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FORMULATION AND EVALUATION OF GLIBENCLAMIDE CUBOSOMAL ORAL CAPSULES

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ABSTRACT

An attempt was made to investigate the potential of Cubosomes as lipid nanocarrier to improve the drug release action of the Glibenclamide. Glibenclamide Cubosomes were prepared by Top-Down approach employing GMO as lipid phase vehicle, Poloxamer-407 as a stabiliser and distilled water as the aqueous phase. The resultant cubosomal dispersion was characterised by Encapsulation efficiency, in-vitro drug release, particle size, zeta potential, FTIR and SEM. Optimised Cubosomal formulation (G7) showed a maximum drug release of 83% in 7 hours, entrapment efficiency of 90%, the particle size of 110.3 nm and zeta potential of -41.3 mV. Glibenclamide Cubosomal oral capsules were prepared with optimised Cubosomal dispersion, by employing starch and Aerosil as granulating agents to obtain a wet mass. The wet mass is passed through the sieve no.16 to from granules and dried in hot air oven. The dried granules were filled into the capsules. The granules were evaluated for SEM, zeta potential, flow properties and Invitro drug release. Optimized Cubosomal capsule formulation (CG4) showed a maximum drug release of 51.17 % in 7 Hours, particle size of 2034.9 nm and Zeta potential of -27.6 mV. Invitro release kinetics exhibited the drug release up to 7 hours. Results suggest that GMO cubosomes, as lipid nanovectors in the form of Cubosomal Oral

Capsules could significantly enhance the efficacy and sustain the release when compared to Glibenclamide Tablets (Daonil).

KEYWORDS: Glibenclamide, GMO (Glyceryl Mono Oleate), Top-Down approach, Oral Capsules, Cubosomal Injection.

INTRODUCTION

Cubosomes are nano particles which are self assembled liquid crystalline particles of certain surfactants with proper ratio of water with microstructure.[1] Instead of the solid particles usually encountered, the cubosomes are the liquid crystalline particles with a solid- like rheology. Their size ranges from 10-500 nm in diameter and appear to be dots, squared shaped, slightly spherical and each dot corresponds to the presence of pore size 5-10 nm.[2] Cubosomes are typically produced by high energy dispersion of bulk cubic phase, followed by colloidal stabilization using the polymeric surfactants. Cubosomes constitute alternate delivery systems that offer the possibility to develop targeted therapeutic agents with improved bioavailability, biodistribution, and pharmacokinetics and safety profiles.[3]

Cubosomes need to be kinetically stabilized by steric means to prevent flocculation of the dispersion and thus improve the shelf life. Steric stabilization of such colloidal particles is typically achieved by addition of block copolymers and Pluronics are the most widespread class of steric stabilizers available in the market for lyotropic liquid crystalline particles. Structurally, these are formed from the amphiphilic building blocks, which mimics biological membranes that can be used as carrier for hydrophilic, hydrophobic and amphiphilic drugs.[4] Cubosomes have great potential in formulating nano-sized particulate systems for oral delivery owing to their best advantages such as high drug pay-load with high internal surface area, low viscosity and bio-compatibility.[5]

Glibenclamide is an oral hypoglycemic agent, prescribed for the treatment of subjects with Type-II Diabetes Mellitus. It belongs to the class of Sulfonylurea's. Glibenclamide is practically insoluble in water, but highly permeable (Class-II) according to the Biopharmaceutics Classification System (BCS).[6] Glibenclamide is usually initiated with a dose of 2-5 mg. The biological half life of Glibenclamide is 5-6 hours. To reduce the frequency of administration and to improve the patient compliance, a once daily sustained release formulation of Glibenclamide is desirable. The present study was involved to develop sustained release Glibenclamide Cubosomal Oral Capsules.

MATERIALS AND METHODS

Glibenclamide was kindly gifted by Inga Laboratories P. Ltd, Mumbai. Glyceryl Monooleate (GMO) was a gift sample from the Mohini Organics P. Ltd, Mumbai. Kolliphor P407 (Poloxamer-407) was a gift sample from the BASF India Limited, Navi Mumbai. Starch and Aerosil were of commercial grade. All other reagents used were of analytical grade.

PREPARATION OF GLIBENCLAMIDE CUBOSOMES

The method employed in preparation of the Glibenclamide cubosomes was Top-Down Approach[7]. Different concentrations of Glyceryl Monooleate (GMO) and Poloxamer 407 as shown in the table 1 and 2 were accurately weighed and heated on the electric water bath at a temperature of 40-50^{o}C until Poloxamer 407 completely dissolves in GMO. The Glibenclamide drug was added to the above solution and mixed well. The obtained clear lipid solution was slowly added to distilled water and subjected to bath sonication for 30 minutes the resultant solution was white opaque dispersion without the presence of any aggregates.

The prepared dispersions were stored in the closed glass vials at room temperature for 48 hours, protected from light and later evaluation was carried out.

TABLE NO. 1: FORMULATIONS OF CUBOSOMES USING VARYING CONCENTRATIONS OF GMO.

Formulation Code	Glyceryl Mono Oleate (GMO) (%W/V)	Poloxamer-407 (%W/W)	Glibenclamide (mg)	Water (%W/V up to 100%)
G1	1	1	10	100
G2	2.5	1	10	100
G3	5	1	10	100
G4	7.5	1	10	100
G5	10	1	10	100
G6	12.5	1	10	100
G7	15	1	10	100
G8	17.5	1	10	100
G9	20	1	10	100
G10	22.5	1	10	100

TABLE NO. 2: FORMULATIONS OF CUBOSOMES USING VARYING CONCENTRATIONS OF POLOXAMER-407.

Formulation Code	Glyceryl Mono Oleate (GMO) (%W/V)	Poloxamer-407 (%W/W)	Glibenclamide (mg)	Water (%W/V up to 100%)
P1	15	0.5	10	100
P2	15	1	10	100
P3	15	1.5	10	100
P4	15	2	10	100
P5	15	2.5	10	100
P6	15	3	10	100

PREPARATION OF GLIBENCLAMIDE CUBOSOMAL ORAL CAPSULES

Optimized Cubosomal formulation G7 (15% GMO and 1% Poloxamer 407) was selected for preparation of capsules. To the optimized Cubosomal formulation, Starch (CG1-CG7) and Aerosil (CG8-CG14) was added separately to obtain a wet mass. Then the wet mass was passed through the sieve no. 16 and granules were obtained. The obtained Cubosomal granules were air dried at room temperature and were filled into the "0" sized capsules. The formulation of Glibenclamide Cubosomal oral capsules were depicted in the table no.3 and 4.

TABLE NO. 3: FORMULATION OF CUBOSOMAL GRANULES USING STARCH.

Formulation Code	Cubosomal Dispersion (ml)	Starch Powder (gm)
CG1	10	1.2
CG2	10	1.4
CG3	10	1.6
CG4	10	1.8
CG5	10	2.0
CG6	10	2.2
CG7	10	2.4

TABLE NO. 4: FORMULATION OF CUBOSOMAL GRANULES USING AEROSIL.

Formulation Code	Cubosomal Dispersion (ml)	Aerosil (gm)
CG8	10	0.2
CG9	10	0.3
CG10	10	0.4
CG11	10	0.5
CG12	10	0.6
CG13	10	0.7
CG14	10	0.8

METHODS FOR OPTIMIZATION OF FORMULATION VARIABLES FOR CUBOSOMES

1. Effect of GMO (Glyceryl Monooleate) Concentration on the formation of Cubosomes.

2. Effect of Poloxamer 407 Concentration on the formation of Cubosomes.

3. Effect of Sonication time on the formation of Cubosomes.

4. Effect of Temperature on the formation of Cubosomes.

EVALUATION OF GLIBENCLAMIDE CUBOSOMES

1. Surface Morphology

The size and shape of the cubosomes was determined using the Optical Microscopy (Olympus CH20i) and the Scanning Electron Microscopy (SEM-Hitachi S 3700N). SEM provides access to the three dimensional Image of the cubosomes. The samples were examined at suitable accelerating voltage of 15-20 kV, at different magnification.

2. Particle size Analysis

The particle size and Zeta potential of cubosomes was determined by dynamic light scattering technique using the Horiba Particle Size Analyzer. Samples were diluted in particle-free purified water and measured at 25^{o}C.

3. Zeta Potential

The Zeta potential of the cubosomes was determined using the Zetasizer (Malvern Instruments). Zeta potential indicates the degree of repulsion between adjacent, similarly charged particles in dispersion system. Zeta potential is the key indicator of the stability of the dispersions. The magnitude of the zeta potential indicates the degree of electrostatic repulsion between adjacent, similarly charged particles in dispersion.

4. Entrapment Efficiency (E.E)

Entrapment efficiency of the cubosomes is defined as the percentage amount of drug which is entrapped by the cubosomes. The Cubosomal dispersions were subjected to the centrifugation (Remi R24 Research Centrifuge) at 15000 RPM for 90 minutes. The resulting solution was then separated and supernatant liquid was collected. The collected supernatant was then diluted appropriately and estimated using UV Visible Spectrophotometer at 233 nm. The percentage of entrapment efficiency was determined from the equation:

$$E.E\% = \frac{(\text{Total Drug}) - (\text{Free Drug})}{(\text{Total Drug})} \times 100$$

5. Invitro Drug Release Studies

Invitro drug release studies were performed using the Franz Diffusion cell i.e. Bi-chambered donor receiver compartment model and this was placed on a magnetic stirrer and temperature was adjusted to 37±0.5^{o}C. One end of the compartment was covered with the Himedia dialysis membrane (Cut-off molecular weight: 12000-14000), which was previously soaked in warm water. Phosphate buffer Saline (PBS) of pH 6.8 was placed in the receptor compartment. Cubosomal formulation was placed on the dialysis membrane, which was in contact with receptor medium. Samples were withdrawn from the receptor compartment at specified time intervals of 1,2,3,4,5,6,7 hours. Replace the receptor medium with the equal amounts of fresh phosphate buffer pH 6.8 after each withdrawal. The samples were analyzed for drug content using a UV Spectrophotometer at 233 nm respectively.

EVALUATION OF GLIBENCLAMIDE CUBOSOMAL ORAL CAPSULES

1. FTIR (Drug-Excipient Compatibility Studies)

Fourier-Transform Infrared Spectroscopy was performed using FTIR Spectrophotometer using the KBr Disc Method. It analyzes the molecular interactions and stability of the formulation between the drug and used excipients.

2. Surface Morphology

The size and shape of the Cubosomal granules was determined using the Optical Microscopy (Olympus CH20i) and the Scanning Electron Microscopy (SEM-Hitachi S 3700N). SEM provides access to the three dimensional Image of the cubosomes. The samples were examined at suitable accelerating voltage of 15-20 kV, at different magnification.

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4. Flow Properties

The Flow properties of the Cubosomal granules were studied by measuring the quality parameters such as Tapped Density, Bulk Density, Hausner's Ratio, Angle of Repose, Compressibility Index or Carr's Index.

4 (a) Angle of repose: It is the parameter related to inter-particulate friction or resistance to movement between the particles. This is the maximum angle possible between surface of pile of powder or granules and the horizontal plane.

A funnel was fixed at a height approximately of 2-4 cm over the platform. The loose granules were slowly passed along the wall of the funnel, till the cone of the granules is formed. The angle is determined by measuring the height of the cone of powder and radius of the heap of powder. The equation for Angle of Repose (θ) is:

$$\theta = Tan^{-1} h/r$$ Where, h= height and r = radius.

4 (b) Bulk Density: Bulk density of granules was determined by pouring gently 1.5 g of granules through a glass funnel into 25 ml of measuring cylinder. The volumes occupied by the samples were recorded.

Bulk Density = Weight of the sample in grams/ Volume occupied by sample

4 (c) Tapped Density: The granules were taken in a measuring cylinder and were tapped 100 times or until there is no change in the volume occupied. It is given by:

Tapped Density = Weight of the sample in grams/ Volume occupied by sample

4 (d) Compressibility Index and Hausner's Ratio

Compressibility Index = (Tapped Density – Bulk Density) / Tapped density * 100

Hausner's Ratio = Tapped Density/ Bulk Density

5. Drug Content

Granules equivalent of 5mg of the drug were accurately weighed and transferred to 100 ml volumetric flask. The solution was made up to the volume with pH 6.8 Phosphate Buffer. The resultant solution was filtered and suitably diluted and analyzed using UV Visible spectrophotometer at 233 nm using pH 6.8 PBS as a blank.

6. Invitro Dissolution Studies

Invitro dissolution test was carried out using USP Type I Dissolution Apparatus. PBS of pH 6.8 was used as dissolution media. 900ml volume of dissolution medium was used along with

a basket speed of 50 rpm was selected. The temperature of the medium was maintained at 37±0.5^{o}C. Aliquots of 5ml were collected at specified time intervals and the same amount of fresh dissolution medium was replaced into the dissolution vessels. Studies were performed for all the Cubosomal oral capsules in pH 6.8 PBS. The collected samples were filtered and suitably diluted to analyze using UV Visible spectrophotometer at 233 nm.

7. Release Kinetics

The optimized Cubosomal oral capsule (CG4) was studied for release kinetics. Coefficient of correlation values were calculated for the linear curves obtained by regression analysis of the plots.

8. Stability Studies

Accelerated stability studies were conducted as per the ICH guidelines at 20^{o}C±2^{o}C at 65% ± 5% Relative Humidity at sampling intervals of 30, 60 and 90 days respectively. The drug content was determined periodically.

RESULTS AND DISCUSSION

1. OPTIMIZATION OF FORMULAION VARIABLES

1 (a) Effect of Glyceryl Monooleate concentration on the formation of cubosomes

Glibenclamide Cubosomes were prepared with the varying concentrations of Glyceryl Mono Oleate from 1% to 22.5% and it was observed that as the concentration of the GMO increases the solubility of the Glibenclamide was also increased due to the structural integrity and the incorporation of drug into the Cubosomal structure.

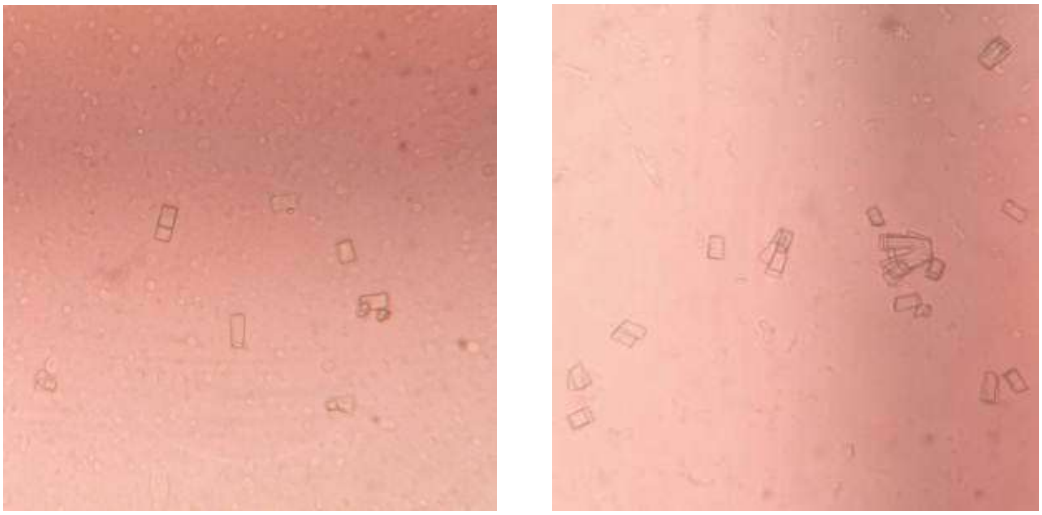


Figure No. 1 & 2: Effect of Glyceryl Monooleate concentration on the formation of Cubosomes.

From the Figure No. 1 and 2 it was observed that in the concentration range of 10% to 22.5% the cubosomes were obtained. Below 10% and above 22.5% GMO spherical shaped cubosomes were obtained rather than cubic structures.

1 (b) Effect of Poloxamer 407 on the formation of cubosomes

Various formulations were prepared using P 407 in concentration range of 0.5% to 3 %. Increase in the P 407 concentration above 1.5% leads to rod shaped or rectangular cubosomes. Decrease in P 407 concentration below 1% leads to formation of spherical vesicles.

1% P 407 concentration (P2) is optimum for cubosomes formation with entrapment efficiency of 90.54% and Drug release of 83.01%. Increase in Poloxamer 407 concentration decreases the entrapment efficiency and drug release.

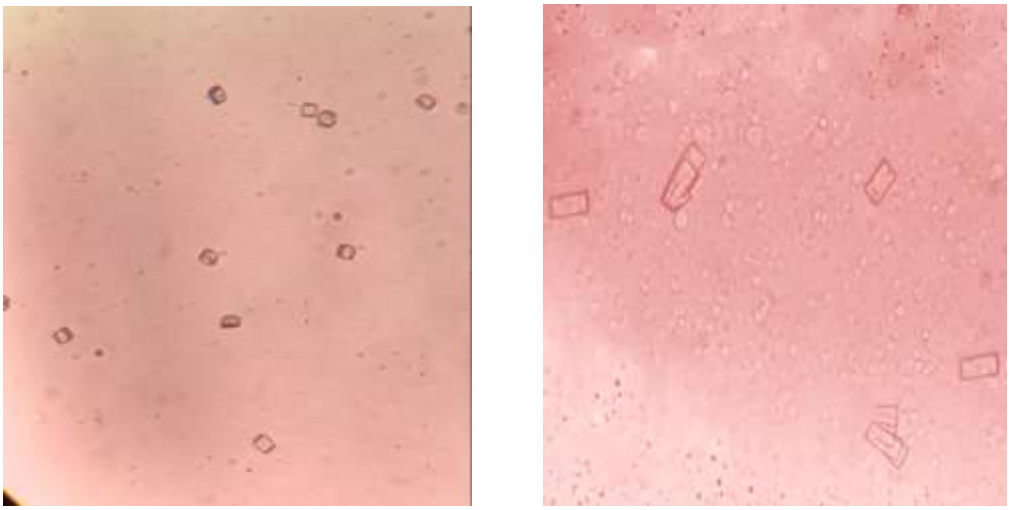


Figure No. 3 & 4: Effect of Poloxamer 407 on the formation of Cubosomes.

From the Figure No. 3 and 4 it was observed that in the concentration range of 1% to 3% the cubosomes were obtained. Below 1% and above 3% P407 rod shaped cubosomes were obtained rather than cubic structures.

1 (c) Effect of Temperature on the formation of cubosomes

Formulations were subjected to varying temperatures ranging from 40^{o}C to 70^{o}C. At and below 40^{o}C no cubosomes were formed. At 45^{o}C partial cubic structures were obtained. The optimum temperature for Cubosome formation is 50^{o}C to 60^{o}C. Beyond the temperature of 70 ^{o}C no cubic structures were formed.

Figure No. 5, 6, 7: Effect of Temperature on the formation of Cubosomes.

Figure No. 8, 9, 10: Effect of Temperature on the formation of Cubosomes.

4.3.4 Effect of Sonication time on the formation of cubosomes

Cubosomal formulations were subjected to sonication for a period ranging from 15 to 40 minutes. Below 20 minutes of sonication time no cubosomes were formed. The optimum sonication time for the formation of cubosomes is 25 to 40 minutes.

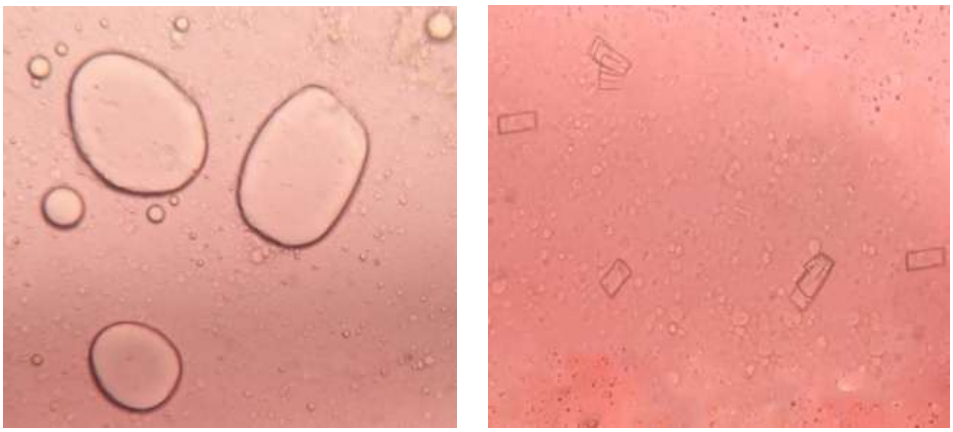


Figure No. 11 & 12: Effect of Sonication time on the formation of Cubosomes.

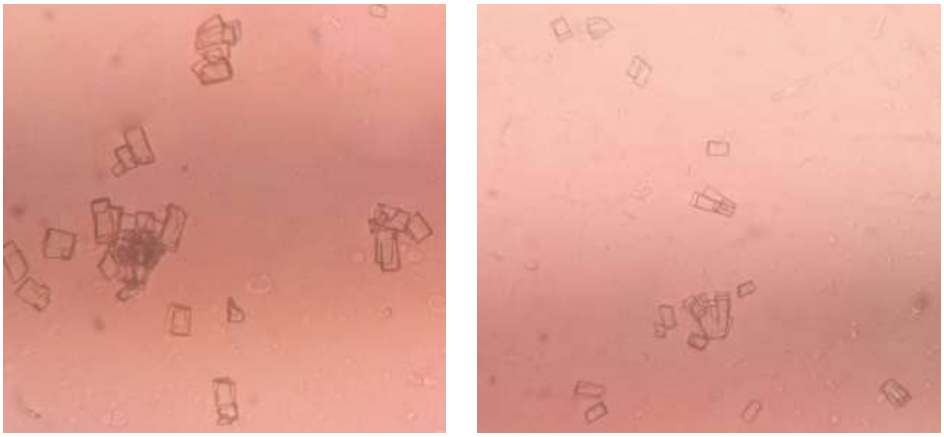


Figure No. 13 & 14: Effect of Sonication time on the formation of Cubosomes.

2. EVALUATION OF GLIBENCLAMIDE CUBOSOMES

2 (a) Surface Morphology

The surface morphology of the cubosomes was determined using scanning electron microscopy and from Figure No. 15 and Figure No. 16 it was observed that the obtained cubosomes have a smooth surface and were cubic shaped.

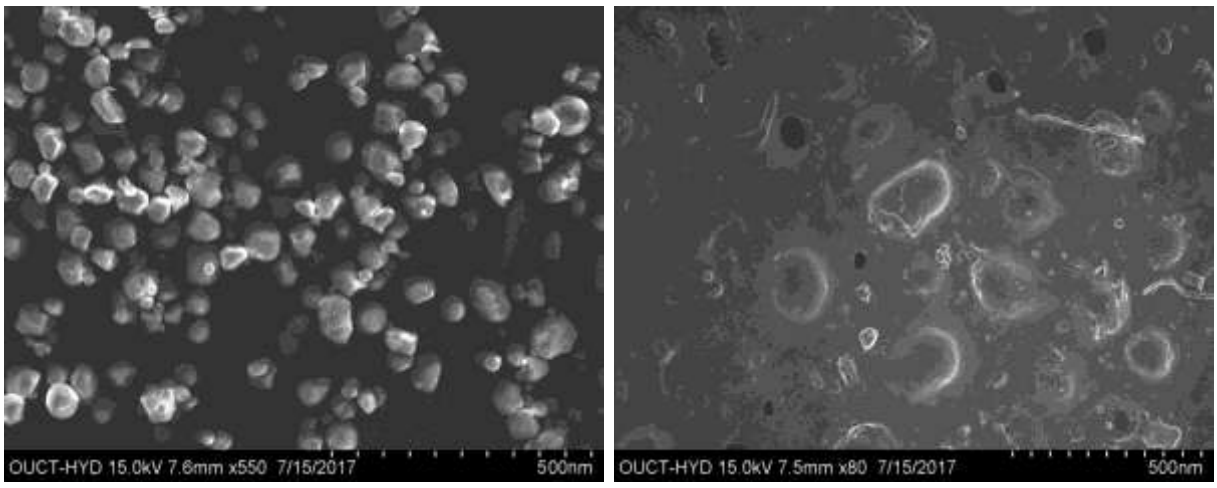


Figure No. 15 & 16 Scanning Electron Microscopic Images of Glibenclamide Cubosomes.

2 (b) Particle size Analysis

Glibenclamide Cubosomes with varying concentrations of GMO and the P407 were subjected to the particle size analysis. The particle size of Glibenclamide Cubosomes should be in the range of 10 to 500 nm.

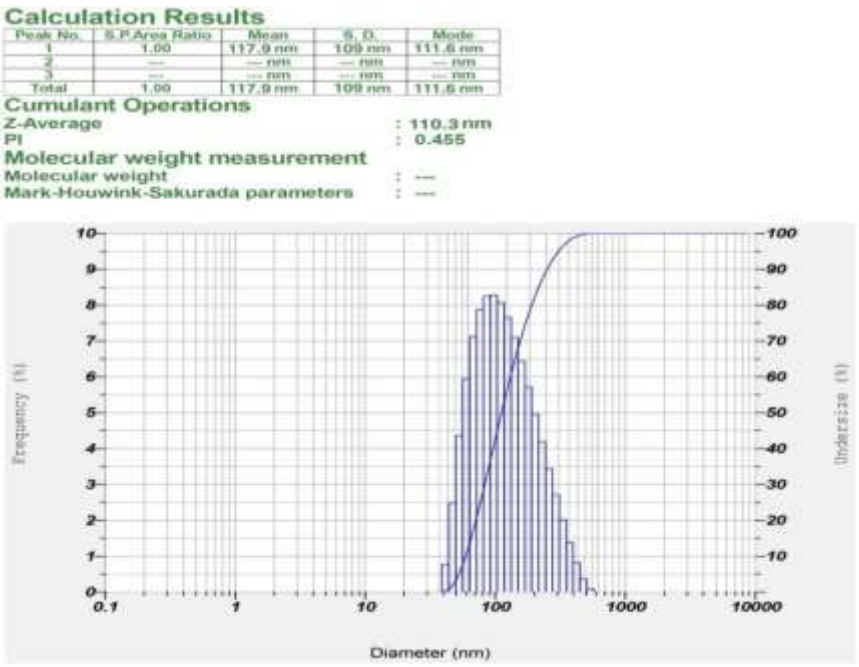


Figure No. 17: Particle Size of Glibenclamide Cubosomes.

From the above figure no. 17 it was found that the average particle size of the Glibenclamide Cubosomes was 110.3 nm which states that the obtained cubosomes were with the good structural integrity to deliver the Glibenclamide for longer time.

2 (c) Zeta Potential

Glibenclamide Cubosomes with varying concentrations of GMO and the P407 were subjected to the Zeta Potential. The Zeta Potential of Glibenclamide Cubosomes should be greater than ±30mV.

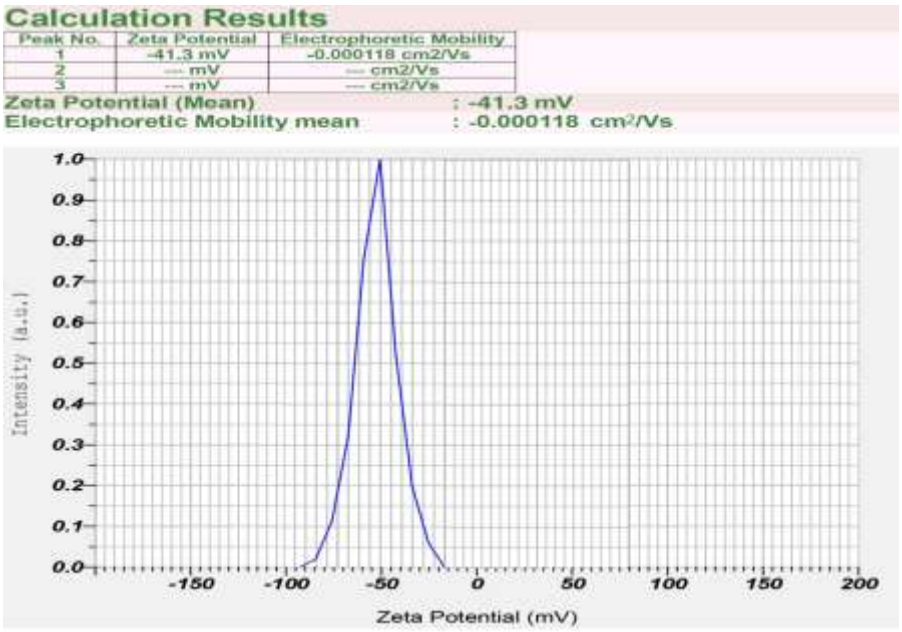


Figure No. 18: Zeta Potential of Glibenclamide Cubosomes.

From the above figure no. 18 the Zeta Potential of the Glibenclamide Cubosomes was found to be -41.3mV, which in turn states that the formulated Glibenclamide Cubosomes were stable in the dispersion and thereby withstand the conditions of varying pH while delivering the drug within the body.

2 (d) Entrapment Efficiency (E.E)

Glibenclamide Cubosomes with varying concentrations of GMO and the P407 were subjected to Ultra Centrifugation to determine the Entrapment Efficiency. This helps in determining the percentage amount of the drug that has been incorporated or entrapped within the cubosomes.

Table No. 5: Drug Entrapment Efficiency of Glibenclamide Cubosomes.

Formulation Code	%EE±SD	Formulation Code	%EE±SD
G1	80.23±2.51	P1	77.05±2.36
G2	84.72±1.25	P2	89.72±1.69
G3	77.59±1.87	P3	80.88±1.06
G4	80.10±0.25	P4	80.10±0.89
G5	83.36±0.98	P5	77.06±0.96
G6	88.87±1.02	P6	74.97±1.24
G7	90.54±1.69		
G8	88.17±1.54		
G9	85.28±1.39		
G10	78.73±1.75		

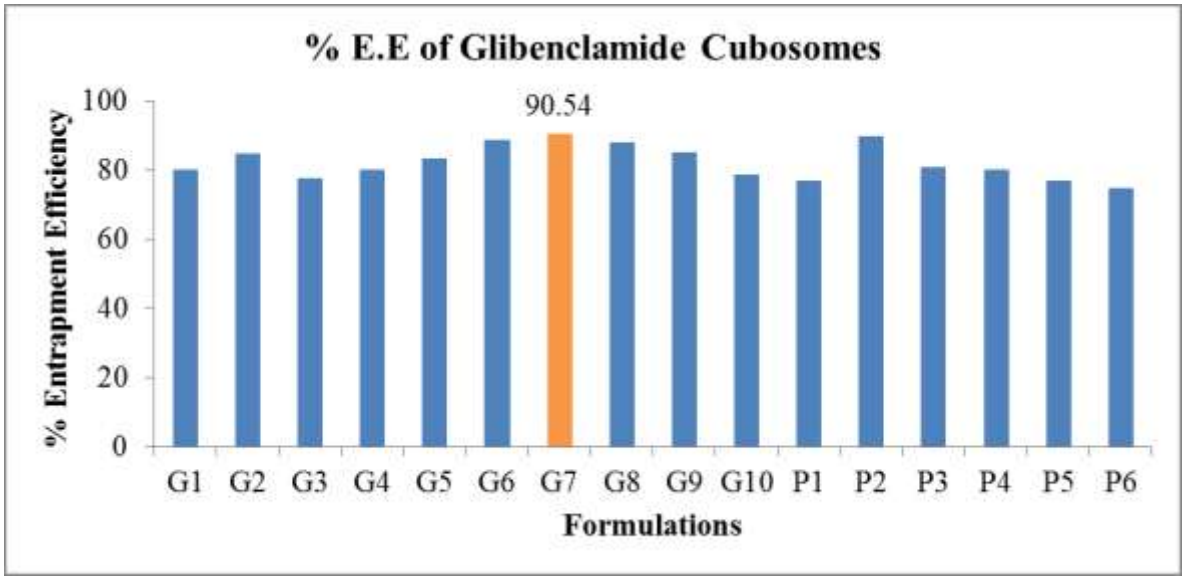


Figure No. 19: Entrapment Efficiency of Glibenclamide Cubosomes.

From the Figure no. 19 and Table no. 5 it was found that among all the Glibenclamide Cubosomal formulations, the Cubosomal formulation (G7) with 15% GMO and 1% P407 has the highest entrapment efficiency of 90.54% when compared with the remaining

formulations. Higher entrapment efficiency helps in incorporating the larger amounts of drug and good intricate liquid crystalline structure for the delivery of the drug.

Hence, the Glibenclamide Cubosomal formulation (G7) was selected for further studies to formulate into the Glibenclamide Cubosomal Oral Capsules.

2 (e) Invitro Drug release Studies

Glibenclamide Cubosomes with varying concentrations of GMO and the P407 were subjected to Invitro Drug release. Invitro drug release studies were performed for all formulations and formulation G7 was optimized. The percentage drug release profiles of various formulations are shown in the Figure no. 20, 21, 22 respectively.

Table No. 6: Invitro Drug release profile of Glibenclamide Cubosomes (G1-G6).

Time (Hrs)	%DRG1±SD	%DRG2±SD	%DRG3±SD	%DRG4±SD	%DRG5±SD	%DRG6±SD
0	0	0	0	0	0	0
1	4.81±1.25	6.40±2.10	5.61±0.98	2.88±1.56	3.36±2.36	3.61±0.86
2	10.57±1.41	16.50±0.56	10.73±1.24	6.98±2.35	7.88±1.56	9.53±2.35
3	16.50±3.22	25.16±0.79	23.07±0.96	13.87±0.36	14.93±2.41	22.51±2.54
4	24.83±2.36	33.33±1.74	37.52±1.53	21.89±3.54	21.50±0.56	36.93±0.12
5	34.29±1.73	41.50±0.23	46.79±0.54	31.82±0.69	31.28±0.39	49.43±0.56
6	45.67±3.25	51.92±3.12	56.73±1.36	45.44±1.89	40.06±1.25	60.81±0.89
7	57.53±1.35	64.90±2.16	74.51±2.69	60.83±1.32	55.44±1.57	69.79±1.54

With Increased concentrations of Poloxamer 407, drug release was found to be decreased. Formulation G7 showed highest drug release of 83.01% and hence it was formulated into the Cubosomal oral capsules as phase separation was observed in the remaining formulations.

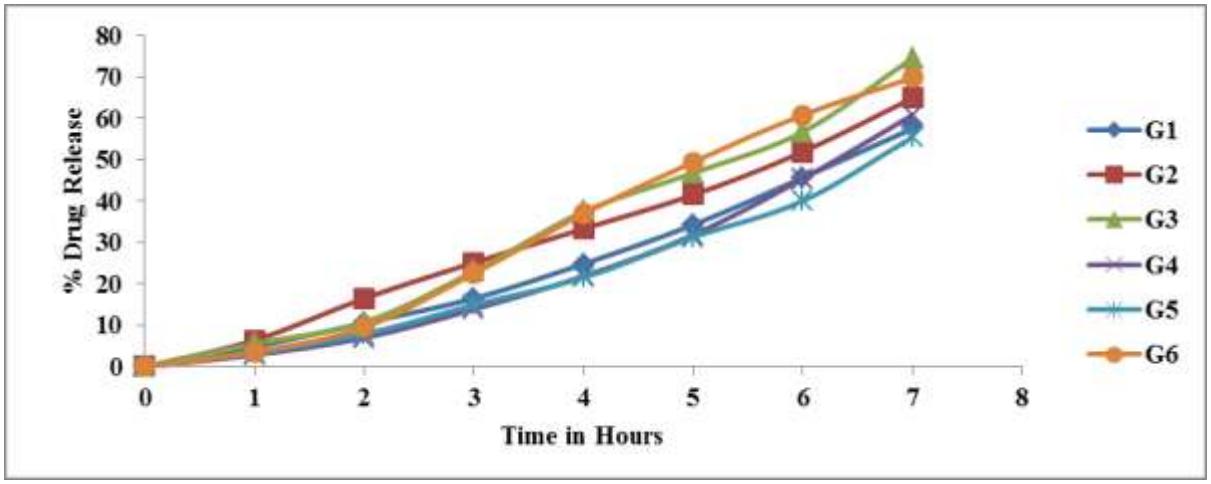


Figure No. 20: Invitro Drug release profile of Glibenclamide cubosomes G1-G6 in PBS pH 6.8.

Figure no. 20 clearly depicts that as the concentration of the GMO increases there is an increase in the solubility of the Glibenclamide and increase in the Invitro drug release as well. From the formulations G1-G6 the concentration of the GMO is gradually increasing and the rate of the drug release is also increasing.

Table no. 7 depicts the various formulations of Glibenclamide cubosomes with varying concentrations of GMO from 15% to 22.5% and the formulation G7 shows higher drug release when compared with rest of the formulations where there is phase separation. Hence the formulation G7 was selected for further studies and to formulate into the Glibenclamide Cubosomal Oral Capsules.

Table No. 7: Invitro drug release profile of Glibenclamide Cubosomes (G7-G10).

Time (Hrs)	%DRG7±SD	%DRG8±SD	%DRG9±SD	%DRG10±SD
0	0	0	0	0
1	6.4±1.23	7.13±0.31	6.24±1.36	5.6±2.01
2	18.10±0.56	14.24±1.23	9.26±1.65	10.7±31.56
3	30.28±3.26	20.81±2.36	16.47±0.54	23.07±0.25
4	40.22±4.21	33.79±1.89	36.02±1.76	37.50±1.36
5	50.32±0.98	45.49±2.31	44.51±2.31	46.79±2.54
6	63.62±0.65	64.88±0.56	59.90±0.49	59.13±3.15
7	83.01±1.26	77.22±1.42	71.44±2.65	69.39±1.30

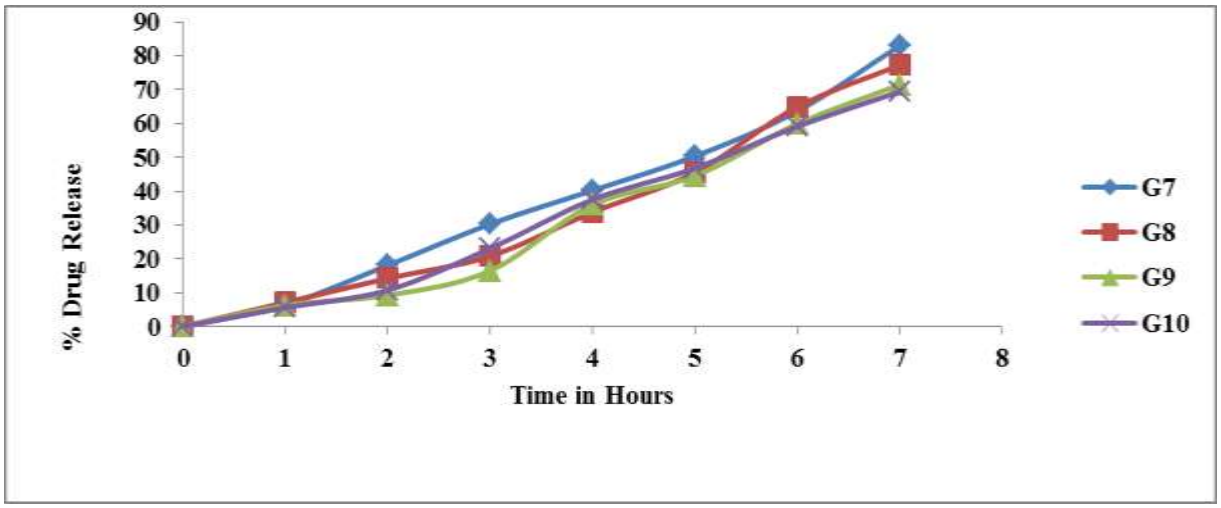


Figure No. 21: Invitro Drug release profile of Glibenclamide cubosomes G7-G10 in PBS pH 6.8.

Figure no. 21 clearly depicts that as the concentration of the GMO increases there is an increase in the solubility of the Glibenclamide and increase in the Invitro drug release as well. From the formulations G7-G10 the concentration of the GMO is gradually increasing and the rate of the drug release is also increasing.

Table No. 8 depicts the various formulations of Glibenclamide cubosomes with varying concentrations of Poloxamer 407 from 1% to 3% and as the concentration of the Poloxamer 407 increases from P1-P6, there is a decrease in the rate of the drug release and the structural integrity of the cubosomes. The Cubosomal structure changes from the cubical to the rod-shaped with the increase in the P407 concentration as well.

Table No. 8: Invitro Drug release profile of Glibenclamide Cubosomes (P1-P6).

Time (Hrs)	%DRP1±SD	%DRP2±SD	%DRP3±SD	%DRP4±SD	%DRP5±SD	%DRP6±SD
0	0	0	0	0	0	0
1	5.60±1.32	7.22±0.65	4.48±1.24	4.00±2.15	4.80±2.35	7.25±1.63
2	17.14±0.36	16.66±0.19	14.51±2.36	12.82±0.96	16.98±0.36	13.46±0.74
3	29.16±2.35	25.32±2.31	30.06±3.65	20.03±1.79	27.40±0.98	21.47±2.10
4	45.35±0.98	34.29±1.05	41.76±0.24	29.00±0.54	36.85±1.45	29.80±0.41
5	55.76±1.24	51.44±2.65	54.74±1.24	36.69±0.61	48.81±1.73	41.50±1.35
6	66.02±0.34	66.18±0.79	66.60±1.35	55.44±1.56	60.57±2.21	56.25±1.79
7	75.96±0.78	81.57±0.95	79.26±0.68	67.30±1.35	74.03±0.49	72.75±2.03

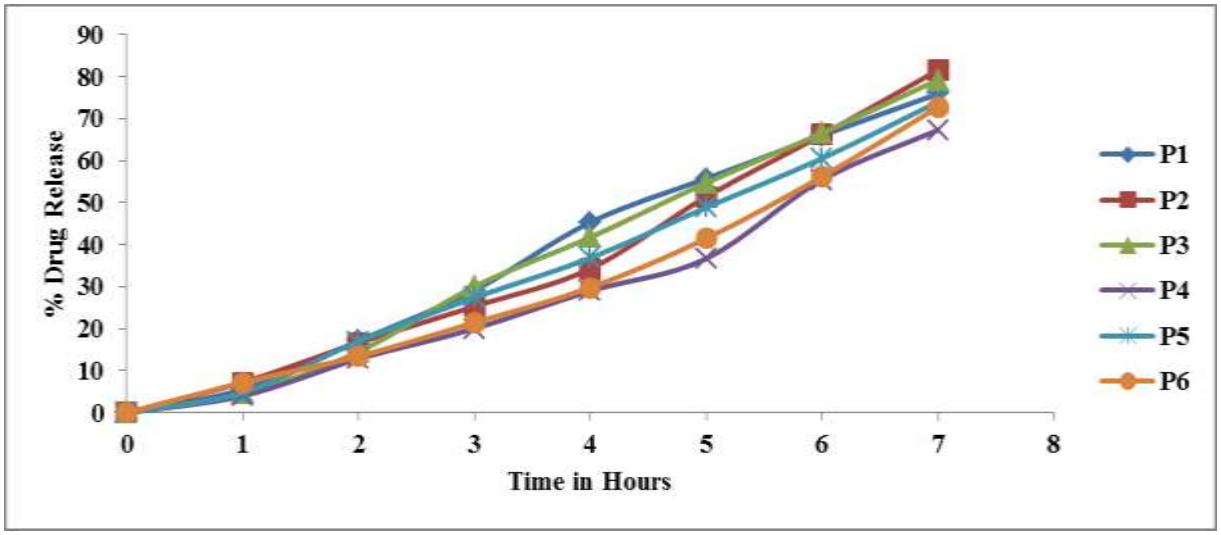


Figure No. 22: Invitro Drug release profile of Glibenclamide cubosomes P1-P6 in PBS pH 6.8.

3. EVALUATION OF GLIBENCLAMIDE CUBOSOMAL ORAL CAPSULES

3 (a) FTIR (Drug-Excipient Compatibility Studies)

The interaction study between the drug and Excipients as well as optimized formulation was evaluated using IR spectrophotometer. Various formulations of Glibenclamide cubosomes containing the varying concentrations of Glyceryl Mono Oleate and Poloxamer-407 were analyzed.

The Glibenclamide Cubosomal Oral Capsules were analyzed for the Drug-Excipient compatibility studies with varying concentrations of Starch and Aerosil. The results are depicted below in the figure no. 4.3 and 4.4 and Table 4.2 and 4.3 respectively.

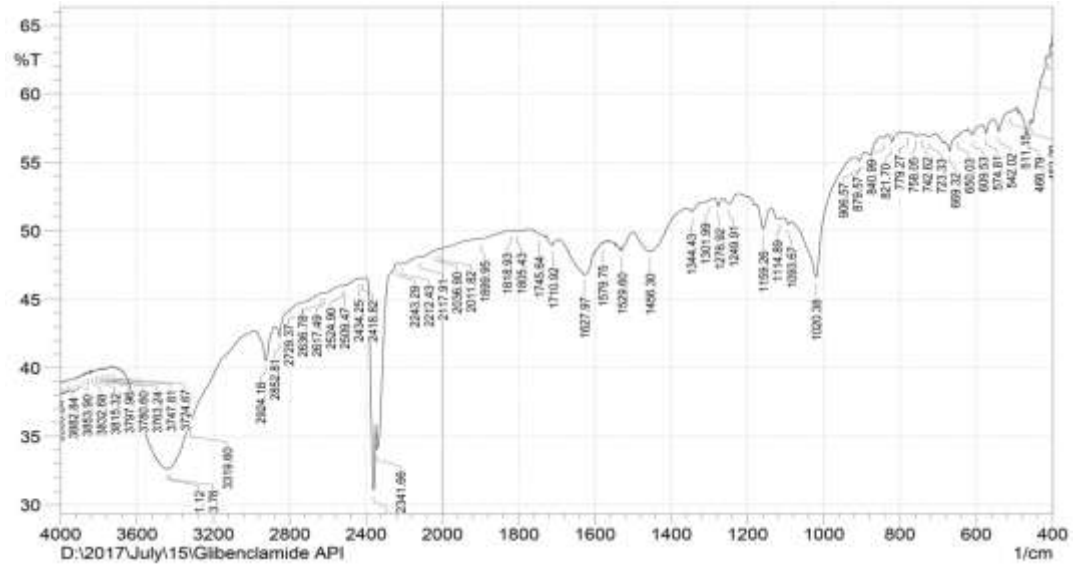


Figure No. 23: FTIR of Glibenclamide Pure Drug (API).

Table No. 9: Characteristic IR peaks of Glibenclamide Pure Drug (API).

Functional Group	Reported Value (cm^{-1})	Observed Value (cm^{-1})
N-H	3500-3300	3319.60
C-H	2950-2850	2924.18
C=O	1820-1670	1818.93
S=O	1300-1150	1276.92
C-Cl	800-600	669.32

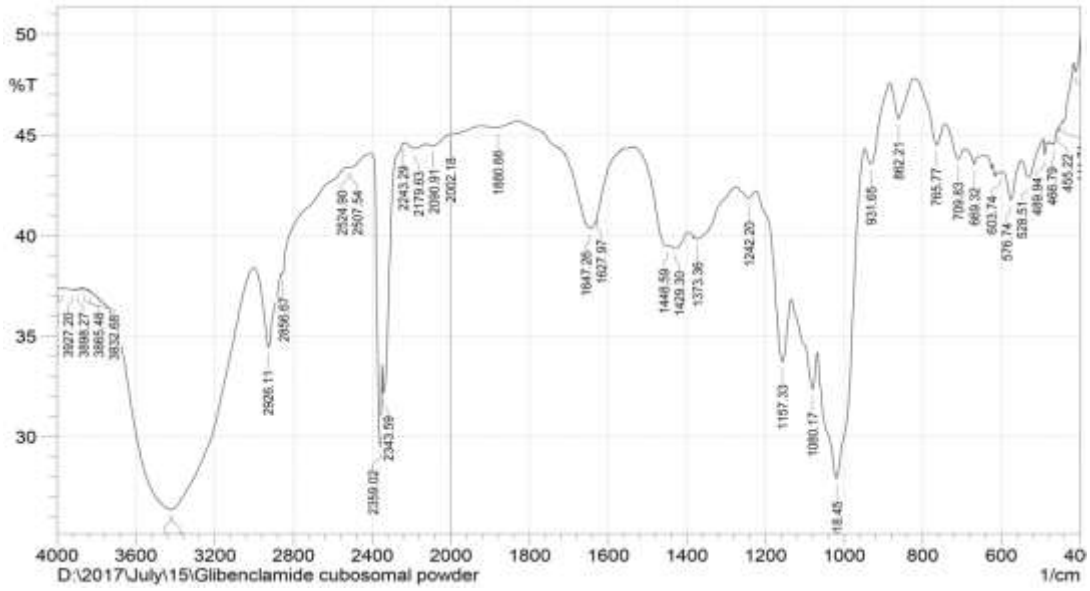


Figure No. 24: FTIR of Glibenclamide Cubosomes.

Table No. 10: Characteristic IR peaks of Glibenclamide Cubosomes.

Functional Group	Reported Value (cm^{-1})	Observed Value (cm^{-1})
N-H	3500-3300	3421.83
C-H	2950-2850	2926.11
C=O	1820-1670	1647.26
S=O	1300-1150	1242.20
C-Cl	800-600	765.77

Similar peaks were observed in spectra of different combinations of Excipients and in optimized formulation, along with absence of interference peaks indicating there is no unwanted reaction between Glibenclamide and other Excipients used in the study.

From the above figures 23, 24 and Tables 9, 10 it can be inferred that there was no appearance or disappearance of any characteristic peaks. This shows that there were no interactions between the drug and Excipients used in the preparation of Glibenclamide Cubosomal Oral capsules.

3 (b) Surface Morphology

The surface morphology of the Cubosomal granules was determined using scanning electron microscopy (Hitachi S-3700N).

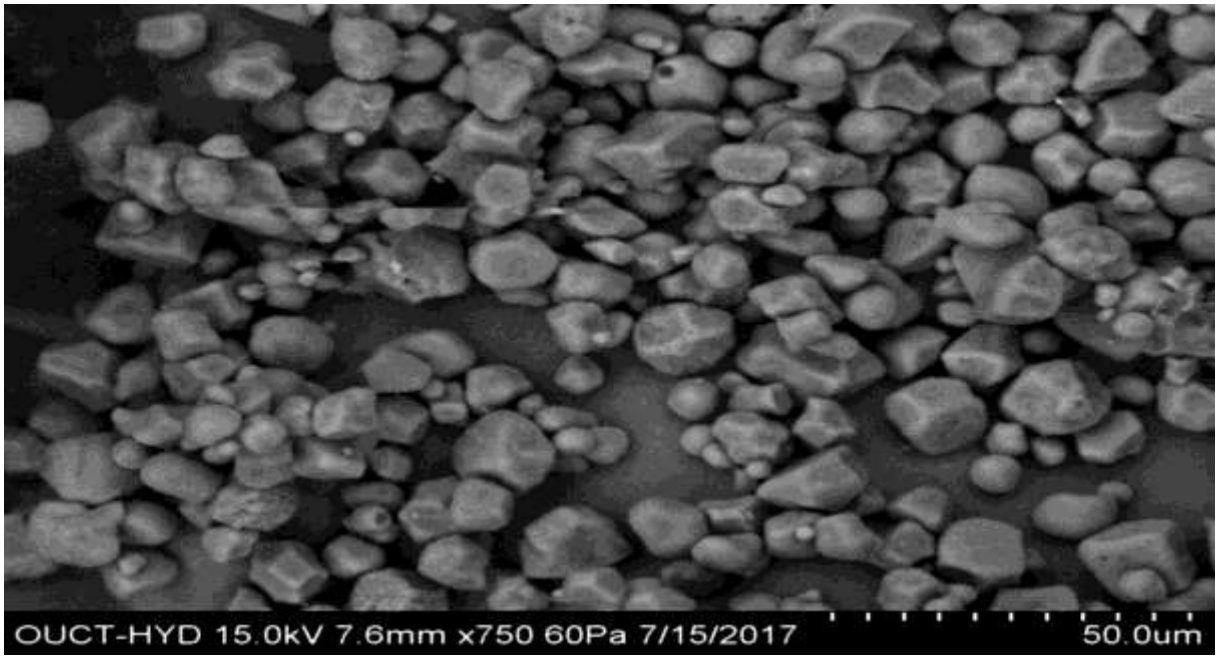


Figure No. 25: Scanning Electron Microscopic images of Glibenclamide.

Cubosomal Granules

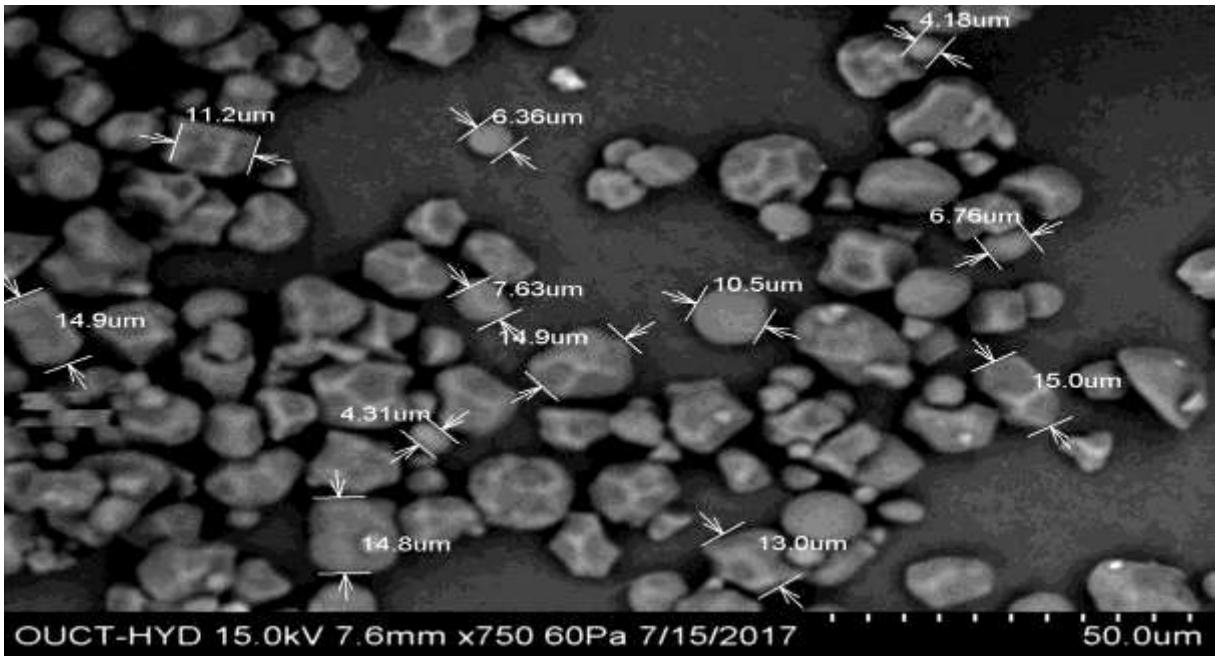


Figure No. 25: Scanning Electron Microscopic images of Glibenclamide Cubosomal Granules.

Various Glibenclamide Cubosomal Oral Capsules filled with granules with varying concentrations of Starch and Aerosil were subjected for the surface morphology using the Scanning Electron Microscope.

The Cubosomal starch granules were prepared with varying concentrations of Starch from 1.2-2.4 grams and Cubosomal Aerosil granules were prepared with varying concentrations of Aerosil from 0.2-0.8 grams.

From the Figure no. 25 and 26 it was found that the surface morphology of the Glibenclamide Cubosomal Granules was ranging from 4-15 micrometers. This helps in determining the flow properties of the granules such as Angle of Repose, Bulk Density, Tapped Density, Compressibility Index and Hausner's Ratio.

The granules were found to be free flowing with no intra and inter particulate friction and can be efficiently suitable to be filled into the capsules.

3. Particle size Analysis

Glibenclamide Cubosomal granules with varying concentrations of Starch and the Aerosil were subjected to the particle size analysis.

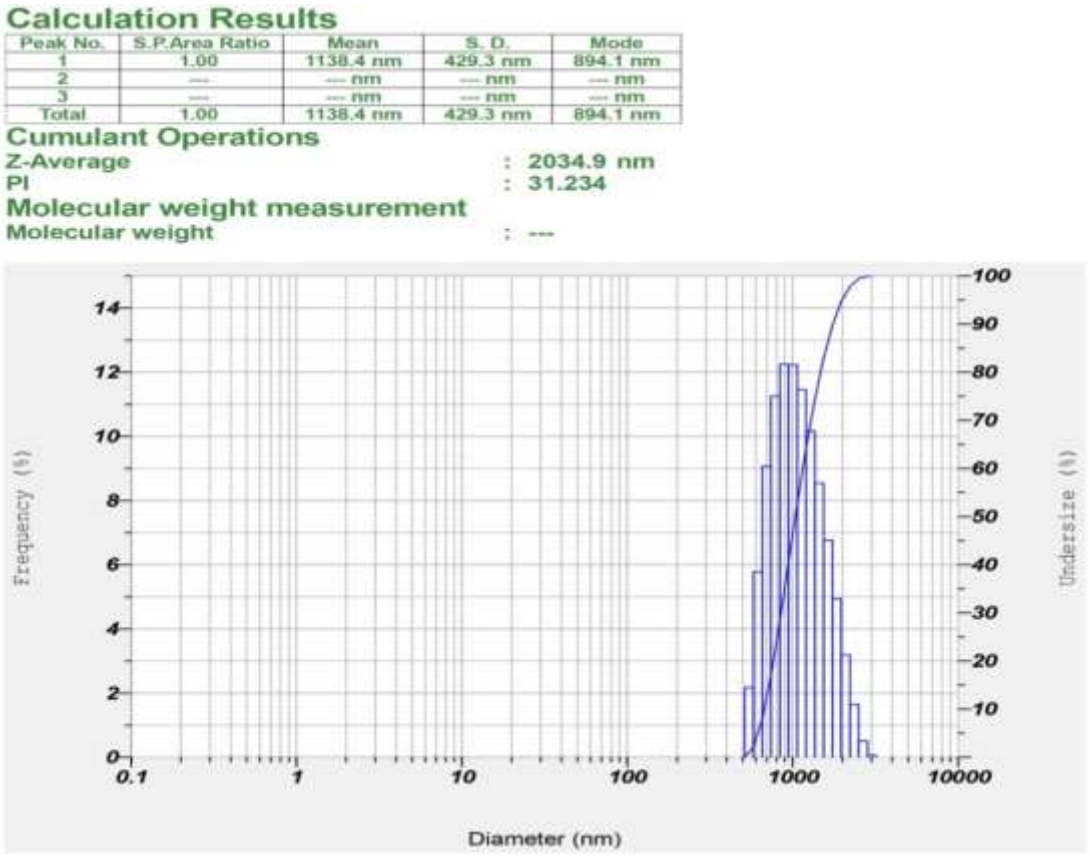


Figure No. 27: Particle Size of Glibenclamide Cubosomal Granules.

From the Figure no. 27 it was found that the particle size of the Glibenclamide Cubosomal Granules was 2034.9 nm.

This helps in determining the flow properties of the granules such as Angle of Repose, Bulk Density, Tapped Density, Compressibility Index and Hausner's Ratio.

The granules were found to be free flowing with no intra and inter particulate friction and can be efficiently suitable to be filled into the capsules.

4. Flow Properties

Flow properties of the optimized Glibenclamide Cubosomal granules formulation CG4 was shown in the below Table no. 11. The Glibenclamide Cubosomal granules were subjected to Angle of Repose, Bulk Density, Tapped Density, Hausner's Ratio and Compressibility Index.

Table No. 11: Flow properties of the Glibenclamide Cubosomal Granules.

Cubosomal Formulation	Angle of Repose	Bulk Density	Tapped Density	Hausner's Ratio	Carr's Compressibility Index
CG-1	26.56	0.41	0.48	1.16	13.7
CG-2	30.96	0.44	0.47	1.06	6.25
CG-3	29.68	0.42	0.48	1.16	13.8
CG-4	29.89	0.39	0.45	1.15	13.15
CG-5	28.81	0.41	0.47	1.15	13.51
CG-6	31.46	0.37	0.42	1.13	12.19
CG-7	27.74	0.48	0.54	1.10	9.67
CG-8	27.74	0.17	0.18	1.09	8.33
CG-9	33.02	0.2	0.23	1.15	13.33
CG-10	31.46	0.25	0.27	1.06	6.25
CG-11	26.12	0.31	0.36	1.14	12.5
CG-12	29.94	0.33	0.4	1.23	18.69
CG-13	30.01	0.3	0.37	1.21	17.39
CG-14	30.45	0.32	0.44	1.38	28

From the above Table no. 11 depicting the flow properties of the optimized Glibenclamide Cubosomal granule formulation (CG4), bulk density was 0.39gm/ml, tapped density was 0.45 gm/ml, Carr's Index was 13.15, Hausner's ratio was 1.15 and the values showed low intra particulate friction between the granules. The angle of repose was found to be 29.89 indicating the good flow properties of the granules. The granules were found to be free flowing and showed suitability to be filled into the capsules.

From the table depicting the flow properties of the optimized Glibenclamide Cubosomal granule formulation (CG4), bulk density was 0.39gm/ml, tapped density was 0.45 gm/ml, Carr's Index was 13.15, Hausner's ratio was 1.15 and the values showed low intra particulate friction between the granules. The angle of repose was found to be 29.89 indicating the good flow properties of the granules. The granules were found to be free flowing and showed suitability to be filled into the capsules.

5. Drug Content

Glibenclamide Cubosomal Oral Capsules with varying concentrations of Starch and Aerosil were subjected to the Drug Content. It helps in determining the amount of the drug present within the Glibenclamide Cubosomal granules.

Table No. 12: Percentage Drug content of the Glibenclamide Cubosomal Oral Capsules.

Formulation	% Drug Content	Formulation	% Drug Content
CG1	75.40	CG8	74.86
CG2	87.71	CG9	81.28
CG3	85.02	CG10	84.02
CG4	88.73	CG11	79.67
CG5	74.33	CG12	80.21
CG6	79.14	CG13	76.47
CG7	75.93	CG14	82.35

From Table no. 12 it was found that the drug content of the Glibenclamide Cubosomal granules was decreasing with the increasing concentrations of the Starch and Aerosil as well. The drug content of the optimized Glibenclamide Cubosomal Oral Capsules formulation CG4 was found to be 88.7%.

6. Invitro Dissolution Studies

The drug release studies were performed using the USP Type I Dissolution Apparatus with below mentioned parameters:

Dissolution Apparatus : USP Type I (Basket)

Dissolution Medium : Phosphate Buffer Saline pH 6.8

Volume of Dissolution Medium : 900ml

Temperature : 37±0.2^{o}C

Table No. 13 and Figure no. 28 depicts the Invitro Dissolution profile of the Glibenclamide Cubosomal Oral Capsules from the formulation CG1-CG7 which are formulated using varying concentrations of the Starch. It was observed that as the concentration of the starch increases the drug release was decreased and the optimized formulation CG4 was showing a release of 51.17% at the end of 7 Hours compared to all the formulations.

Table No. 13: Invitro Dissolution profile of the Glibenclamide Cubosomal Oral Capsules (CG1-CG7).

Time (Hrs)	%DRCG1 ±SD	%DRCG2 ±SD	%DRCG3 ±SD	%DRCG4 ±SD	%DRCG5 ±SD	%DRCG6 ±SD	%DRCG7 ±SD
0	0	0	0	0	0	0	0
1	7.78±1.23	6.25±0.69	6.32±2.58	5.34±0.69	6.85±1.23	5.82±0.35	4.86±0.64
2	10.25±2.36	9.85±1.23	12.68±0.85	13.65±1.27	11.22±0.79	14.14±3.21	10.24±0.51
3	22.47±0.25	20.98±0.34	20.05±0.64	20.53±3.65	22.47±3.61	25.89±1.57	17.59±2.36
4	28.44±3.65	26.25±2.12	26.00±1.69	26.12±1.54	26.98±0.34	31.89±0.41	21.1±1.54
5	42.90±1.47	40.56±0.97	39.95±2.35	40.95±2.36	40.12±2.57	37.59±0.69	32.07±2.57
6	46.46±0.69	47.65±1.82	45.04±1.52	48.96±0.51	44.58±1.63	40.23±2.37	37.59±0.97
7	49.61±1.27	50.71±2.36	48.69±3.61	51.17±0.64	47.74±0.86	44.34±1.98	41.69±1.82

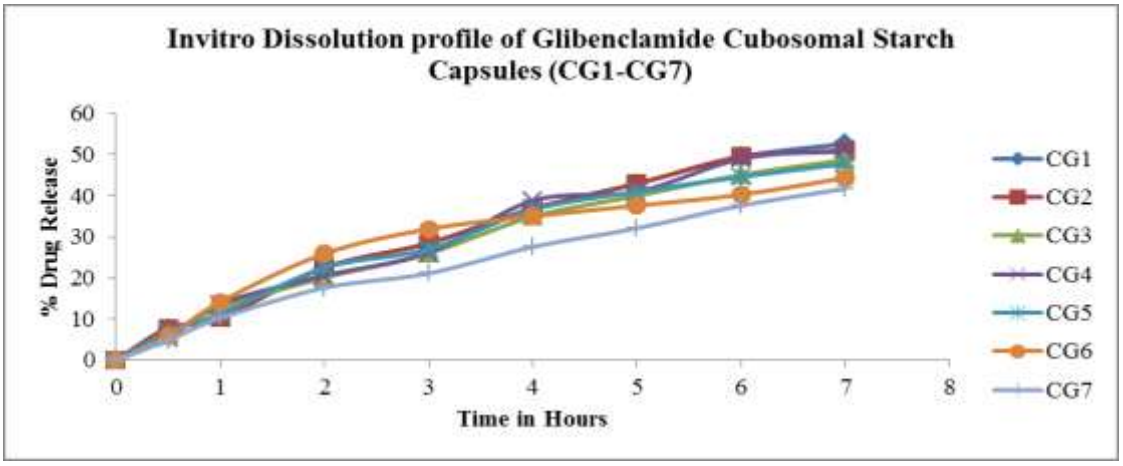


Figure No. 28: Invitro Dissolution profile of Glibenclamide Cubosomal Oral Capsules (CG1-CG7).

Glibenclamide Cubosomal Oral Capsule CG-4 shows higher drug release when compared with the other formulations and it was optimized for the further studies including the release kinetics and stability studies.

Table No. 14: Invitro Dissolution profile of the Glibenclamide Cubosomal Oral Capsules (CG8-CG14).

Time (H)	%DRCG8 ±SD	%DRCG9 ±SD	%DRCG10±SD	%DRCG11±SD	%DRCG12±SD	%DRCG13±SD	%DRCG14±SD
0	0	0	0	0	0	0	0
1	12.96±0.98	12.61±1.24	10.25±1.65	13.26±2.37	11.22±1.27	11.91±2.37	10.21±2.35
2	21.53±1.25	19.8±0.39	22.47±2.37	25.89±1.65	23.40±0.58	17.61±3.92	15.59±1.24
3	37.95±2.69	34.90±2.09	36.80±1.42	34.92±0.96	36.85±1.37	33.81±0.41	26.54±3.12
4	37.95±0.75	34.98±1.24	36.81±0.58	34.92±1.14	36.85±2.34	33.81±1.78	26.54±0.67
5	40.13±0.36	38.21±0.65	40.95±2.34	37.59±3.24	40.92±3.41	37.41±2.32	31.07±2.34
6	46.40±1.24	45.12±1.96	44.58±1.15	40.15±2.09	44.50±1.74	41.25±0.91	35.59±1.17
7	50.24±0.47	47.20±2.07	48.5±1.78	44.91±1.52	46.20±0.34	43.91±3.64	40.63±0.58

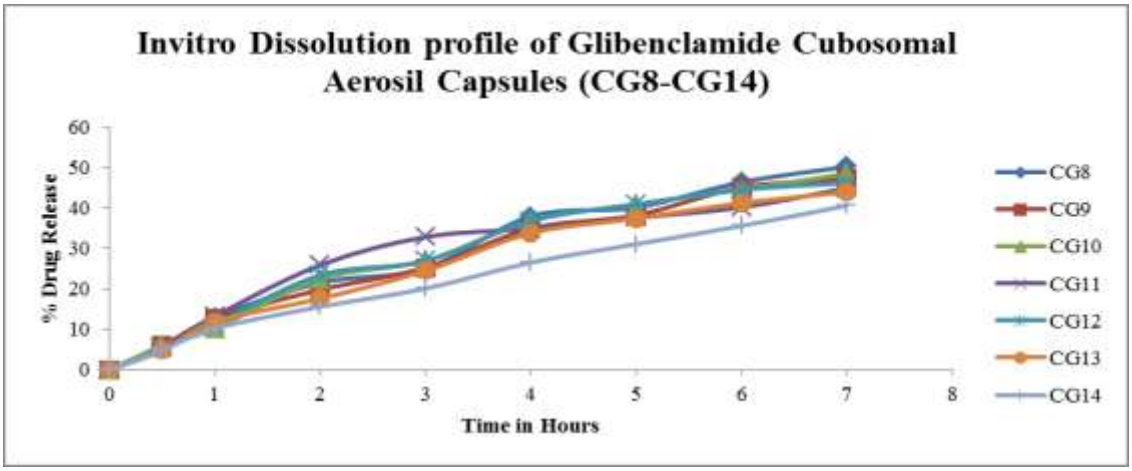


Figure No. 29: Invitro Dissolution profile of Glibenclamide Cubosomal Oral Capsules (CG8-CG14).

Table no. 14 and Figure no. 29 shows the dissolution profiles of the Glibenclamide Cubosomal formulations with varying concentrations of Aerosil i.e. CG7-CG14.

From the Invitro Dissolution studies it was observed that the Glibenclamide Cubosomal Oral Capsules formulated with the varying concentrations of Starch (CG1-CG6) shown a greater drug release profiles when compared with the Glibenclamide Cubosomal Oral Capsules formulated with varying concentrations of Aerosil (CG7-CG14).

Comparison of Invitro Dissolution studies of Optimized Glibenclamide Cubosomal Oral Capsules with the Invitro Dissolution studies of the Marketed Glibenclamide Tablets:

The drug release of the optimized Glibenclamide Cubosomal oral capsules are compared with the drug release of the Marketed Glibenclamide tablets i.e. Daonil® Glibenclamide Tablets 5mg IP (Sanofi).

It was found that the drug release of the optimized Glibenclamide Cubosomal oral capsules exhibits the sustained action and the drug release was 51.17% in 07 hours than the Marketed Glibenclamide Tablets, which was found to be 95.70 in 04 hours.

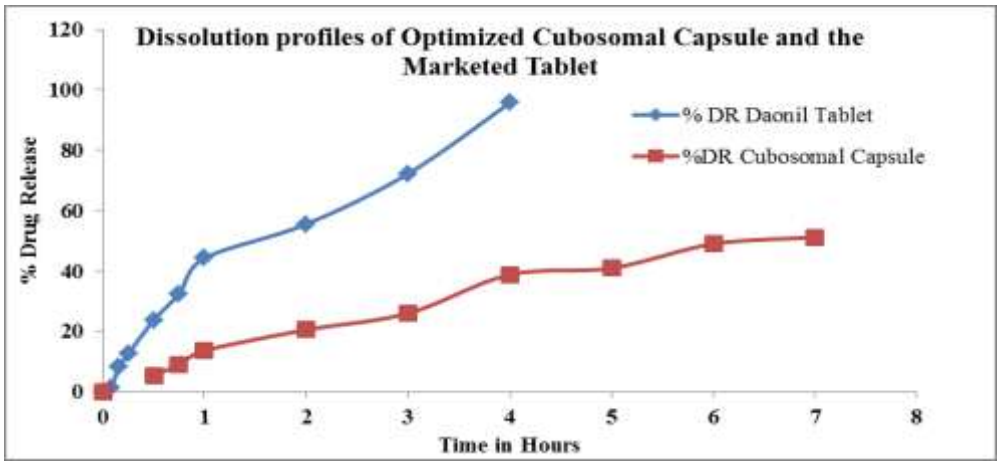
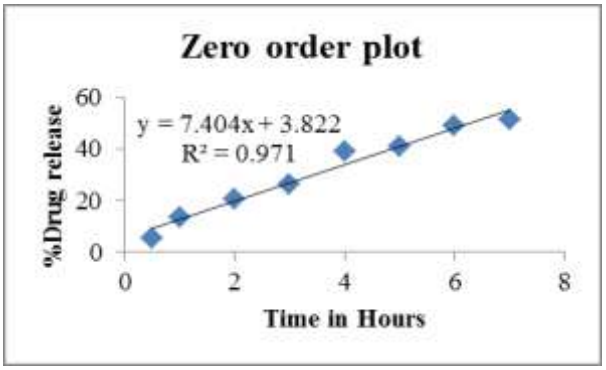


Figure No. 30: Invitro Dissolution profile of Optimized Glibenclamide Cubosomal Oral Capsules and the Marketed Glibenclamide Tablets.

7. Release Kinetics

The mechanism of Glibenclamide Cubosomal release from capsules was studied by fitting the data obtained from Invitro release studies into zero-order, first-order, Higuchi's, Hixson Crowell, KorseMeyer-peppas kinetic models. Obtained values of correlation coefficient are given in **Table No. 15** It was found that optimized formulation CG4 showed Zero order drug release.

Kinetic Model	R^2 Values
Zero Order	0.971
First Order	0.952
Higuchi	0.948
Hixson Crowell	0.913
KorseMeyer-peppas	0.895



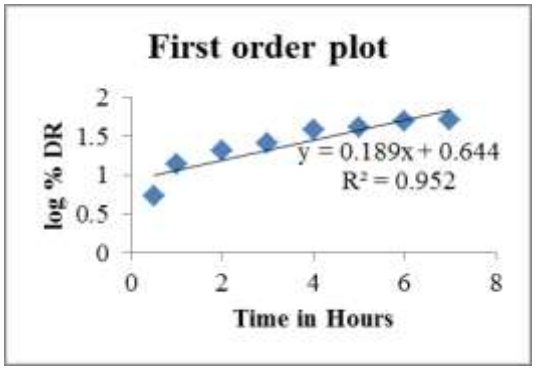
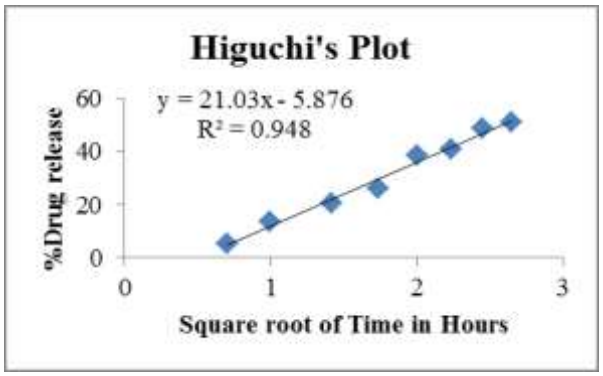


Figure No. 31 and 32: Zero Order Plot and First Order Plot.



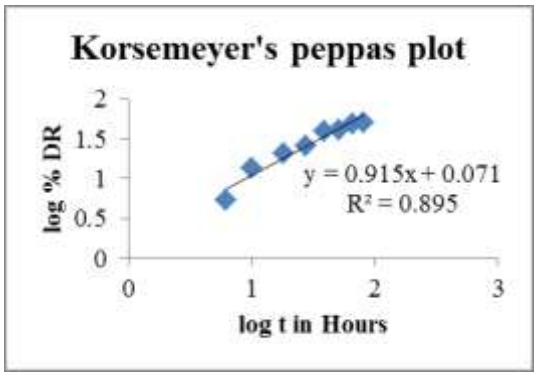


Figure No. 33 and 34: Higuchi's Plot and Korsemeyer's peppas Plot.

8. Stability Studies

Drug content and drug release values are analyzed periodically as per ICH guidelines through accelerated stability studies for optimized capsule formulation CG4 and given in

Table No. 16: Release Kinetics of Optimized Glibenclamide Oral Capsules.

Stability Conditions	% Drug Content CG4 Number of Months		
	1	2	3
25°C±2°C/60% RH	88.73	87.26	87.15

At fixed time intervals, % Drug content determination of these formulations shows that there was no significant change. At the first month of storage the drug content was found to be 88.73% and at the end of the third month it was found to be 87.15% at 25°C and 60% RH. Thus we may conclude that the drug does not undergo degradation on storage.

ACKNOWLEDGEMENTS

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CONCLUSION

An attempt was made to develop Glibenclamide cubosomes using the GMO and Poloxamer 407 as the stabilizer and evaluated for the compatibility and morphological studies. Formulation G7 containing 1% Poloxamer 407 and 15% GMO concentration was optimized based on the drug release, good entrapment efficiency and greater stability. The optimized Cubosomal formulation was again formulated into the capsules by the addition of starch and Aerosil as adsorbing agents to obtain the sustained release action over the time. Formulation CG4 containing the starch showed cubic structure with higher drug release of 51.17% at the end of the 7 hours in pH 6.8 PBS and was found to be stable than other formulations. Thus it can be concluded that cubosomes are efficient in obtaining the increased drug release action with the Glibenclamide.

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