.07 mV. The entrapment efficiency across 9 batches varied from 63.12% to 80.15. The entrapment efficiency of optimized batch was found to be 80.15%. In-vitro drug release for these batches was observed between 68.5% and 88.58% in 8hr. The application of Cubosome proved the potential for ocular delivery of Acetazolamide over the conventional Tablet formulations and Ocular drug delivery for Acetazolamide has been successfully developed.

Keywords: Cubosome, Ocular, Acetazolamide.

1. **Introduction:** Acetazolamide (ACZ) is used in the treatment of glaucoma, with the aim of lowering IOP, especially in emergency cases. It exerts its action by inhibiting the activity of carbonic anhydrase in the ciliary processes of the eye and consequently decreases the production of the aqueous humor. Despite its potent action in glaucoma management, a high oral dose (500 mg) is needed to achieve its therapeutic effect by carbonic anhydrase inhibition in the peripheral system. Subsequently, the broad range of systemic side effects is associated with ACZ that interfere with patient convenience. The most common side effects are diuresis, renal failure, vomiting, anorexia, central nervous system depression, and metabolic acidosis. The topical formulations for ACZ are not available in the market until now as it has poor solubility in water and limited corneal permeability which resulted in low ocular bioavailability, causing an inadequate amount of the drug to reach the ciliary body. Numerous studies tried to improve ACZ ocular bioavailability to develop an effective topical formulation. Among these studies is the formulation of ACZ in aqueous solutions containing cyclodextrins or penetration enhancers and polymeric suspension. Other studies employed nanotechnology-based approaches through vesicular preparations as niosomal and liposomal dispersions, modified vesicles nanoparticles, dendrimers and nanoemulsion. Cubosomes have gained growing attention as ophthalmic nanocarriers in the last few years due to their biocompatibilities and bioadhesive properties. In addition, cubosomes are characterized by their high loading capacity for many drugs of different hydrophilicities. They also enable targeted and controlled drug release, enhance drug stability, improve transcorneal permeability, prolong corneal retention, and are prepared by simple techniques at low cost.

2. MATERIALS AND METHODS

2.1 Materials: Acetazolamide was purchased from Dhamatec, Mumbai. Glyceryl monooleate, Polaxamer407 Research lab, Mumbai. Carbopol934 Molychem and Triethanolamine Propyleneglycol Loba chemical, Mumbai. All the material used for formulation of Cubosome gel are of Analytical grade.

Preformulation Studies

3.1 Physical characteristics

By visual examination, the drug was identified for physical characters like color, texture and odour etc.

3.2 Solubility

Solubility studies of Acetazolamide were carried out in water, Methanol, Aceton, Dimethyl Sulfoxide. 10 mg of drug was dissolved in each solvent separately and solubility was determined qualitatively.

3.3 Melting Point

A Small quantity of drug was placed into a fusion tube. That tube was placed in digital melting point apparatus. Then the melting point was recorded.

3.4 Determination of λ_{max} and calibration curve:

Determination of λ max: 141°C The absorbance maxima were found using a UV Spectrophotometer.10 milliliter flask with 10 mg drug and a 7.4 pH buffer at the proper strength. That solution was diluted to 50 milliliters from five milliliters using phosphate buffer 7.4 (100 mcg/ml). 10 milliliters was added to a stock solution. a different flask. The remedy was diluted using 100 ml of pH buffer 7.4 (10 mcg/ml). Implementing a UV Spectrophotometer, the solution's maximum acetazolamide concentration. Acetazolamide wavelength reaches its maximum at 265nm.

3.5 FTIR Spectroscopy

The aim of FT-IR study is to determine purity of drug and study drug-excipient compatibility. FTIR spectroscopy can be used to investigate and predict any possible physiochemical interaction between different components in a formulation and therefore it can be applied for the selection of suitable compatible excipients while selecting the ingredients, we would choose those which are stable, compatible, cosmetically and therapeutically acceptable. The aim of the present study was to find out the possible interaction between Glyceryl monooleate, Polaxamer407. Carbopol934 Molychem and Triethanolamine Propyleneglycol and the drug Acetazolamide and to identify the compatibility between the drug and other excipients. 10 mg of the sample put in FTIR plate surface and touch senser using OPUS Software in Bruker ALPHA II FTIR Spectrophotometer.

3.6 Differential scanning calorimetry (DSC)

The DSC studies carried out to observe the thermal behavior of pure drug were carried out using differential scanning calorimeter (STAR SW 12.10). Sample of about 5 mg was placed in a 50µl perforated aluminum pan and sealed. Heat runs for each sample were set from 5°C to 300°C using nitrogen as purging gas and samples were analyzed.

4. Characterization of Cubosomal Gel

Was employed to produce loaded cubosomal dispersions composed of a GMO bulk phase and Poloxamer 407 were accurately weighed, melted at 60°C to form a homogeneous lipid phase, and acetazolamide was incorporated with continuous stirring.

Preheated distilled water (70°C) was added dropwise under constant stirring to form a two-phase system, which was allowed to equilibrate for 24 hours to promote self-assembly.

The dispersion was then homogenized at 8000–10,000 rpm and sonicated for 15 minutes to reduce particle size and stabilize the cubosomes, was maintained under ambient conditions protected from light, and used for subsequent gel formulation.

4.1 Experimental Design

Utilizing Design Expert software 12, statically assess the outcomes of the primary composite design. Two independent factors were studied: glyceryl monooleate (X1) and poloxamer 407

(X2); their effects on Entrapment effectiveness and particle size were found to be studied as dependent variables. 9 runs come from the factorial

Formulation Code	Factor1(X1)	Factor2(X2)	Response1(Y1)	Response2(Y2)
	A: GMO	Stirring Rate	Particle Size	EE
	ml	RPM	nm	%
F1	0	-1	157.5	69.73
F2	-1	-1	160	66.12
F3	1	-1	169	63.12
F4	0	0	134.2	78.37
F5	-1	0	136.9	76.48
F6	1	0	145.6	72.23
F7	-1	1	126	75.58
F8	0	1	124.6	80.15
F9	1	1	132.7	74.42

5. Characterization of Acetazolamide Cubosome

5.1 Entrapment efficacy

The effectiveness of trapping of cubosomal formulation varied between 66.12% and 80.15%. Table 8.15 and show how efficient each of the nine formulations is entrapping the drugs. Poloxamer 407 and Glyceryl Monooleate were combined to create a cubosome with a high entrapment efficiency. Among the formulations tested, the F8 formulation exhibited the highest entrapment efficiency. The F8 formulation showed that cuboosomal vesicles may be produced by the proper ratios of Glyceryl Monooleate and Poloxamer 407.

5.2 Particle size The optimal Mean Diameter that has a considerable impact on the solution's stability, accessibility, and organoleptic properties. the reactive substance's capacity to stabilize and release. that are used in formulation are impacted by the critical particle size dispersion.

5.3 Zeta potential The nature and composition of the environment, as well as the surface charge of the particle, anything adsorbed to the interface and nature and composition of the surroundings, all influence the zeta potential. A zeta sizer containing zeta cells and gold-plated polycarbonate cells can be used to calculate the surface charge of the cubosome. The zeta potential is crucial in determining the stability of cubosome.

5.4 Transmission Electron Microscopy The drug has been successfully kept inside the vesicle, as shown by a TEM study of a cubosome containing acetazolamide-loaded cubosomes, which also reveals a smooth surface structure. Particles having a mean curvature of zero have cubic forms. Hexagonal vesicles were seen in tiny numbers, nevertheless. Particle sizes were substantially separated from one another and in the nano range.

5.5 In-vitro drug Release: The dialysis membrane-based diffusion trials took 8 hours for all of the formulations to finish. sample employing a double-beam UV spectrophotometer, analysis was done. An illustration of the release for batches is shown diagrammatically in the picture. This study examined the release in vitro of a formulation of Acetazolamide-Encapsulated Cubosomal gel using simulated tear fluid with a pH of Effective drug release-maintained vesicles with a distinct biphasic rhythm are reported to exist in nanoparticles.

6. RESULTS AND DISCUSSION

6.1 Preformulation Studies

Tests	Procedure	Observations
Colour	Visual observation	yellow-white
Odor	Smelling by nose	Odourless
Physical appearance	Visual observation	Crystalline Powder
Melting Point	Capillary Method	258°C

6.2 Solubility

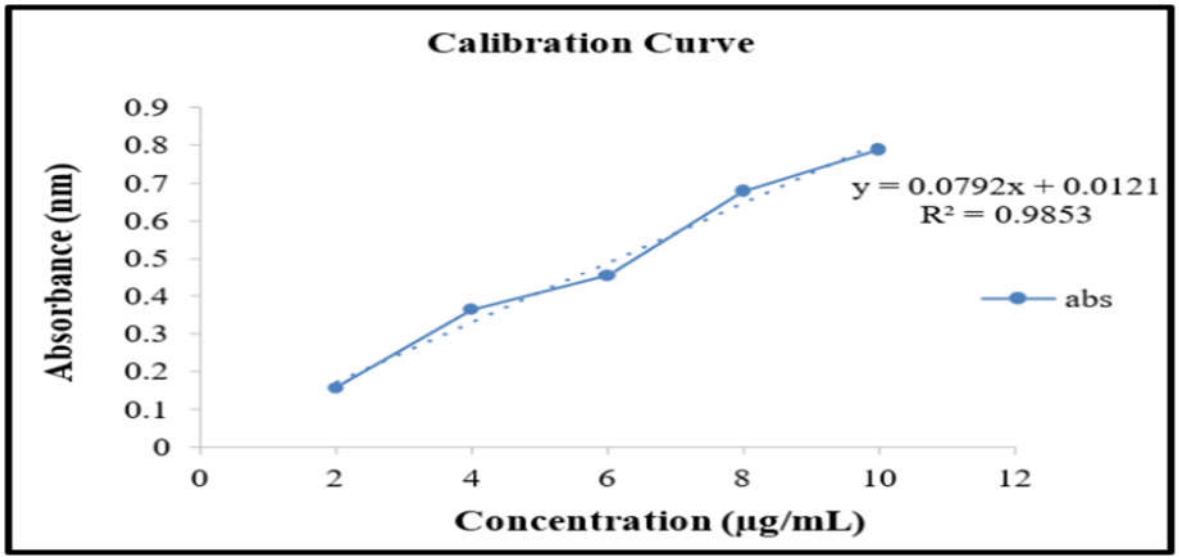
Sr. No.	Name of Solvent	Solubility data
1	Water	Insoluble
2	Methanol	Slightly soluble
3	Aceton	Slightly soluble
5	Dimethyl Sulfoxide	Soluble

6.3 Determination of ʎmax and calibration curve:

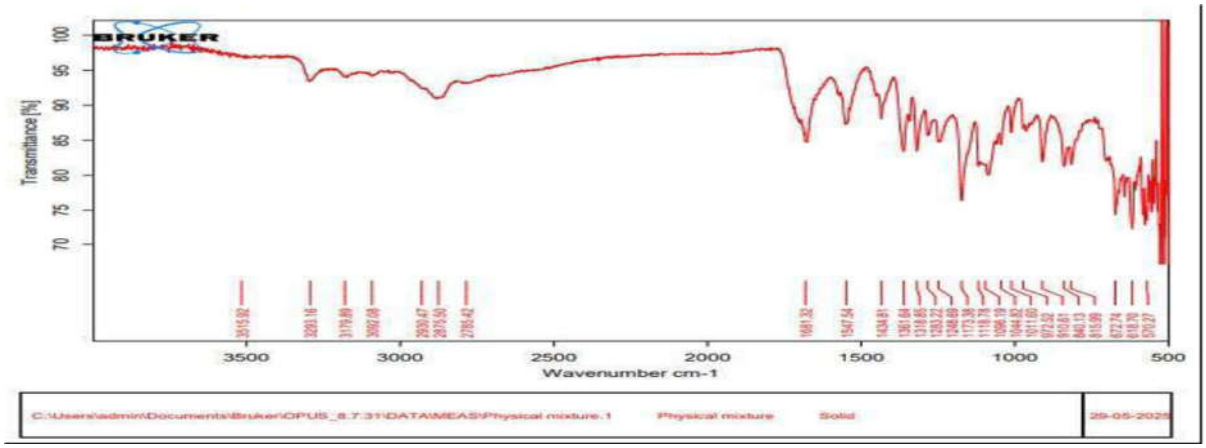
Determination of λ max: 141°C The absorbance maxima were found using a UV Spectrophotometer.10 milliliter flask with 10 mg drug and a 7.4 pH buffer at the proper strength. That solution was diluted to 50 milliliters from five milliliters using phosphate buffer 7.4 (100 mcg/ml). 10 milliliters was added to a stock solution. a different flask. The remedy was diluted using 100 ml of pH buffer 7.4 (10 mcg/ml). Implementing a UV Spectrophotometer, the solution's maximum acetazolamide concentration. Acetazolamide wavelength reaches its maximum at 265nm.

6.3 Calibration Curve of Acetazolamide

Sr. No.	Concentration (µg/ml)	Absorbance
1	0	0
2	2	0.155
3	4	0.361
4	6	0.453
5	8	0.678
6	10	0.788



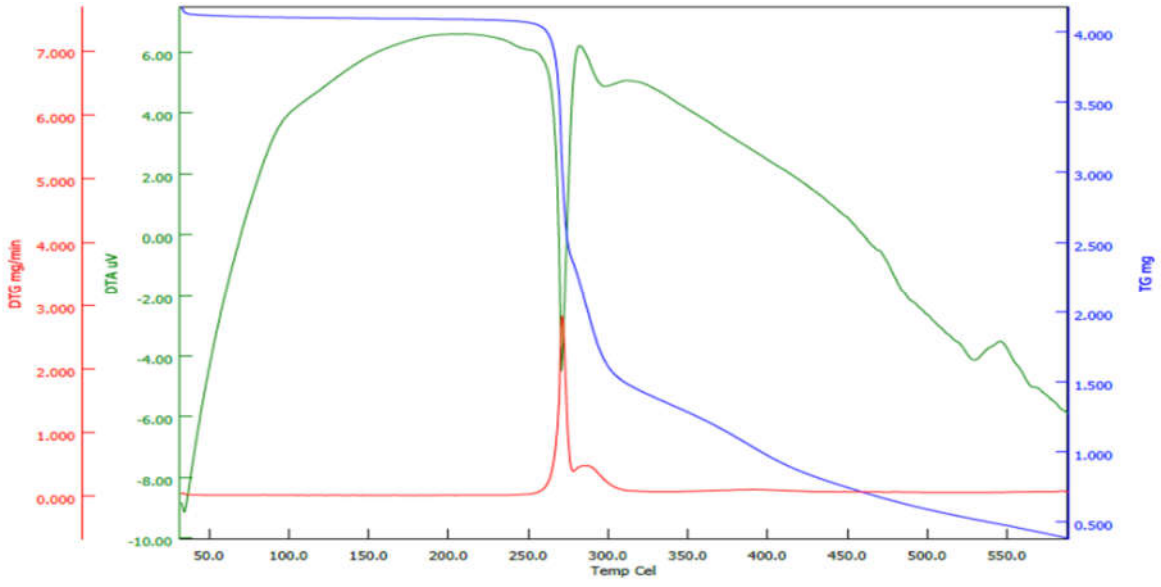
6.4 FT-IR Analysis



FTIR Spectra of Finished product

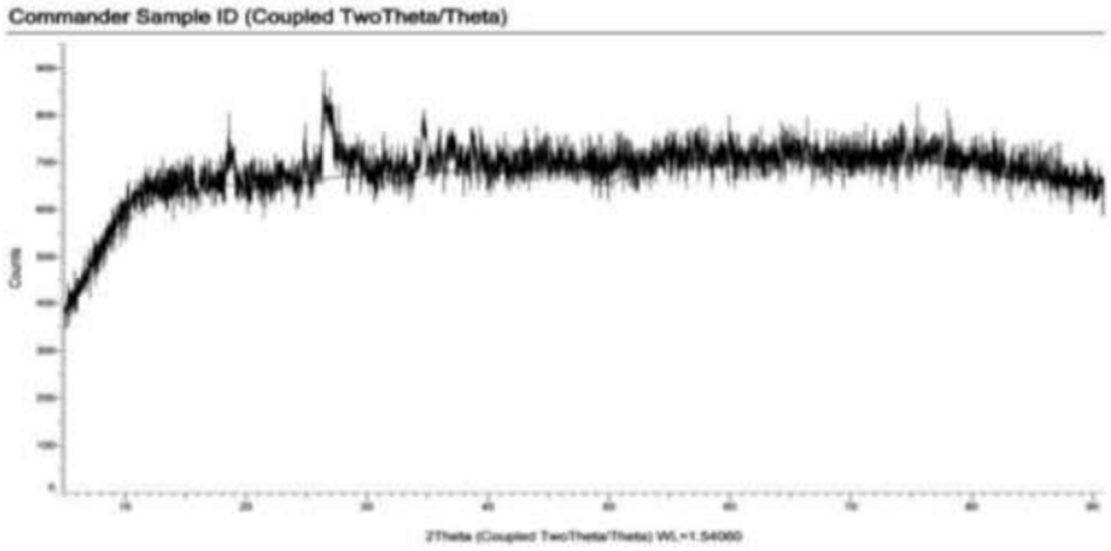
6.5 Differential Scanning Calorimetric Studies:

The given thermal analysis image is a combined TG–DTG–DTA thermogram, often obtained from simultaneous thermal analysis (STA). The TG–DTG–DTA thermogram shows that the sample remains thermally stable up to about 250 °C with no significant weight loss. A sharp decomposition event around 260–280 °C is observed, indicated by a major weight loss in the TG curve and a prominent DTG peak. The corresponding DTA peak confirms a thermal degradation process at this temperature. Gradual weight loss at higher temperatures suggests further breakdown and residue formation, indicating good thermal stability of the sample below its decomposition temperature.



6.6 Diffraction of X-rays (XRD)

X-ray diffraction (XRD) pattern recorded in coupled θ/2θ (Bragg– Brentano) mode. The diffractogram was plotted with the diffraction angle (2θ) on the abscissa and the diffraction intensity (counts per second) on the ordinate. Multiple sharp peaks indicate the sample is predominantly crystalline with ordered atomic planes. The low-angle peaks (~6–10°) suggest large lattice spacing or layered crystal packing. Each peak corresponds to a specific crystal plane and acts as a fingerprint for the material. XRD alone does not confirm identity or solubility unless matched with a reference database.



6.7 Drug Entrapment Efficiency:

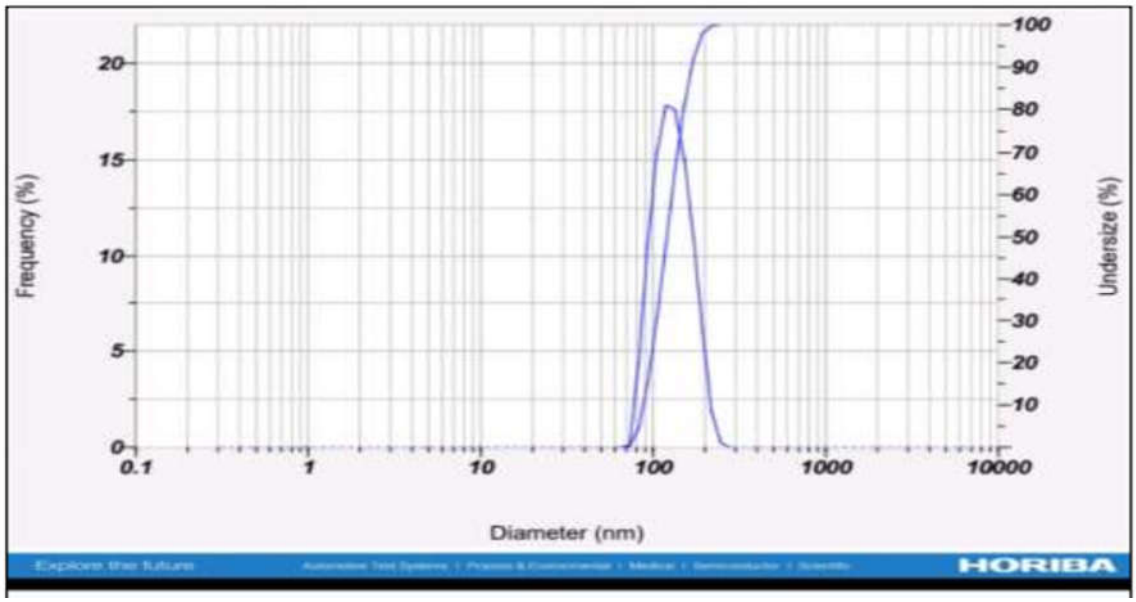
The effectiveness of trapping of cubosomal formulation varied between 66.12% and 80.15%. Table 8.15 and show how efficient each of the nine formulations is entrapping the drugs. Poloxamer 407 and Glyceryl Monooleate were combined to create a cubosome with a high entrapment efficiency. Among the formulations tested, the F8 formulation exhibited the highest entrapment efficiency. The F8 formulation showed that cuboosomal vesicles may be produced by the proper ratios of Glyceryl Monooleate and Poloxamer 407.

Drug Entrapment Efficiency

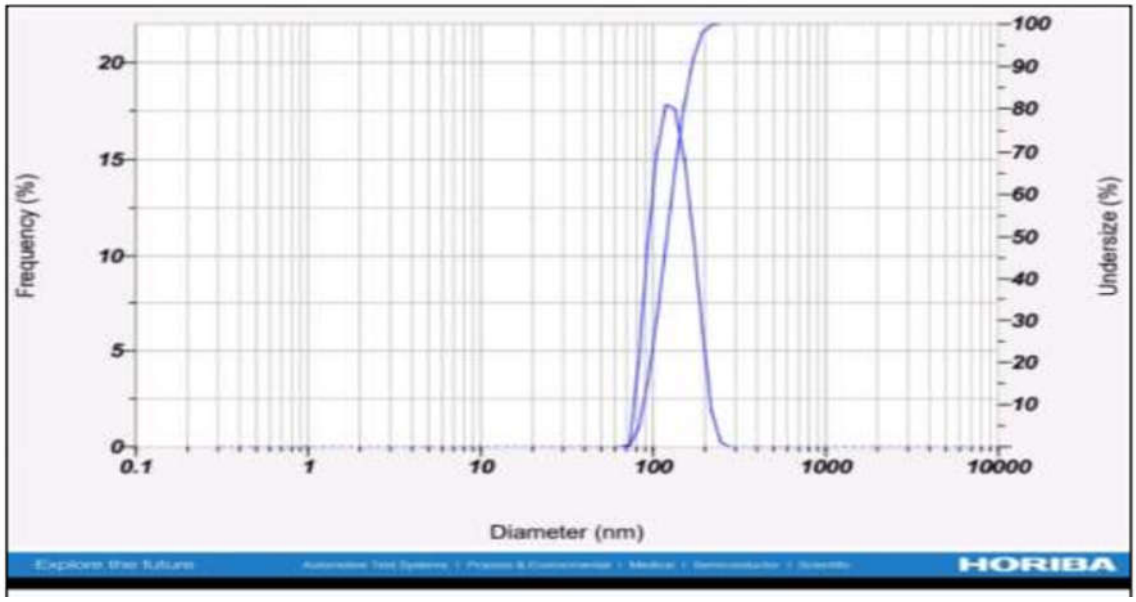
Batch. No	Entrapment Efficacy
F1	69.73
F2	66.12
F3	63.12
F4	78.37
F5	76.48
F6	72.23

F7	75.78
F8	80.15
F9	74.42

Particle Size of Batch F8



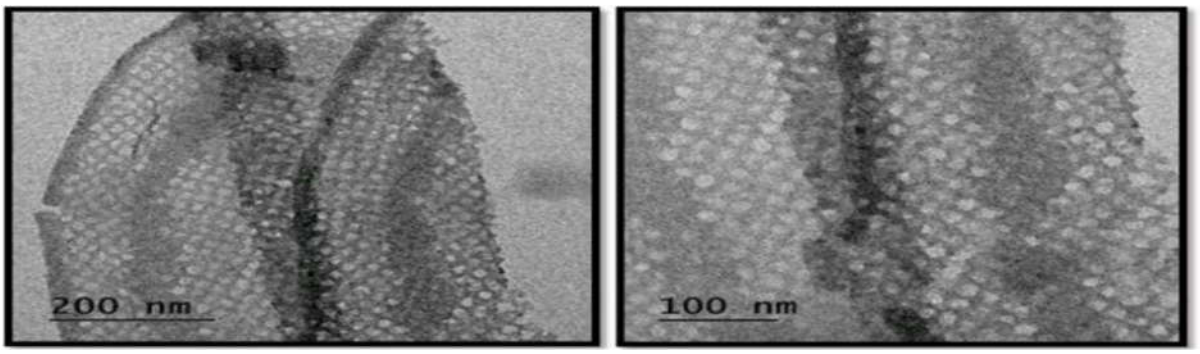
Zeta Potential of Batch F8



6.8 Transmission Electron Microscopy

The drug has been successfully kept inside the vesicle, as shown by a TEM study of a cubosome containing acetazolamide-loaded cubosomes, which also reveals a smooth surface structure.

Particles having a mean curvature of zero have cubic forms. Hexagonal vesicles were seen in tiny numbers, nevertheless. Particle sizes were substantially separated from one another and in the nano range.



Transmission Electron Microscopy

EVALUATION OF ACETAZOLAMIDE LOADED CUBOSOMAL GEL

6.9 Measurement of pH:

Ocular formulations with pH readings between6.4 to 7.4 are generally well tolerated and do not cause irritation to the eye, as they are within the normal physiological range of tear fluid pH.

Measurement of pH

Formulation code	pH
F1	6.32
F2	6.08
F3	6.42
F4	6.25
F5	6.01
F6	6.75
F7	5.87
F8	6.22
F9	5.91

6.10 Fluid Consistency

The viscosity of the gel was measured using a Brookfield viscometer at specified shear rates. It was observed that the gel exhibited a formulation increased with a higher concentration of polymer used in the preparation of Cubosomes. This indicates that polymer content plays a significant role in enhancing the gel's viscosity

Filuid Consistency

Formulation code	Viscosity (Cps)
F1	5073.5
F2	5234.9
F3	4909.3
F4	5077.7
F5	5160.5
F6	5292.4
F7	5347.1
F8	5519.3
F9	5203.7

6.11 Spreadability:

The calculation was performed using the following formula: sprediability

$$X = \frac{ML}{T}$$

T is the time measured in seconds needed to separate the slides, L is the length (in cm) of the glass slides, and M is the weight (in grames) attached to the top slide. The Cubosome loaded gel whose spreadability varies from 7.1 to 8.5 g cm/s.

Spreability

Formulation code	Spreadability(gm.cm/sec)
F1	7.3

F2	7.1
F3	7.9
F4	7.5
F5	8.2
F6	8.5
F7	7.7
F8	7.9
F9	8.0

6.12 Drug content:

The drug content gels were found to % to % respectively. Table 9.14: The number of drugs in Cubosomal gel.

Drug Content of Cubosome Loaded Gel

Formulation code	Spreadability(gm.cm/sec)
F1	7.3
F2	7.1
F3	7.9
F4	7.5
F5	8.2
F6	8.5
F7	7.7
F8	7.9
F9	8.0

6.13 Stabilities studies:

The steadiness of the acetazolamide-encapsulated cubosomal gel was evaluated by storing the formulations at three different conditions: refrigerated room temperature (25°C ± 2°C), 4°C ± 2°C, and accelerated temperature (between 40°C and 2°C) with At 75% relative humidity for a period of one month. After storage, the drug content was found to be 94.45%, 93.23%, and 88.52% at 4°C, 25°C, and 40°C, respectively, indicating minimal degradation under refrigerated and room temperature conditions, with a slight reduction at elevated temperature. The pH values of the gels remained relatively stable, measured at 7.3, 7.2, and 6.9 at 40°C, 25°C, and 4°C, respectively, which meets the standard criteria for ophthalmic products. These findings demonstrate that the cubosomal gel formulation possesses adequate stability in terms of both physical and chemical at least a month under the tested storage conditions, supporting its suitability for ocular administration.

Drug Content of Cubosome Loaded Gel

Parameter	After 1 Month at 25°C	After 1 Month 36°C	After 1 Month 40°C
pH	7.2	7.3	6.9
Content of Drugs	92.23	94.45	88.52

6.14 In-vitro drug Release

The dialysis membrane-based diffusion trials took 8 hours for all of the formulations to finish.

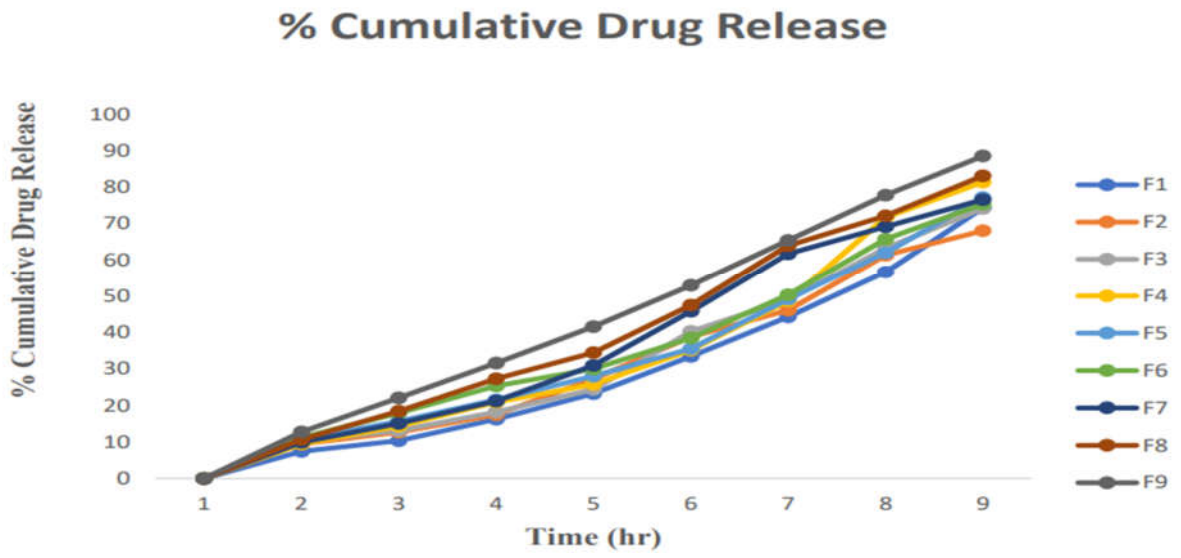
sample employing a double-beam UV spectrophotometer, analysis was done. An illustration of the release for batches is shown diagrammatically in the picture.

This study examined the release in vitro of a formulation of Acetazolamide-Encapsulated Cubosomal gel using simulated tear fluid with a pH of Effective drug release-maintained vesicles with a distinct biphasic rhythm are reported to exist in nanoparticles.

The drug's spread from the particles with relation to the surrounding water phase as a result of drug entrapment initial burst action. On the other hand, 1 hour after manufacturing, the percentage of medication released from the cubosome formulation varies from 7.45 to 12.77 percent.

In-Vitro Drug Release

TIME (Hr)	F1%	F2%	F3%	F4%	F5%	F6%	F7%	F8%	F9%
0	0	0	0	0	0	0	0	0	0
1	7.45	9.45	10.24	9.46	10.64	11.22	9.91	10.68	12.77
2	10.31	12.63	13.10	14.33	15.66	17.90	15.08	18.43	22.08
3	16.23	17.49	18.18	20.86	21.54	25.31	21.20	27.29	31.83
4	23.22	27.22	24.35	25.75	28.03	29.95	30.09	34.38	41.65
5	33.38	38.81	40.20	35.17	35.51	38.40	45.73	47.4	52.9
6	44.22	46.04	48.94	48.58	49.12	50.25	61.67	63.51	65.42
7	56.62	61.31	63.32	71.74	62.02	65.79	69.16	72.14	77.86
8	74.35	68.05	74.19	81.41	77.18	76.60	76.60	83.15	88.58



In-Vitro Drug Release

7. Conclusion:

The study successfully demonstrates that acetazolamide-loaded cubosomal gel can be effectively formulated using glyceryl monooleate and poloxamer 407.

The optimized formulation possessed nanoscale particles along with excellent drug entrapment, good physicochemical stability, and sustained drug release, all of which are desirable for ocular drug delivery.

The cubosomal gel system has the potential to enhance ocular improve bioavailability, minimize dosing frequency, and support patient compliance compliance in glaucoma therapy. Hence, cubosomal gel represents a novel and efficient ocular drug delivery approach for acetazolamide and warrants further in-vivo and clinical investigations.

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