Original Article

Development And In-Vitro Characterization Of Liposomal Embedded In-Situ Ocular Gel Of Dorzolamide Hydrochloride For The Treatment Of Glaucoma

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Abstract

Background: Liposomes is the most common and well-designed nano-carriers for drug targeting delivery. They have improved therapies for a range of biomedical applications by the help of stabilization therapeutic compounds, to overcome obstacles to cellular and tissue up-take, and improve bio-distribution of drug to target sites in vivo.

Objective: The drug is released from liposomes through diffusion technique. The main objective for this drug delivery system was to improve bioavailability and enhance the contact resistance time of drug in the eye.

Method: Reverse phase evaporation was used to successfully create liposomes, which were then analyzed for morphology, particle size, drug entrapment effectiveness, and content.

Results: The invitro studies shows that this dosage form holds great promise for drug release. As a result, liposomes design showed nearly spherical vesicles. The EE % and DC % could reach up to 88.9% and 92.1% respectively. In vitro release studies show a lipid concentration increase prolong release of drug from liposomes.

Conclusion: A dorzolamide sample's melting point, IR scan, and UV scan were all carried out. According to the findings of the studies mentioned above, the medication is called dorzolamide. Following completion of the Preformulation trial, it was discovered that the medication is stable in all PH. In analytical tests, the phosphate buffer pH 5.6, pH 7.4, and pH 6.8 were used to calibrate the dorzolamide curve. It was determined from the investigation that the standard plot was appropriate for use in other analytical studies. Liposome batches demonstrate that integration effectiveness increased as polymer concentration was raised. Scanner electron microscopy was used to characterize the surface of the produced liposomes.

KEYWORD: Ocular Drug Delivery, Liposomes, Nanoparticles, In-situ ocular gel, Glaucoma, Dorzolamide Hydrochloride

INTRODUCTION

Ophthalmic formulations are sterile in dosage, it is free from foreign matter, suitably designed in the form that can be used for outer side of eye (topical), can be administer inner side (intraocular) or by cavity of the eye or it can be used with an device which is used as ophthalmic for treatment. [1]

Eye is very important organ and unique. Drug delivery through ophthalmic route is very sensitive job to the researcher's because its identical and different anatomy which can resist the absorption of drug into deep tissues of eye. There are various drug delivery systems available for ophthalmic use such as eye drops, eye ointments, eye lotions, eye suspensions but these preparation and formulation when administered into the eye. The ophthalmic dosage previously manufactured have a side effect that they can be loss their viscosity and resistance time in eye because they are soluble in tear. This problem can be solved out by designing the drug as a

formulation that undergoes instant in situ gel formation when administered into eye cavity. In situ gels was developed to raise the residence time of the drug in eye cavity and to get better resistance, bioavailable drug and get relaxation from multi time dosing into eye**.[2]**

For regulate the drug in use of eye cavity the delivery systems have to maintain many characteristics like Sterility, Iso-tonicity, better penetration from eye muscle, Have less protein binding, less soluble in tear, Easiness in installation and removal, Good rheological properties, good resistance time of formulation in eye cavity. Mainly Corneal absorption is used by the drug to absorb into the ocular system it more effective than other route such as scleral and Maidenhead pericardium l absorption by which the blood vessel can absorb the drug from absorption route and lastly general solution occurs of drug in body. The drug which absorbs by this route with the mechanism that is paracellular diffusion and transcellular diffusion. Mostly the ophthalmic drug or drug used for the treatment of eye is lipophilic for better absorption because the nature of eye ball is lipophilic in nature due to this nature the drug can be absorbed easily. But some of the drugs are hydrophilic in nature they can be absorb by paracellular diffusion. The properties of the drug that can affect the bioavailability drug are as follows soluble, size, shape, pH etc. [3]

GLAUCOMA: Glaucoma is a disease that is related to ocular system in which the infected person may be blindness. This disease is common for blindness. In is disease the liquid from the anterior chamber may not be release or drain due to this more than the required fluid is accumulate in the chamberer of the eye and the accumulate fluid may create pressure on the retinal and optic nerve. Increase pressure on eye effect the eye. The disease is medic able when known early. It can also be defined as **kala motia.** It is very in older persons.

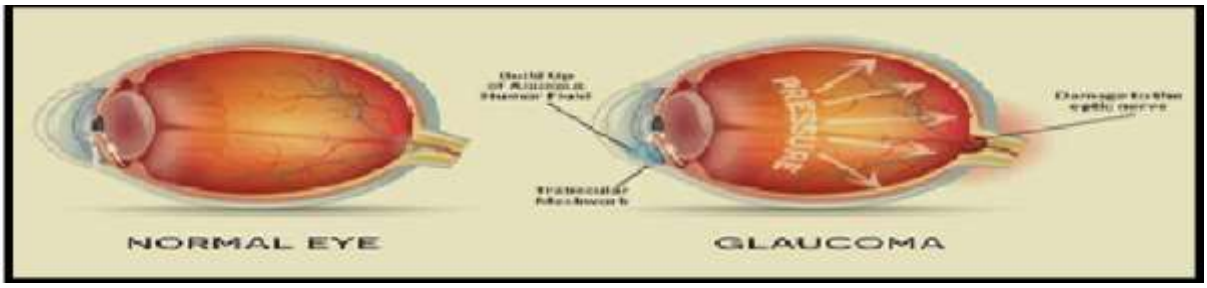


Figure 1: Structure of Normal Eye and Glaucoma Eye

OCULAR DRUG DELIVERY SYSTEM.

EYE DROPS: Eye drops are the formulation that are saline in nature. These are the formulation that can be used to heal ocular disease through topical route of administration. Eye drops are the formulation that can be used topically for the treatment of inner segment of eyes because the required concentration of drug cannot reach to the post chamber of ocular ball by topical ocular route.

SPRAYS: Eye spray are the formulation that are used in the treatment of eye disorder mainly used for pupil dilation and cycloplegics examination. Eye sprays are not common in formulation and not commonly used for the treatment of eye disorder, but for some patients it is recommended by doctors for the treatment of mydriasis use single salt or in combination.

OCCULAR INSERT: Ocular insert are new developed drug delivery system that can show controlled release of drug when compared to other forms of drug preparation.

NANOSUSPENSION: Nanosuspensions are poorly soluble drug the drug can be in suspended form in nano emulsion formulation in equilibrium dispersion. Nano emulsion have an advantage they can increase the bioavailability of drug and also it can reduce the irritation to the eye.

LIPOSOMES: The formulation that is a unique drug delivery method is called a liposome. Drug bioavailability and solubility can both be improved. They can be formulated by sonication, reverse phase evaporation and other methods in which dispersion of phospholipids occurs, some other methods are solvent injection methods etc.

GEL FORMULATIONS: Gel are the formulation that are mostly used in eye treatment. This formulation can reduce the drain of the drug from the cavity due to it has increased the viscous nature hence of drug so it can increase bioavailability of drug and their formulation. These are the formulation they can change their nature of state with effect in temperature, etc.

Table 1: Polymers used for ocular liposomes system

Polymer	Origin
Soy phosphatidylcholine (phospholipid 85G)	Natural
Egg lecithin	Natural
Cholesterol	Natural
Chitosan	Natural

MATERIAL USE:

Table 2: List of material use

Sr. No.	Materials
1	Dorzolamide
2	Chitosan
3	Egg lecithin
4	Chloroform
5	Di ethyl ether

DENTIFICATION OF DRUG

FTIR Analysis

"FTIR" was used to determine the conformation of the original drug, the polymer, and the interactions between the polymer and the drug. In this test, the sample was measured, combined with KBr, and put under 100 kg/cm of pressure to produce pallets.

STATEMENT

The Dorzolamide liposomes were prepared by using Egg lecithin, Chloroform, Di ethyl ether.

FORMULATION

Different type of polymer used for preparation of ocular liposomes. Drug polymer ratio was varied for each formulation. Thus 5 formulations were prepared and chosen for further studies.

PRESETATION OF PROBLEMS

Various parameter affects the preparation of liposomes mixture of drug-polymer and pellets were recorded.

UV Spectroscopic Method

The wavelength of maximum absorbance of dorzolamide were determine by scanning of solution within the range 200 nanometers to 400 nanometer using UV spectrophotometer. The scan of drug was compared with scan of drug given in standard books and other literature.

ANALYTICAL METHOD

Preparation of media:

Preparation of pH 5.6 phosphate saline buffer: 8.5 ml of concentrated HCl ad was take and dilute with distilled water up to 1000 ml.

Synthesis of buffer pH6.8: Take 50 milliliter 0.2 M KH_2PO_4 and add 22.4ml of 0.2 molar NAOH were add & volume make up to 200 millimeter by distill water. (IP 2010)

Preparation of pH 7.4 buffer: Take 0.2 Molar KH_2PO_4 add 39.1 millimeter of 0.2 Molar NAOH was added and volume make up to 200ml by distill water. (IP 2010)

Scanning of drug: To create a solution with a concentration of 1 mg/ml, 100 mg of the medication were accurately weighed and dissolved in 100 ml of various media (pH 5.6 phosphate saline buffer, pH 6.8 phosphate buffer, distil water, pH 7.4 phosphate buffer). Additional suitable dilutions were created using various solutions and scanned. To obtain absorption at the maximum wavelength, use a UV-visible spectrophotometer between 200 and 400 nm.

Preparation of standard plot: 100miligram of dorzolamide dissolved in 100ml of different media to make a solution of 1 mg/ml.

Preparation of working standard: The different conc. of drug 5-25 µg/ml was prepared from stock solution of different media. Then absorbance of each conc. at lambda max was taken and slope, intercept and correlation coefficient were calculated.

PREFORMULATION STUDIES: Preformulation studies was first step for the design and development of dosage form from a new chemical entity, Preformulation studies mans the investigation of physical and chemical properties of a new chemical entity or when combined with different excipients.

Solubility of drug: The medication was added to a modest amount (2 ml) of various medium to create a saturated solution. A sample of 0.5 ml of the saturated solution was obtained, filtered, and the absorbance was measured. The coefficient of soluble medication in various media was estimated from the absorbance using the regression equation of the standard plot.

Determination of partition coefficient: The drug was dissolved in 30 ml of pH 7.4 phosphate buffer to create a saturated solution, and the absorbance was measured after the appropriate dilution. 30ml of n-octanol was then added to the separated funnel after the saturated solution had been poured into it. This amalgam was shaken for 30 minutes, followed by 20 minutes of side-keeping, to separate the two layers. Finally, the AQ. layer was removed, and the readings were recorded.

Determination of dissociation constant: Dissociation constant was determined by ph. titration method by calculating the half neutralization point .05 M drug solution was prepared. The initial ph of 0.05 M solution was noted and this drug solution was titrated against pH 5.6 phosphate saline buffer and a graph was plotted between volume of HCL added in ml versus subsequent changes in ph after addition of HCL solution.

Drug-Excipient compatibility study: A certain amount of drug and polymer used in the formulation were mixed and stored at ambient temperature for a period of 3 week. These samples were analyzed by FTIR.

Table 3: Drug-Excipient compatibility

Sample	Mixture	Ratio
D1	Drug: Egg lecithin	1:1
D2	Drug: Chitosan	1:1

FORMULATION DEVELOPMET

Preparation of liposomes: Dorzolamide artificial vesicle was prepared by the method reverse phase evaporation by use of various ingredients.

Preparation of organic solvent using polymer: First egg lecithin and chloroform were mixed properly in a container by the use of magnetic stirrer with hot plate, organic solvent dried than it make a layer of polymer and then film was dissolved in another organic solvent and drying.

Loading of drug solution: Aqueous solution containing drug mixed in beaker containing dried film to hydrate the film and shaking it manually to get liposomes in gel like form.

Table 4: Composition of Formulation

Sr. No.	INGREDIENT	Quantity (mg)				
		F1	**F2**	**F3**	**F4**	**F5**
1	Dorzolamide	50	50	50	50	50
2	Egg lecithin	100	200	250	300	500
3	Chitosan	5	10	10	20	30
4	Diethyl ether	10	10	10	10	10
5	Ethanol	20	20	20	20	20
6	chloroform	10	20	20	10	10
7	Gellan gum	0.5	0.5	0.7	0.7	0.7
8	Sodium alginate	0.3	-	0.3	-	0.3
9	Methyl cellulose	0.3	0.4	0.5	0.5	0.5

Table 5: Parameter

Sr. No.	PROCESS PARAMETER	SET
1	Temperature	50
2	Magnetic stirrer speed (rpm)	60

EVALUATION PARAMETERS

Prepared liposomes were evaluated for yield, surface morphology, drug content and vesicle shape.

Percentage yield: Batches of liposomes were evaluated for percentage yield. The percentage yield of various batches of liposomes was calculate by the help of this formula.

% yield = practical yield / theoretical yield X 100

Drug content: Take the sample about 50mg of dorzolamide liposomes into 50 ml volumetric flask containing buffer pH7.4 and makeup volume up to the mark with the same buffer. From the stock solution prepared required dilutions and absorbance was taken at 253nm.

In-vitro release profile: The in vitro release of dorzolamide for liposomal synthesis was determined by membrane diffusion method. In brief, the presoaked dialysis bag containing 0.5 ml of liposomal formulation was suspending in beaker containing 20ml of "PBS (pH 7.4)" and stirred at 37±0.5℃. at predetermined time desired amount of sample was withdrawn from the beaker and analyzed b UV spectrophotometer.

Size of the vesicles: The morphology, size and size distribution of vesicles were analyzed by field emission scanning electron microscopy (SEM, ZEISS

FINDINGS, RESULTS AND DISCUSSIONS

IDENTIFICATION OF DRUGS

Melting point: It was discovered that dorzolamide had a melting point of 284.4°C.

Fourier Transform Infrared (FTIR) Spectroscopy Analysis: In IR study it was found that the peaks obtained in sample were similar as in the reference spectra of dorzolamide from IP. The scan shows identical peaks in same wavenumber range as shown in reference spectra. So, it was anticipated that sample was found pure dorzolamide and suitable for use further studies.

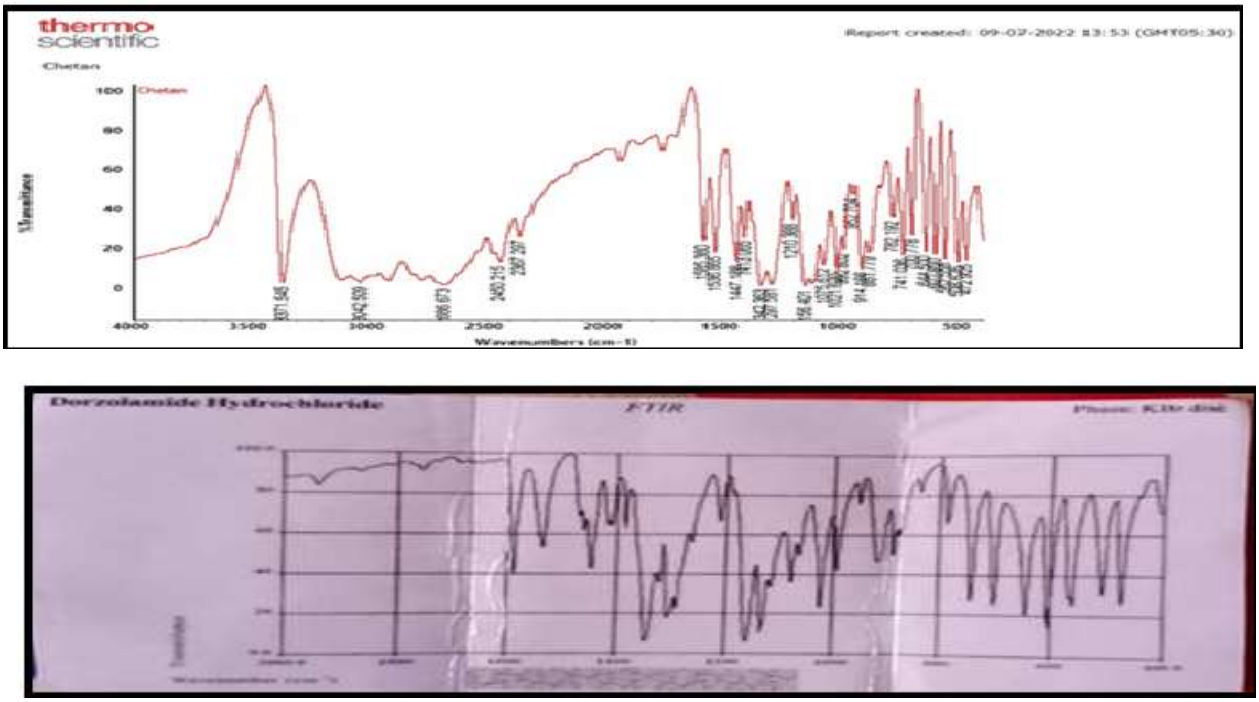


Figure 2: IR Spectra of dorzolamide sample and reference sample from IP

ANALYTICAL STUDIES

Scanned graph of drug solution: The lambda max of solution dorzolamide is showed in figure :

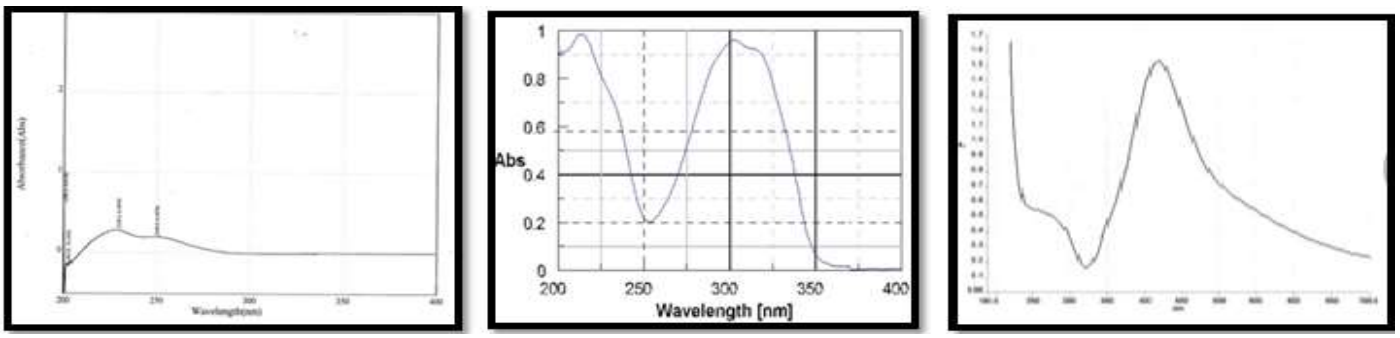


Figure 3: Scanning of dorzolamide in pH 7.4 phosphate buffer, pH 5.6 saline buffer and pH 6.8 phosphate buffer.**:** Absorbance value of solution are as follows in table

Solvent	Observed value	Reported value
pH 5.6 phosphate saline buffer	253	253
pH6.8 phosphate saline buffer	240.1	240
pH7.4 phosphate saline buffer	253.3	253

Standard plot: The standard plot of dorzolamide was prepared in pH 5.6 saline phosphate buffer, pH 6.8phosphate buffer, pH 7.4 phosphate buffer. The absorbance is given in table 6.3, 6.4, 6.5 and standard plot in fig 6.6, 6.7, 6.8.

00Table 7: Standard plot in pH 7.4 phosphate buffer

Concentration in (microgram per litter)	Absorbance			
	A_1	A_2	A_3	average±SD
2	.0662	.0660	.0660	.0661±.0001
4	.1190	.1190	.1190	.1190±.0
6	.1768	.1764	.1766	.1766±.0002
8	.2346	.2345	.2347	.2346±.0001
10	.2875	.2877	.2876	.2876±.0001
12	.3469	.3463	.3466	.3466±.0003

Table 8: Table for plot in pH 6.8

Concentration in (microgram)	Absorbance			
	A_1	A_2	A_3	average±SD
2	.0650	.0652	.0651	.0651±.0001
4	.0982	.0981	.0980	.0981±.0001
6	.1423	.1422	.1421	.1422±.0002
8	.2010	.2018	.2020	.2016±.0005
10	.2238	.2239	.2240	.2239±.0001
12	.2861	.2860	.2862	.2861±.0001

Table 9: Table for standard plot in pH 5.6 phosphate buffer

Concentration in (microgram)	Absorbance			
	A_1	A_2	A_3	average±SD
2	.0560	.0562	.0561	.0561±.001
4	.0901	.0901	.0901	.0901±.0
6	.1226	.1228	.1224	.1226±.0002
8	.1915	.1917	.1916	.1916±.001
10	.2135	.2135	.2135	.2135±.0
12	.2262	.2258	.2260	.2660±.002

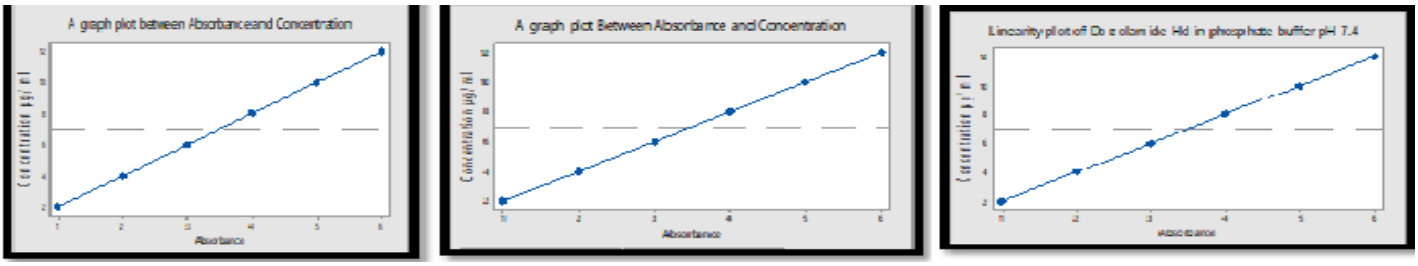
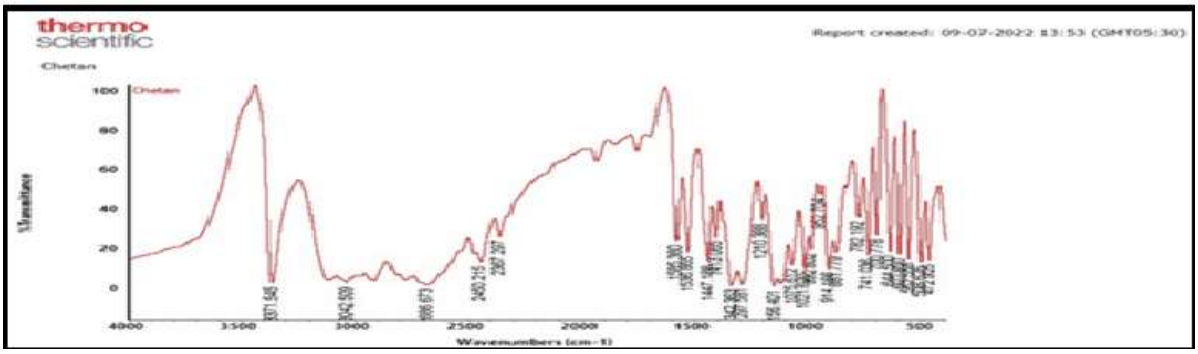


Figure 4: Standard pot of dorzolamide in pH 5.6 phosphate saline buffer, pH 6.8 phosphate buffer and pH 7.4 buffer.

PREFORMULATION STUDIES

Determined drug solubility: Solubility of dorzolamide were determine in different-different solution such as pH 5.6 phosphate saline buffer, pH6.8, pH7.4. By the help of solubility study was performed it showed that the molecule is highly soluble through in pH7.4 phosphate buffer.

Interaction study of drug and molecule: The FTIR analyses of physical mixture of dryg-polymer are represented in Figure6.8



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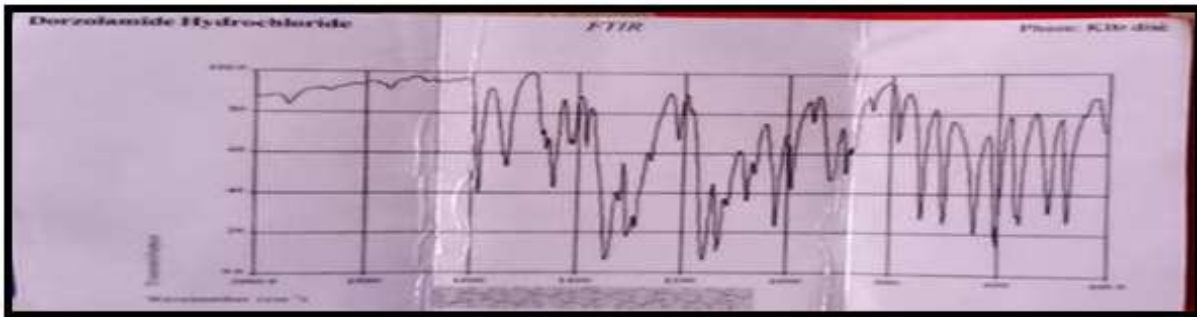

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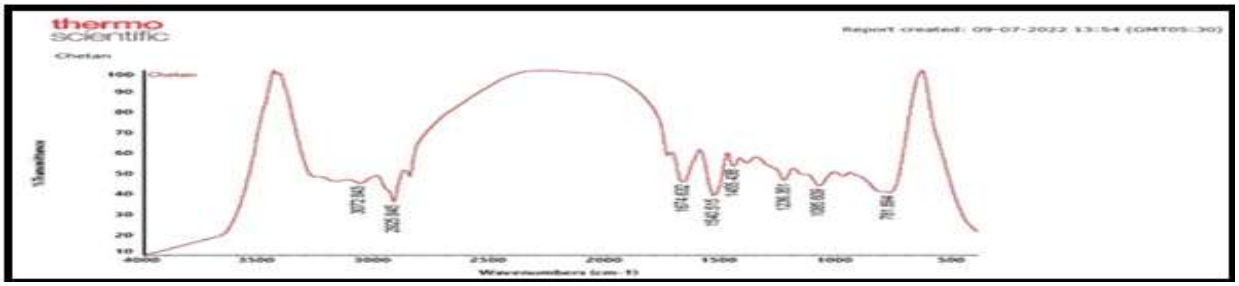

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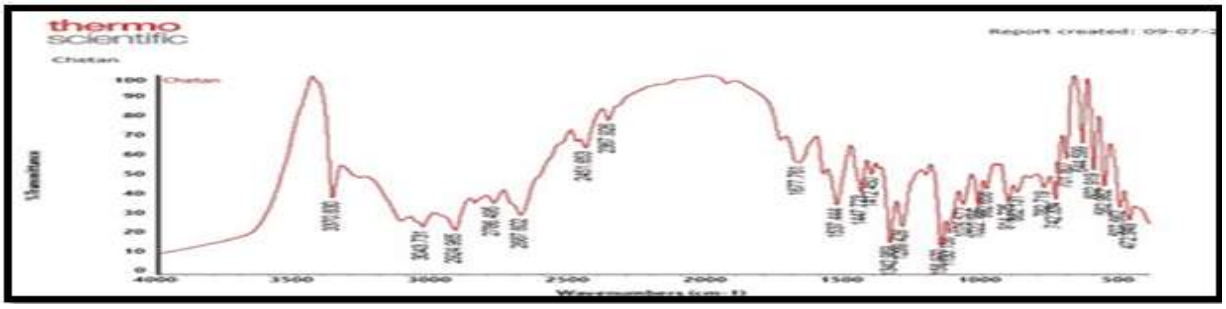

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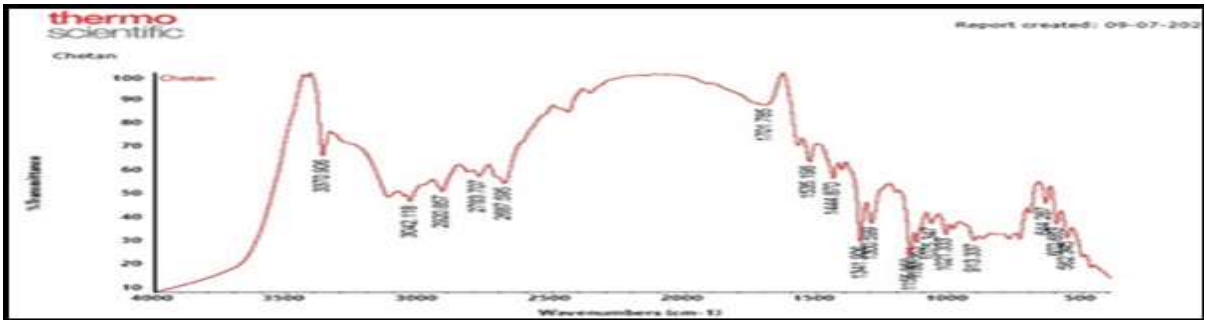

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Figure 5: FTIR spectra of (1) Dorzolamide HCl Sample, (2) Dorzolamide reference from IP, (3) Egg lecithin, (4) Physical mixture of drug and polymer, (5) Chitosan.

Solution stability studies: This study was done at -20℃ , 4℃ and 25℃ temperature at pH 5.6 phosphate buffer saline. The study was carried out for 7 days.

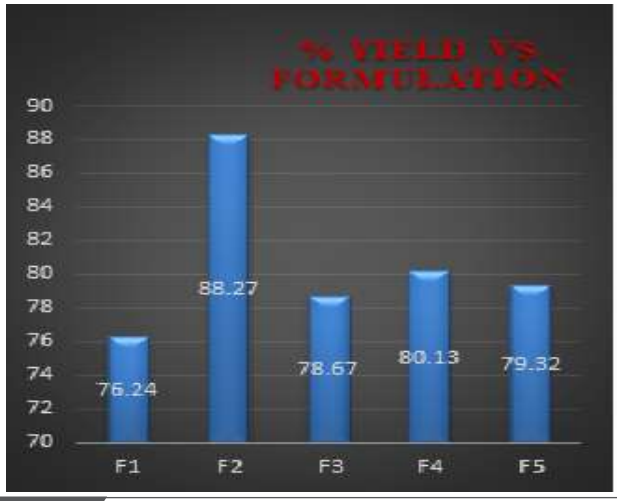
Figure 6: Solution stability study of drug in different media

The solution stability study of drug indicated that drug was found to be stable. The drug shows higher stability in pH 6.8 phosphate buffer, about 33.2% drug remained after 5 days.

EVALUATION OF LIPOSOMES

Table Evaluation results

Batch Formulation	% Yield	%Drug loading
F1	78.22	76.24
F2	86.57	88.27
F3	75.43	78.67
F4	71.34	80.13
F5	80.11	79.32



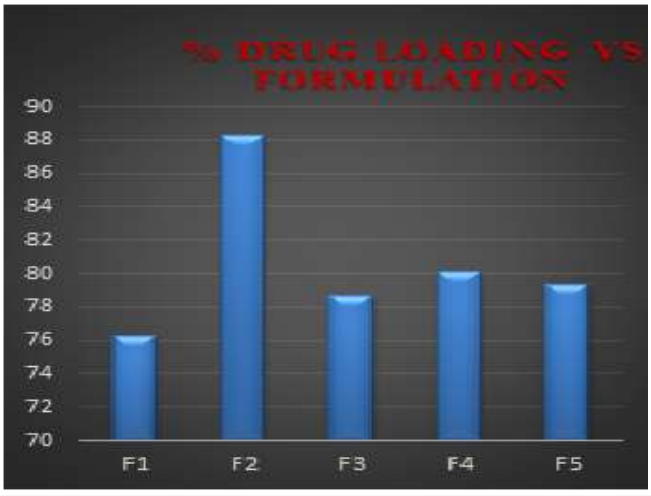


Figure 7: % drug loading and % yield distribution

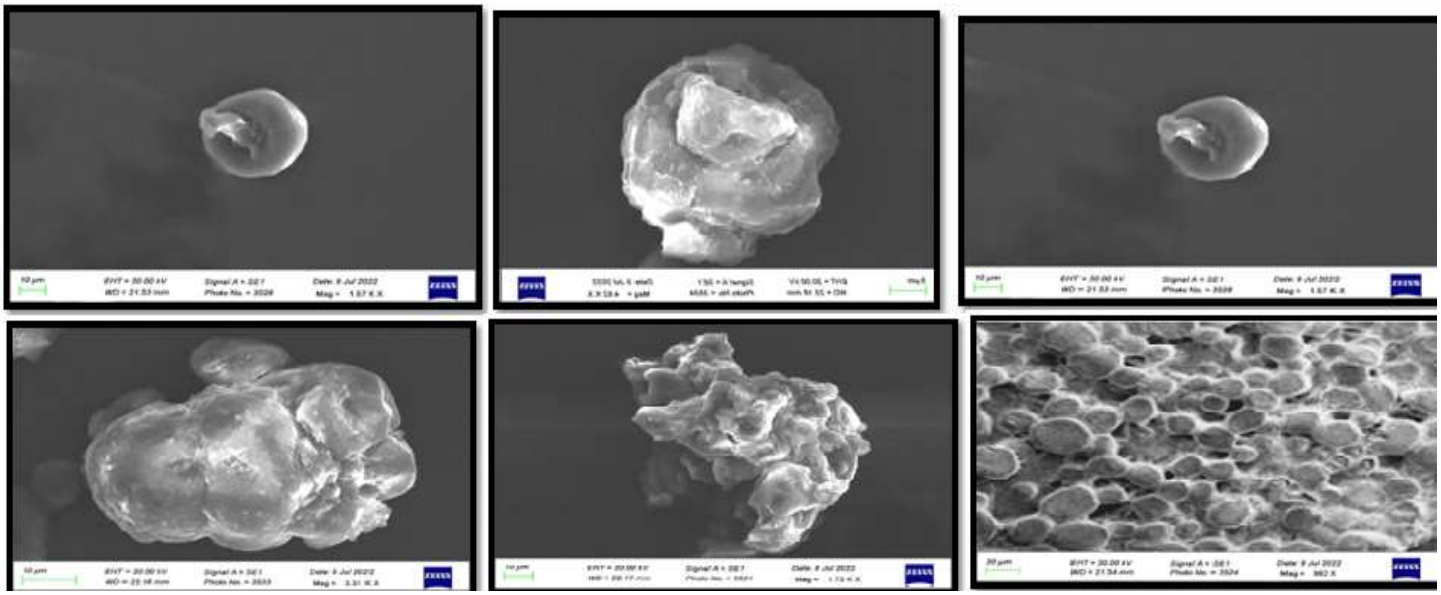
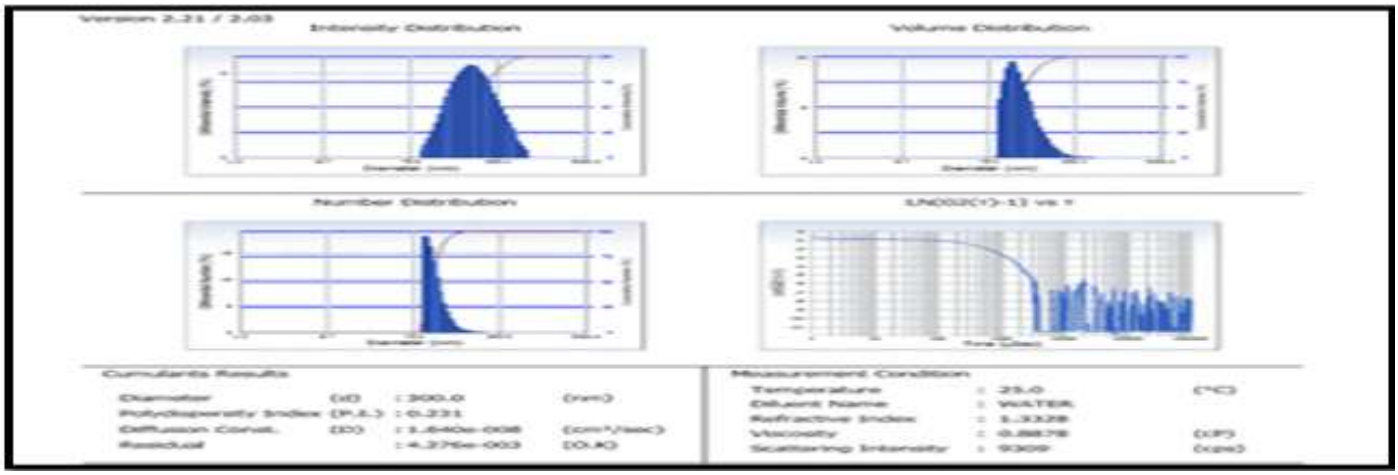


Figure 8: Scanning electron microscopy of formulated liposomes.



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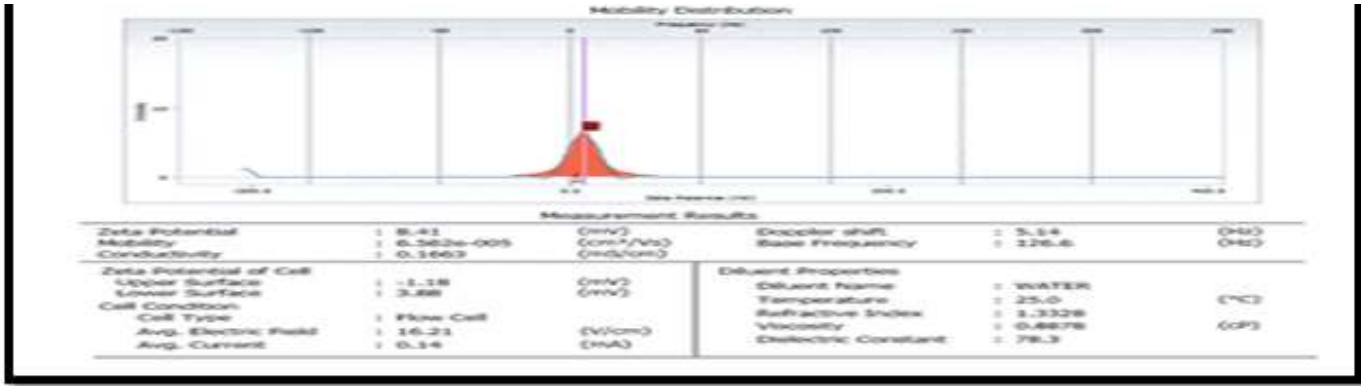

[B]

Figure 09: [A] Polydispersity Index of prepared liposomes [B] Zeta Potential of prepared liposomes

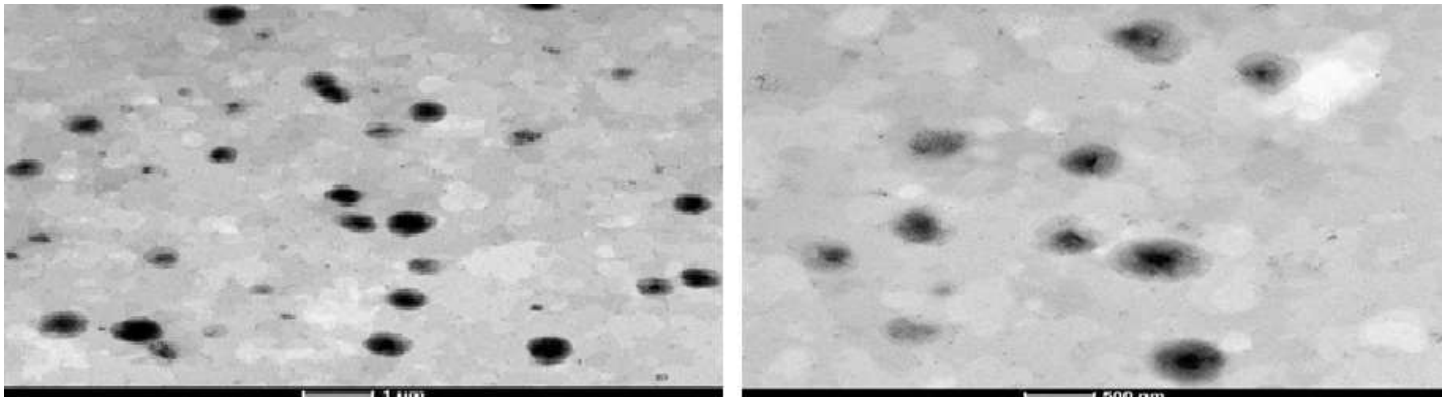

Figure 10: TEM images of prepared liposomes.

In Vitro drug release studies: In vitro drug release profile of prepared liposomes was examined by dialysis sac with 150 ml of phosphate buffer saline pH 7.4 containing 7% v/v propylene glycol and 25 % v/ methanol at 37°C followed the method published before. Liposomal concentration equivalent to 2 mg disperses in 1 ml of bicarbonate buffer placed in dialysis bag. Control bag were prepared and analyzed. The sac was hung inside the flask with the help of rod glass to dip the bag in solution. The flask was place on magnetic stirrer and stirred at 100 rpm at 37°C. Sample was collected at every half hour and assayed spectrophotometrically for drug content at 253.

Time (min.)	F1	F2	F3	F4	F5
0	0	0	0	0	0
30	20.23	18.23	16.23	18.23	18.23
60	24.7	23.7	18.7	33.7	27.7
90	30.66	31.66	26.66	37.66	38.66
120	49.78	45.78	35.78	54.78	54.78
150	67.45	72.45	52.45	69.45	60.45

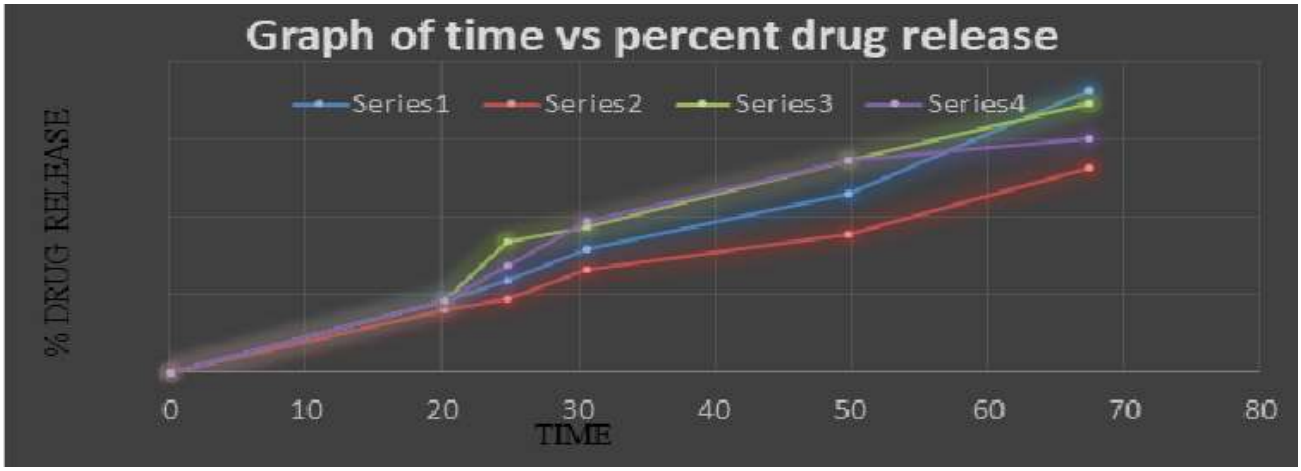

Figure 10: Drug release curve of each formulation.

IMPLEMENTATION AND CONCLUSION

The optic nerve in your eye might become damaged by the disease glaucoma. With time, it becomes worse. It frequently has to do with an increase in pressure inside your eye. As a rule, glaucoma runs in families. Typically, you don't develop it until much later in life. A carbonic anhydrase inhibitor called dorzolamide is used to treat glaucoma. But the issue with dorzolamide is how long it takes for the drug to take effect in the eye. Therefore, liposome synthesis lengthens its resistance period to enhance bioavailability.

A dorzolamide sample's melting point, IR scan, and UV scan were all carried out. The medicine in question is dorzolamide, according to the findings of the studies mentioned above. Following completion of the Preformulation trial, it was discovered that the medication is stable in all PH during analytical experiments, the calibration