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PREPARATION AND EVALUATION OF MUCOADHESIVE NANOPARTICLE OF AN ANTIHYPERTENSIVE AGENT

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ABSTRACT
Diltiazem HCl (DTZ) is an antihypertensive agent that antagonizes the action of beta-1 receptor. DTZ when given orally is well absorbed from the gastrointestinal tract and is subject to an extensive first-pass effect. DTZ undergoes extensive metabolism in which only 2% to 4% of the unchanged drug appears in the urine. Drugs which induce or inhibit hepatic microsomal enzymes may alter DTZ disposition. It has been reported that the absolute bioavailability of DTZ when given orally is 30-40%. The biological half-life of DTZ is 4-6 hour and the main site of absorption is proximal small intestine.
The reduced bioavailability of DTZ may be because of transportation of dosage form from the region of absorption window to site where it is less absorbed. Therefore there was a need to increase gastroretention time of dosage form so that drug would be available at the site of absorption and results in improved bioavailability. A mucoadhesive nanoparticle delivery system was envisioned for DTZ as such a system when administered would adhere on the gastric mucosa for a prolong period of time and the drug would be available at the main site of absorption i.e. proximal small intestine resulting in enhanced bioavailability.
Keywords: Bioavailability, mucoadhesive nanoparticle, gastric mucosa, antihypertensive agent.

INTRODUCTION

Diltiazem HCl (DTZ) is an antihypertensive agent that antagonizes the action of beta-1 receptor. DTZ when given orally is well absorbed from the gastrointestinal tract and is subject to an extensive first-pass effect. DTZ undergoes extensive metabolism in which only 2% to 4% of the unchanged drug appears in the urine. Drugs which induce or inhibit hepatic microsomal enzymes may alter DTZ disposition[1]. It has been reported that the absolute bioavailability of DTZ when given orally is 30-40%. The biological half-life of DTZ is 4-6 hour and the main site of absorption is proximal small intestine[2].

The reduced bioavailability of DTZ may be because of transportation of dosage form from the region of absorption window to site where it is less absorbed. Therefore there was a need to increase gastroretention time of dosage form so that drug would be available at the site of absorption and results in improved bioavailability. A mucoadhesive nanoparticle delivery system was envisioned for DTZ as such a system when administered would adhere on the gastric mucosa for a prolong period of time and the drug would be available at the main site of absorption i.e. proximal small intestine resulting in enhanced bioavailability

MATERIALS AND METHODS

FTIR Study

Drug sample was vacuum dried for 12 hours before IR studies. Drug (5mg) was mixed with potassium bromide (100mg) and compressed into pellets. The IR spectrum was taken in CDRI, Lucknow. The observed peaks were reported for functional groups.

Quantitative Estimation of Drug

Drug was estimated in the range of 1-10 mcg/ml and 2-20 mcg/ml for diltiazem respectively in water (pH 7.0), PBS (pH 7.4) and SGF (pH 1.2)

PBS (pH 7.4): Disodium hydrogen phosphate 2.38 g, potassium dihydrogen phosphate 0.19 g, sodium chloride 8.0 g were dissolved in sufficient distilled water and volume was made up to 1 liter. The pH was adjusted to 7.4 prior to quantitative estimation.

SGF (pH 1.2): Sodium Chloride 2.0 g and 7.0 ml of hydrochloric acid were dissolved in sufficient distilled water and was made upto 1 liter. PH was adjusted to 1.2 prior to use.

Construction of calibration curve of Diltiazem HCl (DTZ)

1. Preparation of Calibration Curve in Distilled Water: 100mg of accurately weighed DTZ was dissolved in minimum quantity of distilled water (20 ml). The volume was made up to 100 ml with distilled water to give standard solution (1000 mcg/ml). From the standard solution, a stock solution was prepared to give a concentration of 10 µg/ml in distilled water. Aliquots of 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 ml of stock solution was pipetted out into 10ml volumetric flask. The volumetric was made up to the mark with distilled water. These dilutions give 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 µg/ml concentration of DTZ respectively. The absorbance of prepared solution of DTZ in distilled water was measured at 237nm in Shimadzu UV-1700 spectrophotometer against an appropriate blank.

2. Preparation of Calibration Curve in Phosphate buffer Saline (pH 7.4): Same procedure was followed as given above by using Phosphate Buffer Saline pH 7.4 in place of distilled water.

3. Preparation of Calibration Curve in Simulated Gastric Fluid (pH 1.2) without pepsin: Same procedure was followed as given above by using Simulated Gastric Fluid (pH 1.2) without pepsin in place of distilled water.

Method of Preparation

Ditiazem HCl nanoparticles were prepared by cross linking method. Diltiazem HCl (100 mg) was accurately weighed and dissolved in the specified concentrations of chitosan solution (in 0.1% acetic acid). Specified quantity of Pluronic F-68 (50-250 mg) was added as a stabilizer to the above solution and stirred continuously with the help of magnetic stirrer for 40 minutes (600-1100 rpm). During the stirring the specified concentration of sodium TPP solution was added drop wise in the drug/polymer solution in specified volume ratio. Then formulation was centrifuged for 30 minutes at 12000 rpm and 4^0 C, the supernatant was removed and the pellets were resuspended and centrifuged three times in distilled water to

remove unentrapped drug. Finally pellets were suspended in distilled water and freeze dried using 5% glucose solution as a cryoprotecter and powder was stored in vials.

Particle Size and Particle Size Distribution [10]

Particle diameter and particle size distribution were determined using the particle size analyzer (Malvern Rasterizer, Malvern Instruments Ltd., Malvern, UK). For analysis Nano-suspensions were diluted five times with filtered (0.45µm) bi-distilled water.

Entrapment Efficiency [11]

For determination of drug entrapment, the amount of drug present in the clear supernatant after centrifugation was determined (w) by UV spectrophotometer at 254 nm. A standard calibration curve of drug was plotted for this purpose. The amount of drug in supernatant was then subtracted from the total amount of drug added during the preparation (W). Effectively, (W-w) will give the amount of drug entrapped in the particles.

Then percentage entrapment of a drug is obtained by using following equation

% Drug Entrapment = (W-w) × 100 / W

Drug Loading [12]

The DTZ content in the nanoparticles was determined by pulverizing the ACV-loaded nanoparticles (10mg) followed by immersing them in 100ml simulated gastric fluid (SGF, pH 1.2, without enzymes) with agitating at room temperature for 12 h. After filtration through a 0.45µm membrane filter (Millipore), the drug concentration was determined spectrophotometric ally at the wavelength of 237 nm. The filtered solution from the empty nanoparticles (without DTZ) was taken as blank. All samples were analyzed in triplicate and the drug loading (DL) was calculated according to the following equation:

$$DL\ (\%) = \frac{WD \times 100}{WT}$$

Where, DL: drug loading;

WD: the weight of the drug loaded in the nanoparticles;

WT: the total weight of the nanoparticles.

Drug Release Study [13]

The in vitro drug release studies were performed by dialysis membrane diffusion technique using glass tube of 10 cm length open at its both ends having 2.5 cm diameter. The dialysis membrane of 12,000 MWco (Spectra poor, Sigma, USA) was used for release study, because it retains NPs and allows free drug to diffuse in the release media. The lower end of the glass tube was covered with the pretreated membrane to keep the nanoparticulate formulation on the donor side. The NPs (equivalent to 10 mg of DTZ) were placed in donor compartment by dispersing in 3 ml of SGF (pH 1.2) where the drug was allowed to freely diffuse over the receptor compartment containing 100 ml of SGF (pH 1.2). The entire system was kept at 37±0.5 ◦C with continuous magnetic stirring at 100 rpm. Samples of 5 ml were withdrawn at predetermined time intervals (0.5, 1, 2, 3, 4, 5, 6, 12 and 24 hours) and replaced with fresh SGF. The withdrawn samples were suitably diluted to carry out UV Spectrophotometric analysis at 254 nm.

Treatment of Dialysis bag: -Tubing of dialysis membrane was washed in running water for 3-4 hours to remove glycerin (humectants). Treated with 0.3 % (w/v) solution of sodium sulfide at 80^0C for 1minute to remove sulfur compounds, washed with hot water (60^0C) for 2 minutes, acidified with acid 0.2% solution of sulfuric acid for 1 minutes and rinse with hot water for 2 min to remove acid.

1. Cumulative percent drug released versus time (zero-order kinetic model).
2. Log cumulative percent drug remaining versus time. (first-order kinetic model).
3. Cumulative percent drug released versus square root of time (Higuchi's model).
4. Log cumulative percent drug released versus log time (Korsmeyer-Peppas equation)

Measurement of Bioadhesive Strength [14]

Bioadhesive properties of nanoparticles were evaluated by Texture analyzer (M/s TA. XT. Plus, Stable Microsystem,UK) using porcine gastric mucosa. Stomach of pig was obtained immediately after slaughter at local slaughterhouse. The stomach was washed with fresh water to remove non-digested food from stomach then placed in SGF at 4^0C (used within 6 h). The membrane was then attached both on the base of texture analyzer and to the stainless steel probe (using two sided adhesive tape), probe is then fixed to the mobile arm of the texture analyzer. The 10 mg of nanoparticulate formulation was placed on the membrane placed on lower surface moistened with 1 mL of SGF. The mobile arm (with attached membrane) was lowered at a rate of 0.5 mm s^{-1} until contact with the formulation was made. A contact force of 10 g was maintained for 500 s, after which the probe was withdrawn from the membrane. After the adhesive bond has formed, the force (weight) required to separate the bond was recorded as mucoadhesive strength.

Particle Size Measurement by Microscopy based Technique [15]

Particle size of optimized formulation was evaluated by Transmission Electron Microscopy (TEM).TEM (H7500; Hita-chiLtd., Tokyo, Japan) was used for determination of shape and size of GNPs. The aqueous dispersion (one drop) was placed over a 400-mesh carbon-coated copper grid followed by negative staining with phosphotungstic acid solution (3%w/v, adjusted to pH 4.7 with KOH) and placed at the accelerating voltage of 95 kV.

RESULT AND DISCUSSION

The preliminary study showed that diltiazem is a white to off-white crystalline powder with a bitter taste. It is freely soluble in water, methanol, chloroform and soluble in 0.1 N NaOH, Simulated gastric fluid (pH1.2) and Phosphate buffer Saline (pH 7.4). The melting point was in the range of 210-213^0 C which is in compliance with the standard value of 213^0C as per Indian Pharmacopoeia. Partition coefficient value (log P) of diltiazem HCl were found to be 1.44 in n-octanol/water system and 1.68 in n-octanol/PBS (pH 7.4) which indicates the lipophilic nature of diltiazem HCl.

From the IR data of the formulation it is clear that functionalities of drug have remained unchanged including intensities of the peak. This suggests that during the process of formulation polymer has not reacted with the drug to give rise to reactant products. So it is only physical mixture and there is no interaction between them which is in favor to proceed for formulation. To study the effect of polymer on the properties of nanoparticles formulation F1 to F5 were formulated. It was found that the particle size of the formulations were in the range of 151-858.4 nm, PDI were in range 0.302 -0.875,entrapment efficiency were found in range 28.55-59.8%, loading efficiency were found in range 50-75% and practical yield were found in range 23.97-37.80%. It was found that on increasing the concentration of chitosan (0.05% to 0.25%w/v) particle size and entrapment efficiency increases while practical yield decreases.

Formulation F3 containing 0.15% of chitosan showed satisfactory results such as particle size of 151nm, PDI 0.398, entrapment efficiency 52.7%, drug loading 72% and practical yield 32.16% among the F1-F5 formulations.

To study the effect of cross linking agent sodium TPP on the properties of nanoparticles formulation F6 to F10 were formulated. It was found that the particle size of the formulations were in the range of 462.7-724.9 nm, PDI were in range 0.189-0.300, entrapment efficiency were found in range 63.4-85.7%, loading efficiency were found in range 63.38-82.61% and practical yield were found in range 31.45-52.40%. It was found that on increasing the concentration of Sodium TPP (0.05% to 0.25%w/v) particle size and entrapment efficiency increases while practical yield decreases. Formulation F8 containing 0.15% of Sodium TPP solution showed satisfactory results such as particle size of 547.6 nm, PDI 0.300entrapment efficiency 69.5%, drug loading 64.1% and practical yield 31.45% among the F6-F10 formulations.

To study the effect of amount of Pluronic F-68 of nanoparticles formulation F11 to F15 were formulated. It was found that the particle size of the formulations were in the range of 322.9-563.1 nm, PDI were in range 0.21-0.659, entrapment efficiency were found in range 63.4-84.8%, loading efficiency were found in range 43.38-76.61% and practical yield were found in range 28.75-53.72%. It was found that on increasing the amount of Pluronic F-68 (50-250mg) particle size and entrapment efficiency increases while entrapment efficiency decreases. Formulation F14 was containing 200mg of Pluronic F-68 showed satisfactory results such as particle size of 322.9 nm, PDI 0.21 entrapment efficiency 66.95%, drug loading 64.11% and practical yield 32.39% among the F11-F15 formulations.

To study the effect of the variation in the volume ratio of chitosan solution (105%w/v) and sodium TPP solution (0.15%) on the properties of nanoparticles formulation F16 to F20 were formulated. It was found that the particle size of the formulations were in the range of 286-1011 nm, PDI were in range 0.5-0.814, entrapment efficiency were found in range 67.85-86.6%, loading efficiency were found in range 43.38-76.61% and practical yield were found in range 28.75-53.72%. It was found that different volume ratio reduced particle size and entrapment efficiency increases. Formulation F20 containing 1:3 (7.5 ml Chitosan solution:22.5ml sodium TPP solution) showed satisfactory results such as particle size of 286 nm, PDI 0.5 entrapment efficiency 67.85%, drug loading 63.16% and practical yield 33.3% among the F16-F20 formulations.

To study the effect of the stirring speed on the properties of nanoparticles formulation F21 to F25 were formulated. It was found that the particle size of the formulations were in the range of 508.9-764.2 nm, PDI were in range 0.367-0.738, entrapment efficiency were found in range 67.85-86.6%, loading efficiency were found in range 63.16-87.33% and practical yield were found in range 29.42-32.30%. It was found that increasing in stirring speed reduces the particle size and satisfactory effect on entrapment efficiency. FormulationF24 (1000) rpm showed satisfactory results such as particle size of 508.9 nm, PDI 0.367 entrapment efficiency 84.8%, drug loading 82.95% and practical yield 29.40% among the F16-F20 formulations.

In vitro release data for mucoadhesive nanoparticles of diltiazem HCl formulation were subjected to goodness of fit test by linear regression analysis according to zero order and first order kinetic equation, Higuchi's and Korsmeyer-Peppas model to ascertain the mechanism of drug release. The results of linear regression analysis including regression coefficients are summarized in table 48 and plots shown in figure 14 to 32.

It was observed from the above data that the formulations have displayed r^2 value for zero order release kinetics in the range of 0.996 to 0.707. The r^2 values for first order release kinetics were in the range of 0.998 to 0.889. The r^2 values in Higuchi's release kinetic model were in the range of 0.691-0.947. In Peppas release kinetic model the r^2 value observed in all formulations from 0.783-0.965 and value of n were in the range of 0.4592 to 0.7432. Formulation F1-F5 showed the goodness of fit in zero order release kinetic model and from F6-F25 showed the best fit in first order kinetic model (F17 and optimized formulation F24 showed the goodness to fit in zero order release kinetic model). From the result of Peppas release kinetic model it was found that all the formulations showed non-fickian diffusion mechanism for the drug release.

The determination of mucoadhesive strength was based on the measurement of shear stress required to break the adhesive bond between a mucosal membrane and the formulation. The formulation is sandwiched between two mucosal membranes fixed on flexible supports in the assemblies for a sufficient period of time. After the adhesive bond has formed, the force (weight) required to separate the bond was recorded as mucoadhesive strength. The mucoadhesion strength of all the formulations was found satisfactory. The optimized formulation F24 showed satisfactory mucoadhesive strength of 7.2 gm.

The TEM characterization revealed that the nanoparticles were spherical in shape. However, some variation in size distribution was observed in the TEM image, which might be attributed to an uncontrolled charge neutralization process involved between oppositely charged chains occurring during the formation of nanoparticles at specific pH.

CONCLUSION

- The physical appearance and melting point of drugs were found concordant with that mentioned in I.P. (2007) and Merck Index (2001), which shows purity of sample. The IR spectrum of drugs was satisfactory.
- Solubility studies in different solvents at room temperature suggested that Diltiazem HCl was freely soluble in water, methanol and chloroform, soluble in 0.1 N HCl, slightly soluble in ethanol, sparingly soluble in simulated gastric fluid (pH1.2) and phosphate buffer saline (pH7.4).
- The Partition coefficient value of Diltiazem HCl were found to be 01.44 in n-octanol/water system and 1.68 in n-octanol/PBS (pH 7.4) which indicates the lipophilic nature of Diltiazem HCl. Spectrophotometric method of analysis of Diltiazem HCl showed λ_{max} at 237 nm respectively in distilled water , PBS (pH 7.4)and SGF (pH1.2). A straight line with correlation coefficient very near to one indicated that the drugs follow beer's law within the specified concentration range.
- From the result of FTIR spectra it was concluded that drug and excipients are compatible with each other.
- It was found that the formulation F1-F25 had the particle size in the range of 56.3-1011 nm, PDI 0.18-1, Entrapment efficiency was in the range 34.8-89.3%,

loading efficiency 46.84-88.51%, practical yield 7.97-53.72% and mucoadhesive strength was found in the range 3.59-7.82 gm.

- The optimized formulation F24 formulated with 0.15 % of chitosan solution, 0.15 % sodium TPP solution at the ratio of 1:3(7.5 ml : 22.5 ml), 200 mg of Pluronic F-68 added and stirring speed of 1000 rpm. The optimized formulation F24 showed particle size of 508.9 nm, PDI 0.659, entrapment efficiency 89.3%, loading efficiency 80.21%, practical yield 29.40% and mucodhesive strength of 7.42 gm.
- Formulation F24 appears suitable for further pharmacodynamic and pharmacokinetic studies to evaluate clinical safety in suitable animal and human models.

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Table 1: Solubility profile of Diltiazem HCl

S.No.	Medium	Solubility Profile	Parts of Solvent
1.	Water	Freely soluble	1-10
2.	Methanol	Freely soluble	1-10
3.	Ethanol	Slightly soluble	100-1000
4.	0.1 N Hydrochloric Acid	Soluble	10-30
5.	0.1 N Sodium Hydroxide	Sparingly Soluble	30-100
6.	Acetone	Very slightly soluble	1000-100000
7.	Simulated Gastric Fluid (pH 1.2)	Sparingly Soluble	30-100
8.	Phosphate buffer Saline (pH 7.4)	Sparingly Soluble	30-100
9.	Chloroform	Freely soluble	1-10

Table 2: Melting Point

S.No.	Melting point		Result
	Onset	Completion	
1.	210°C	213°C	210-213°C
2.	209.5°C	212°C	
3.	210°C	213.5°	

Table 3: Partition coefficient values of drug

S. No.	Medium	Partition coefficientof drugs (LogP)
1.	n-Octanol : Water	1.44
2.	n-Octanol : PBS pH (7.4)	1.68

Table 4: Important band frequencies in IR spectrum of Diltiazem HCl

S.No	IR Absorption band cm^{-1}	Assignments
1.	3426.3	-OH
2.	3006.0	Cyclic C-H, stretching
3.	2935	Ali- C-H, stretching
4.	2839.4	CH_2 symmetric stretching
5.	2574.7	S-H stretching
6.	1743.9	C=O
7.	1679.5	C=C
8.	1606.8	N-H bend
9.	1582.3 1510.8, 1496 and 1475.3	C-C ring stretching
10.	1411.1-1293.8	C-N stretching of aromatic amines
11.	1293.8	Asymmetric C-O-C stretching
12.	1218.0	C-C stretching

Table 5: Major Peaks observed in the spectrum

Serial No.	Sample Name	Major Peaks(cm^{-1})
1.	Drug (diltiazem)	3447,2931,1677,1511,1029,770
2.	Physical mixture of drug and polymer	3359,2960,1678,1513,1032,768

Table 6: Calibration curve of DTZ at λ_{max} 237nm.

Sl. No.	Conc. (mcg/)	Distilled Water		Phosphate buffer Saline (pH 7.4)		Simulated Gastric Fluid (pH 1.2)	
		Absorbance	Regressed Value	Absorbance	Regressed Value	Absorbance	Regressed Value
1	0	0.000	0.000	0.000	0.000	0.000	0.000
2	2	0.148±0.008	0.136	0.123±0.009	0.117	0.155±0.021	0.132
3	4	0.284±0.011	0.248	0.232±0.029	0.227	0.248±0.009	0.242
4	6	0.355±0.016	0.360	0.332±0.003	0.337	0.356±0.021	0.352
5	8	0.479±0.006	0.472	0.465±0.006	0.447	0.474±0.005	0.462
6	10	0.578±0.014	0.584	0.547±0.003	0.557	0.560±0.015	0.572

Table 7: Statistical Parameters related to standard curve of Diltiazem HCl at λ_{max} at 237nm:

S.No.	Absorption data	Parameters	Values
1.	Standard Curve in Water (pH 7.0)	Beer's Law Range	2-10 mcg/ml
		Regression Coefficient	0.990
		Regressed line equation(y = mx + c)	y = 0.056x+0.024
2.	Standard Curve in PBS (pH 7.4)	Beer's Law Range	2-10 mcg/ml
		Regression Coefficient	0.997
		Regressed line equation(y = mx + c)	y= 0.055x+0.007
3.	Standard Curve in SGF (pH 1.2)	Beer's Law Range	2-10 mcg/ml
		Regression Coefficient	0.996
		Regressed line equation (y = mx + c)	Y=0.055+0.022

Where y is the response, x is the concentration, m is the slope and c is the intercept of a best fit line.

Table 8: Effect of concentration of chitosan on particle size, PDI and size distribution.

Formulation code	Conc. of Chitosan (% w/v)	Average Size(d.nm)	PDI	Size Distribution
F1	0.05	423	0.604	7.1%(15-35nm) 84.4%(100-550nm) 8.5%(7000-8500nm)
F2	0.1	858.4	0.875	8.6%(10-25nm) 91.4%(80-300nm)
F3	0.15	151	0.398	21.3%(4-6nm) 78.7% (60-90nm)
F4	0.2	220.8	0.302	100%(600-1000nm)
F5	0.25	563.1	0.493	100%(400-700 nm)

Table 9: Effect of concentration of chitosan on Entrapment efficiency, Drug loading and Practical yield

Formulation code	Conc. of Chitosan (% w/v)	Entrapment efficiency (%)	Drug loading (%)	Practical Yield (%)
F1	0.05	28.55±1.03	75±1.43	37.80±2.12
F2	0.1	42.85±0.87	76±1.45	28.71±3.03
F3	0.15	52.7±2.01	72±2.01	32.16±2.31
F4	0.2	59.8±1.20	56±1.42	25.80±1.72
F5	0.25	34.6±3.01	50±2.76	23.97±2.61

Table 10: Effect of concentration of sodium TPP on particle size, PDI and Size distribution

Formulation code	Conc. of Na TPP (% w/v)	Average Size(d.nm)	PDI	Size Distribution
F6	0.025	462.7	0.196	7.1%(20-50nm) 84.4%(150-600nm) 8.5%(7000-8000nm)
F7	0.050	500.5	0.277	8.6%(10-25nm) 91.4%(90-200nm)
F8	0.150	547.6	0.300	21.3%(4-8 nm) 78.7%(70-100nm)
F9	0.200	482.1	0.189	100%(500-800nm)
F10	0.250	724.9	0.238	100%(250-550nm)

Table 11: Effect of concentration of sodium TPP on Entrapment efficiency, Drug loading and Practical yield

Formulation code	Conc. of TPP (% w/v)	Entrapment efficiency(%)	Drug loading(%)	Practical yield(%)
F6	0.025	85.7±1.25	82.61±2.45	52.54±1.85
F7	0.050	83.05±2.36	80.41±2.82	49.63±2.10
F8	0.150	79.9±2.89	75.39±3.78	38.34±2.45
F9	0.200	69.65±1.89	64.11±1.84	31.45±1.52
F10	0.250	63.4±3.04	63.38±2.85	30.75±1.24

Table12: Effect of amount of Pluronic F-68 on particle size, PDI and size distribution

Formulation Code	Pluronic F-68 (mg)	Average Size (d.nm)	PDI	Size Distribution
F11	50	549.1	0.506	100%(90-400nm)
F12	75	563.1	0.493	100%(200-550 nm)
F13	100	386.5	0.292	8.6%(10-25nm) 91.4%(90-200nm)
F14	200	322.9	0.21	93.6%(300-800nm)6.4%(7000-8500nm)
F15	250	402.7	0.659	100%(400-600nm)

Table 13: Effect of amount of Pluronic F-68 on Entrapment efficiency, Drug loading and Practical yield

Formulation code	Pluronic F-68 (mg)	Entrapment efficiency(%)	Drug loading(%)	Practical yield(%)
F11	50	84.8±3.45	76.61±1.87	53.72±1.64
F12	75	76.8±2.56	64.41±2.57	52.07±1.43
F13	100	74.1±1.96	62.39±2.91	43.08±2.84
F14	200	66.95±2.16	64.11±1.46	32.39±2.54
F15	250	63.4±1.89	43.38±2.57	28.75±3.64

Table 14: Effect of volume ratio of polymer and Na TPP on particle size, PDI and Size distribution

Formulation code	Volume ratio(CHN Solution: TPP solution)	Average Size(d.nm)	PDI	Size Distribution
F16	4:1(24ml:6ml)	1011	0.814	6%(8-12nm) 94%(300-600nm
F17	3:1(22.5ml:7.5ml)	524.8	0.718	13.7%(80-110nm) 86.3%(500-1100nm)
F18	1:1(15ml:15ml)	697	0.604	100%(300-700nm)
F19	1:2(10ml:20ml)	469.9	0.604	9.6%(20-60nm) 90.4%(200-500nm)
F20	1:3(7.5ml:22.5ml)	286.6	0.5	7.5%(20-45nm) 92.5%(300-600nm)

Table 15: Effect of volume ratio of polymer and Na TPP on Entrapment efficiency, Drug loading and Practical yield

Formulation code	Volume ratio(CHN Solution:TPP solution)	Entrapment efficiency (%)	Drug loading (%)	Practical yield (%)
F16	4:1(24ml:6ml)	86.6±1.98	87.33±1.45	30.84±3.54
F17	3:1(22.5ml:7.5ml)	83.95±2.45	88.51±3.07	29.42±2.74
F18	1:1(15ml:15ml)	81.25±1.62	73±1.79	35.82±2.41
F19	1:2(10ml:20ml)	74.1±3.45	71.16±2.45	32.30±3.47
F20	1:3(7.5ml:22.5ml)	67.85±2.58	63.16±1.54	33.32±2.48

Table 16: Effect of stirring speed on particle size, PDI and Size distribution

Formulation code	Stirring Speed	Average Size(d.nm)	PDI	Size Distribution
F21	700 rpm	446.2	0.53	86.9%(100-550nm) 13.1%(1000-2500nm)
F22	800 rpm	764.5	0.738	14.3%(90-120nm) 85.7%(600-1150nm)
F23	900 rpm	608.6	0.45	8.9%(100- 250nm) 91.1%(500-850nm)
F24	1000 rpm	508.9	0.367	100%(400-650nm)
F25	1100 rpm	722.9	0.651	5.3%(15-45nm) 94.7%(350-600nm)

Table 17: Effect of stirring speed on Entrapment efficiency, Drug loading and Practical yield

Formulation code	Stirring Speed	Entrapment efficiency (%)	Drug loading (%)	Practical yield (%)
F21	700 rpm	86.6±2.53	82.16±2.74	32.68±1.53
F22	800 rpm	84.8±1.51	84.61±2.74	31.08±2.45
F23	900 rpm	81.25±3.45	83.44±2.85	30.20±3.14
F24	1000 rpm	89.3±1.23	80.21±3.47	31.71±1.43
F25	1100 rpm	84.8±1.92	82.95±2.54	29.40±4.68

Drug Release study

Table 18: *In vitro* drug release data of formulation F1

S. No.	Time (hr)	Square Root of Time	Log Time	Cumulative % Drug Release	Log Cumulative % Drug Release	Cumulative % Drug Remaining	Log Cumulative % Drug Remaining
1	0.5	0.7071	-0.3010	14.52	1.16197	85.48	1.93186
2	1.0	1.0000	0.0000	24.65	1.39182	75.35	1.87708
3	2.0	1.4142	0.30103	39.63	1.59802	60.37	1.78082
4	3.0	1.7320	0.47712	55.53	1.74453	44.47	1.64807
5	4.0	2.0000	0.60206	73.09	1.86386	26.91	1.42991
6	5.0	2.2360	0.69897	89.06	1.94968	10.94	1.03902
7	6.0	2.4494	0.77815	100.32	2.00139		
8	12.0	3.4641	1.07918	99.42	1.99747		
9	24.0	4.8989	1.38021	99.31	1.99699		

Table 19: *In vitro* drug release data of formulation F2

S. No.	Time (hr)	Square Root of Time	Log Time	Cumulative % Drug Release	Log Cumulative % Drug Release	Cumulative % Drug Remaining	Log Cumulative % Drug Remaining
1	0.5	0.7071	-0.3010	10.54	1.02284	89.46	1.95163
2	1.0	1.0000	0.0000	23.39	1.36903	76.61	1.88429
3	2.0	1.4142	0.30103	34.90	1.54283	65.1	1.81358
4	3.0	1.7320	0.47712	51.68	1.71332	48.32	1.68413
5	4.0	2.0000	0.60206	69.79	1.84379	30.21	1.48015
6	5.0	2.2360	0.69897	84.98	1.92932	15.02	1.17667
7	6.0	2.4494	0.77815	99.34	1.99712	0.66	-0.18046
8	12.0	3.4641	1.07918	100.2	2.00087		
9	24.0	4.8989	1.38021	100.12	2.00052		

Table 20: *In vitro* drug release data of formulation F3

S. No.	Time (hr)	Square Root of Time	Log Time	Cumulative % Drug Release	Log Cumulative % Drug Release	Cumulative % Drug Remaining	Log Cumulative % Drug Remaining
1	0.5	0.7071	-0.3010	4.16	0.61909	95.84	1.98155
2	1.0	1.0000	0.0000	11.69	1.06781	88.31	1.94601
3	2.0	1.4142	0.30103	20.57	1.31323	79.43	1.89998
4	3.0	1.7320	0.47712	31.43	1.49734	68.57	1.83613
5	4.0	2.0000	0.60206	47.84	1.67979	52.16	1.71734
6	5.0	2.2360	0.69897	64.34	1.80848	35.66	1.55218
7	6.0	2.4494	0.77815	71.76	1.85588	28.24	1.45086
8	12.0	3.4641	1.07918	90.87	1.95842	9.13	0.96047
9	24.0	4.8989	1.38021	99.89	1.99952	0.11	-0.95861

Table 21: *In vitro* drug release data of formulation F4

S. No.	Time (hr)	Square Root of Time	Log Time	Cumulative % Drug Release	Log Cumulative % Drug Release	Cumulative % Drug Remaining	Log Cumulative % Drug Remaining
1	0.5	0.7071	-0.3010	4.98	0.69723	95.02	1.97782
2	1.0	1.0000	0.0000	14.76	1.16909	85.24	1.93064
3	2.0	1.4142	0.30103	24.98	1.39759	75.02	1.87518
4	3.0	1.7320	0.47712	32.89	1.51706	67.11	1.82679
5	4.0	2.0000	0.60206	41.89	1.62211	58.11	1.76425
6	5.0	2.2360	0.69897	57.89	1.7626	42.11	1.62439
7	6.0	2.4494	0.77815	62.78	1.79782	37.22	1.57078
8	12.0	3.4641	1.07918	80.78	1.9073	19.22	1.28375
9	24.0	4.8989	1.38021	100.0	2	0	

Table 22: *In vitro* drug release data of formulation F5

S. No.	Time (hr)	Square Root of Time	Log Time	Cumulative % Drug Release	Log Cumulative % Drug Release	Cumulative % Drug Remaining	Log Cumulative % Drug Remaining
1	0.5	0.7071	-0.3010	3.98	0.59988	96.02	1.98236
2	1.0	1.0000	0.0000	12.08	1.08207	87.92	1.94409
3	2.0	1.4142	0.30103	22.98	1.36135	77.02	1.8866
4	3.0	1.7320	0.47712	33.83	1.5293	66.17	1.82066
5	4.0	2.0000	0.60206	44.65	1.64982	55.35	1.74312
6	5.0	2.2360	0.69897	52.34	1.71883	47.66	1.67815
7	6.0	2.4494	0.77815	55.98	1.74803	44.02	1.64365
8	12.0	3.4641	1.07918	74.90	1.87448	25.1	1.87448
9	24.0	4.8989	1.38021	97.89	1.99074	2.11	0.32428

Table 23: *In vitro* drug release data of formulation F6

S. No.	Time (hr)	Square Root of Time	Log Time	Cumulative % Drug Release	Log Cumulative % Drug Release	Cumulative % Drug Remaining	Log Cumulative % Drug Remaining
1	0.5	0.7071	-0.3010	7.90	0.89763	92.1	1.96426
2	1.0	1.0000	0.0000	20.87	1.31952	79.13	1.89834
3	2.0	1.4142	0.30103	29.45	1.46909	70.55	1.8485
4	3.0	1.7320	0.47712	48.90	1.68931	51.1	1.70842
5	4.0	2.0000	0.60206	59.36	1.77349	40.64	1.60895
6	5.0	2.2360	0.69897	74.45	1.87186	25.55	1.40739
7	6.0	2.4494	0.77815	83.56	1.922	16.44	1.2159
8	12.0	3.4641	1.07918	100.45	2.00195		
9	24.0	4.8989	1.38021	100.14	2.00061		

Table 24: *In vitro* drug release data of formulation F7

S. No.	Time (hr)	Square Root of Time	Log Time	Cumulative % Drug Release	Log Cumulative % Drug Release	Cumulative % Drug Remaining	Log Cumulative % Drug Remaining
1	0.5	0.7071	-0.3010	6.44	0.80889	93.56	1.97109
2	1.0	1.0000	0.0000	17.59	1.24527	82.41	1.91598
3	2.0	1.4142	0.30103	25.78	1.41128	74.22	1.87052
4	3.0	1.7320	0.47712	39.32	1.59461	60.68	1.78305
5	4.0	2.0000	0.60206	52.96	1.72395	47.04	1.67247
6	5.0	2.2360	0.69897	71.82	1.85625	28.18	1.44994
7	6.0	2.4494	0.77815	78.98	1.89752	21.02	1.32263
8	12.0	3.4641	1.07918	100.89	2.00385		
9	24.0	4.8989	1.38021	101.04	2.00449		

Table 25: *In vitro* drug release data of formulation F8

S. No.	Time (hr)	Square Root of Time	Log Time	Cumulative % Drug Release	Log Cumulative % Drug Release	Cumulative % Drug Remaining	Log Cumulative % Drug Remaining
1	0.5	0.7071	-0.3010	8.67	0.93802	91.33	1.96061
2	1.0	1.0000	0.0000	15.01	1.17638	84.99	1.92937
3	2.0	1.4142	0.30103	24.34	1.38632	75.66	1.87887
4	3.0	1.7320	0.47712	36.54	1.56277	63.46	1.8025
5	4.0	2.0000	0.60206	51.56	1.71231	48.44	1.6852
6	5.0	2.2360	0.69897	68.34	1.83467	31.66	1.50051
7	6.0	2.4494	0.77815	73.45	1.86599	26.55	1.42406
8	12.0	3.4641	1.07918	94.01	1.97317	5.99	0.77743
9	24.0	4.8989	1.38021	99.83	1.99926	0.17	-0.76955

Table 26: *In vitro* drug release data of formulation F9

S. No.	Time (hr)	Square Root of Time	Log Time	Cumulative % Drug Release	Log Cumulative % Drug Release	Cumulative % Drug Remaining	Log Cumulative % Drug Remaining
1	0.5	0.7071	-0.3010	5.56	0.74507	94.44	1.97516
2	1.0	1.0000	0.0000	13.34	1.12516	86.66	1.93782
3	2.0	1.4142	0.30103	21.56	1.33365	78.44	1.89454
4	3.0	1.7320	0.47712	33.98	1.53122	66.02	1.81968
5	4.0	2.0000	0.60206	45.86	1.66143	54.14	1.73352
6	5.0	2.2360	0.69897	52.76	1.7223	47.24	1.67431
7	6.0	2.4494	0.77815	67.96	1.83225	32.04	1.50569
8	12.0	3.4641	1.07918	89.78	1.95318	10.22	1.00945
9	24.0	4.8989	1.38021	100.10	2.00043		

Table 27: *In vitro* drug release data of formulation F10

S. No.	Time (hr)	Square Root of Time	Log Time	Cumulative % Drug Release	Log Cumulative % Drug Release	Cumulative % Drug Remaining	Log Cumulative % Drug Remaining
1	0.5	0.7071	-0.3010	3.78	0.57749	96.22	1.98327
2	1.0	1.0000	0.0000	10.70	1.02938	89.3	1.95085
3	2.0	1.4142	0.30103	18.69	1.27161	81.31	1.91014
4	3.0	1.7320	0.47712	30.86	1.4894	69.14	1.83973
5	4.0	2.0000	0.60206	37.65	1.57576	62.35	1.79484
6	5.0	2.2360	0.69897	53.92	1.73175	46.08	1.66351
7	6.0	2.4494	0.77815	62.43	1.79539	37.57	1.57484
8	12.0	3.4641	1.07918	92.43	1.96581	7.57	0.8791
9	24.0	4.8989	1.38021	99.56	1.99808	0.44	-0.35655

Table 28: *In vitro* drug release data of formulation F11

S. No.	Time (hr)	Square Root of Time	Log Time	Cumulative % Drug Release	Log Cumulative % Drug Release	Cumulative % Drug Remaining	Log Cumulative % Drug Remaining
1	0.5	0.7071	-0.3010	5.91	0.77159	94.09	1.97354
2	1.0	1.0000	0.0000	14.56	1.16316	85.44	1.93166
3	2.0	1.4142	0.30103	23.90	1.3784	76.1	1.88138
4	3.0	1.7320	0.47712	34.89	1.5427	65.11	1.81365
5	4.0	2.0000	0.60206	45.87	1.66153	54.13	1.73344
6	5.0	2.2360	0.69897	54.53	1.73664	45.47	1.65772
7	6.0	2.4494	0.77815	67.23	1.82756	32.77	1.51548
8	12.0	3.4641	1.07918	89.78	1.95318	10.22	1.00945

Table 29: *In vitro* drug release data of formulation F12

S. No.	Time (hr)	Square Root of Time	Log Time	Cumulative % Drug Release	Log Cumulative % Drug Release	Cumulative % Drug Remaining	Log Cumulative % Drug Remaining
1	0.5	0.7071	-0.3010	6.66	0.82347	93.34	1.97007
2	1.0	1.0000	0.0000	16.56	1.21906	83.44	1.92137
3	2.0	1.4142	0.30103	26.23	1.4188	73.77	1.86788
4	3.0	1.7320	0.47712	38.56	1.58614	61.44	1.78845
5	4.0	2.0000	0.60206	49.46	1.69425	50.54	1.70364
6	5.0	2.2360	0.69897	61.23	1.78696	38.77	1.5885
7	6.0	2.4494	0.77815	69.57	1.84242	30.43	1.4833
8	12.0	3.4641	1.07918	91.67	1.96223	8.33	0.92065
9	24.0	4.8989	1.38021	99.96	1.99983	0.04	-1.39794

Table 30: *In vitro* drug release data of formulation F13

S. No.	Time (hr)	Square Root of Time	Log Time	Cumulative % Drug Release	Log Cumulative % Drug Release	Cumulative % Drug Remaining	Log Cumulative % Drug Remaining
1	0.5	0.7071	-0.3010	8.98	0.95328	91.02	1.95914
2	1.0	1.0000	0.0000	16.34	1.21325	83.66	1.92252
3	2.0	1.4142	0.30103	29.45	1.46909	70.55	1.8485
4	3.0	1.7320	0.47712	41.57	1.61878	58.43	1.76664
5	4.0	2.0000	0.60206	53.39	1.72746	46.61	1.66848
6	5.0	2.2360	0.69897	62.87	1.79844	37.13	1.56972
7	6.0	2.4494	0.77815	77.34	1.8884	22.66	1.35526
8	12.0	3.4641	1.07918	98.98	1.99555	1.02	0.0086
9	24.0	4.8989	1.38021	99.78	1.99904	0.22	-0.65758

Table 31: *In vitro* drug release data of formulation F14

S. No.	Time (hr)	Square Root of Time	Log Time	Cumulative % Drug Release	Log Cumulative % Drug Release	Cumulative % Drug Remaining	Log Cumulative % Drug Remaining
1	0.5	0.7071	-0.3010	7.09	0.85065	92.91	1.96806
2	1.0	1.0000	0.0000	14.98	1.17551	85.02	1.92952
3	2.0	1.4142	0.30103	29.54	1.47041	70.46	1.84794
4	3.0	1.7320	0.47712	39.67	1.59846	60.33	1.78053
5	4.0	2.0000	0.60206	58.87	1.76989	41.13	1.61416
6	5.0	2.2360	0.69897	68.98	1.83872	31.02	1.49164
7	6.0	2.4494	0.77815	79.51	1.90042	20.49	1.31154
8	12.0	3.4641	1.07918	99.65	1.99848	0.35	-0.45593
9	24.0	4.8989	1.38021	99.99	1.99996	0.01	-2

Table 32: *In vitro* drug release data of formulation F15

S. No.	Time (hr)	Square Root of Time	Log Time	Cumulative % Drug Release	Log Cumulative % Drug Release	Cumulative % Drug Remaining	Log Cumulative % Drug Remaining
1	0.5	0.7071	-0.3010	9.45	0.97543	90.55	1.95689
2	1.0	1.0000	0.0000	21.08	1.32387	78.92	1.89719
3	2.0	1.4142	0.30103	35.43	1.54937	64.57	1.81003
4	3.0	1.7320	0.47712	47.98	1.68106	52.02	1.71617
5	4.0	2.0000	0.60206	64.43	1.80909	35.57	1.55108
6	5.0	2.2360	0.69897	76.89	1.88587	23.11	1.3638
7	6.0	2.4494	0.77815	89.34	1.95105	10.66	1.02776
8	12.0	3.4641	1.07918	100.18	2.00078		
9	24.0	4.8989	1.38021	100.04	2.00017		

Table 33: *In vitro* drug release data of formulation F21

S. No.	Time (hr)	Square Root of Time	Log Time	Cumulative % Drug Release	Log Cumulative % Drug Release	Cumulative % Drug Remaining	Log Cumulative % Drug Remaining
1	0.5	0.7071	-0.3010	8.78	0.94349	91.22	1.96009
2	1.0	1.0000	0.0000	14.90	1.17319	85.1	1.92993
3	2.0	1.4142	0.30103	25.65	1.40909	74.35	1.87128
4	3.0	1.7320	0.47712	37.67	1.576	62.33	1.7947
5	4.0	2.0000	0.60206	46.34	1.66596	53.66	1.72965
6	5.0	2.2360	0.69897	62.45	1.79553	37.55	1.57461
7	6.0	2.4494	0.77815	73.23	1.86469	26.77	1.42765
8	12.0	3.4641	1.07918	98.61	1.99392	1.39	0.14301
9	24.0	4.8989	1.38021	100.09	2.00039	-0.09	

Table 34: *In vitro* drug release data of formulation F22

S. No.	Time (hr)	Square Root of Time	Log Time	Cumulative % Drug Release	Log Cumulative % Drug Release	Cumulative % Drug Remaining	Log Cumulative % Drug Remaining
1	0.5	0.7071	-0.3010	5.67	0.75358	94.33	1.97465
2	1.0	1.0000	0.0000	10.47	1.01995	89.53	1.95197
3	2.0	1.4142	0.30103	28.55	1.45561	71.45	1.854
4	3.0	1.7320	0.47712	38.52	1.58569	61.48	1.78873
5	4.0	2.0000	0.60206	48.51	1.68583	51.49	1.71172
6	5.0	2.2360	0.69897	59.45	1.77415	40.55	1.60799
7	6.0	2.4494	0.77815	71.84	1.85637	28.16	1.44963
8	12.0	3.4641	1.07918	99.34	1.99712	0.66	-0.18046
9	24.0	4.8989	1.38021	100.05	2.00022		

Table 35: *In vitro* drug release data of formulation F23

S. No.	Time (hr)	Square Root of Time	Log Time	Cumulative % Drug Release	Log Cumulative % Drug Release	Cumulative % Drug Remaining	Log Cumulative % Drug Remaining
1	0.5	0.7071	-0.3010	7.67	0.8848	92.33	1.96534
2	1.0	1.0000	0.0000	16.97	1.22968	83.03	1.91924
3	2.0	1.4142	0.30103	24.43	1.38792	75.57	1.87835
4	3.0	1.7320	0.47712	34.76	1.54108	65.24	1.81451
5	4.0	2.0000	0.60206	45.57	1.65868	54.43	1.73584
6	5.0	2.2360	0.69897	59.45	1.77415	40.55	1.60799
7	6.0	2.4494	0.77815	67.43	1.82885	32.57	1.51282
8	12.0	3.4641	1.07918	99.45	1.9976	0.55	-0.25964
9	24.0	4.8989	1.38021	99.76	1.99896	0.24	-0.61979

Table 36: *In vitro* drug release data of formulation F24

S. No.	Time (Hrs)	Square Root of Time	Log Time	Cumulative Percentage Drug Release	Log Cumulative Percentage Drug Release	Cumulative Percent Drug Remaining	Log Cumulative Percent Drug Remaining
1	0.5	0.7071	-0.3010	8.45	0.92686	91.55	1.96166
2	1.0	1.0000	0.0000	16.45	1.21617	83.55	1.92195
3	2.0	1.4142	0.30103	26.45	1.42243	73.55	1.86658
4	3.0	1.7320	0.47712	39.57	1.59737	60.43	1.78125
5	4.0	2.0000	0.60206	39.58	1.59748	60.42	1.78118
6	5.0	2.2360	0.69897	52.56	1.72066	47.44	1.67614
7	6.0	2.4494	0.77815	63.65	1.8038	36.35	1.5605
8	12.0	3.4641	1.07918	98.44	1.99317	1.56	0.19312
9	24.0	4.8989	1.38021	99.56	1.99808	0.44	-0.35655

Table 37: *In vitro* drug release data of formulation F25

S. No.	Time (hr)	Square Root of Time	Log Time	Cumulative % Drug Release	Log Cumulative % Drug Release	Cumulative % Drug Remaining	Log Cumulative % Drug Remaining
1	0.5	0.7071	-0.3010	6.21	0.79309	93.79	1.97216
2	1.0	1.0000	0.0000	12.74	1.10517	87.26	1.94082
3	2.0	1.4142	0.30103	20.45	1.31069	79.55	1.90064
4	3.0	1.7320	0.47712	32.56	1.51268	67.44	1.82892
5	4.0	2.0000	0.60206	43.61	1.63959	56.39	1.7512
6	5.0	2.2360	0.69897	56.52	1.7522	43.48	1.63829
7	6.0	2.4494	0.77815	65.34	1.81518	34.66	1.53983
8	12.0	3.4641	1.07918	99.45	1.9976	0.55	-0.25964
9	24.0	4.8989	1.38021	100.05	2.00022		

Table 38: Regression Analysis Data of Formulations of diltiazem

Formulation code	Zero Order	First Order	Higuchi's Equation	Peppas Equation
F1	y = 16.461x + 5.3602 R² = 0.9934	y = -0.1849x + 2.0955 R² = 0.9226	y = 21.661x + 18.271 R² = 0.6916	y = 0.4592x + 1.5128 R² = 0.7836
F2	y = 16.338x + 2.9194 R² = 0.9965	y = -0.0616x + 1.9898 R² = 0.9778	y = 22.895x + 13.255 R² = 0.7183	y = 0.4935x + 1.4716 R² = 0.8035
F3	y = 12.434x - 1.9413 R² = 0.9916	y = -0.0924x + 2.0549 R² = 0.98	y = 25.13x - 6.3979 R² = 0.8858	y = 0.7193x + 1.1696 R² = 0.8909
F4	y = 10.58x + 1.5864 R² = 0.9897	y = -0.0616x + 1.9898 R² = 0.9778	y = 23.458x - 5.1009 R² = 0.9478	y = 0.555x - 6.0265 R² = 0.9651
F5	y = 9.8825x + 1.6708 R² = 0.9829	y = -0.062x + 2.0512 R² = 0.8119	y = 22.755x - 6.0265 R² = 0.9651	y = 0.6525x + 1.1857 R² = 0.9401
F6	y = 8.73x + 14.721 R² = 0.857	y = -0.1315x + 2.0686 R² = 0.9679	y = 23.729x + 5.8701 R² = 0.8177	y = 0.5383x + 1.3983 R² = 0.8654
F7	y = 8.8652x + 10.755 R² = 0.8946	y = -0.1168x + 2.0711 R² = 0.9542	y = 24.978x - 0.2548 R² = 0.8463	y = 0.608x + 1.3131 R² = 0.8845
F8	y = 4.115x + 23.514 R² = 0.7076	y = -0.1061x + 2.0639 R² = 0.9901	y = 24.183x - 1.0597 R² = 0.8738	y = 0.6391x + 1.2641 R² = 0.8919
F9	y = 4.1993x + 18.944 R² = 0.7814	y = -0.0854x + 2.0516 R² = 0.9898	y = 24.56x - 6.4318 R² = 0.9264	y = 0.6751x + 1.1963 R² = 0.9296
F10	y = 4.1993x + 18.944 R² = 0.7814	y = -0.0964x + 2.1033 R² = 0.9738	y = 25.448x - 10.715 R² = 0.9265	y = 0.7507x + 1.1083 R² = 0.9308
F11	y = 4.132x + 19.853	y = -0.0848x + 2.0444	y = 24.102x - 4.838	y = 0.6402x + 1.2321

	$R^2 = 0.7768$	$R^2 = 0.9926$	$R^2 = 0.9273$	$R^2 = 0.9365$
F12	y = 4.0865x + 22.492 $R^2 = 0.745$	y = -0.0921x + 2.0411 $R^2 = 0.9959$	y = 23.845x - 1.6278 $R^2 = 0.9105$	y = 0.5996x + 1.2903 $R^2 = 0.9261$
F13	y = 8.4709x + 11.683 $R^2 = 0.9099$	y = -0.1686x + 2.2182 $R^2 = 0.9437$	y = 24.03x + 1.1621 $R^2 = 0.8709$	y = 0.5967x + 1.3172 $R^2 = 0.901$
F14	y = 8.7497x + 11.686 $R^2 = 0.8843$	y = -0.1169x + 2.0653 $R^2 = 0.9729$	y = 24.493x + 1.2032 $R^2 = 0.8423$	y = 0.604x + 1.297 $R^2 = 0.899$
F15	y = 8.7323x + 16.916 $R^2 = 0.8272$	y = -0.1564x + 2.0979 $R^2 = 0.939$	y = 23.199x + 9.236 $R^2 = 0.7892$	y = 0.5155x + 1.4325 $R^2 = 0.8512$

Formulation code	Zero Order	First Order	Higuchi's Equation	Peppas Equation
F16	y = 4.1842x + 17.605 $R^2 = 0.7856$	y = -0.0826x + 2.0589 $R^2 = 0.9984$	y = 24.62x - 8.1488 $R^2 = 0.9311$	y = 0.7432x + 1.1254 $R^2 = 0.9087$
F17	y = 8.5247x + 11.021 $R^2 = 0.9022$	y = -0.0939x + 1.9684 $R^2 = 0.8813$	y = 24.228x + 0.1774 $R^2 = 0.8651$	y = 0.611x + 1.3034 $R^2 = 0.8887$
F18	y = 8.4784x + 9.2449 $R^2 = 0.9452$	y = -0.0822x + 2.0172 $R^2 = 0.9863$	y = 24.748x - 2.7367 $R^2 = 0.898$	y = 0.6268x + 1.2739 $R^2 = 0.9173$
F19	y = 8.1726x + 7.0508 $R^2 = 0.9383$	y = -0.1044x + 2.099 $R^2 = 0.9784$	y = 24.97x - 6.6579 $R^2 = 0.9101$	y = 0.7369x + 1.1336 $R^2 = 0.868$
F20	y = 8.1726x + 7.0508 $R^2 = 0.9383$	y = -0.0851x + 2.0597 $R^2 = 0.9901$	y = 24.97x - 6.6579 $R^2 = 0.9101$	y = 0.7053x + 1.1611 $R^2 = 0.929$
F21	y = 8.4619x + 9.3507 $R^2 = 0.9321$	y = -0.1574x + 2.2128 $R^2 = 0.9395$	y = 24.609x - 2.4499 $R^2 = 0.886$	y = 0.6408x + 1.259 $R^2 = 0.9128$
F22	y = 8.6077x + 8.2214 $R^2 = 0.9341$	y = -0.0912x + 2.0428 $R^2 = 0.9784$	y = 25.148x - 4.2325 $R^2 = 0.8877$	y = 0.7029x + 1.2047 $R^2 = 0.8782$
F23	y = 8.3881x + 8.3033 $R^2 = 0.9557$	y = -0.0804x + 2.0233 $R^2 = 0.9743$	y = 24.639x - 3.8748 $R^2 = 0.9$	y = 0.6201x + 1.265 $R^2 = 0.931$
F24	y = 8.0671x + 8.3226 $R^2 = 0.9668$	y = -0.1495x + 2.2186 $R^2 = 0.8998$	y = 24.19x - 4.0781 $R^2 = 0.9161$	y = 0.6068x + 1.2654 $R^2 = 0.9458$
F25	y = 8.5293x + 5.6831 $R^2 = 0.9661$	y = -0.0779x + 2.0351 $R^2 = 0.9767$	y = 25.541x - 7.932 $R^2 = 0.9041$	y = 0.7085x + 1.1708 $R^2 = 0.9248$

Table 39: Mucoadhesive strength of formulations

Formulation Code	Mucoadhesive Strength(g)	Formulation Code	Mucoadhesive Strength(g)
F1	4.56	**F14**	6.98
F2	5.48	**F15**	7.06
F3	6.42	**F16**	5.26
F4	6.08	**F17**	6.45
F5	5.36	**F18**	4.95
F6	3.59	**F19**	6.23
F7	4.18	**F20**	6.83
F8	7.51	**F21**	6.18
F9	6.56	**F22**	7.15
F10	5.62	**F23**	6.45
F11	6.24	**F24**	7.42
F12	5.65	**F25**	7.82
F13	6.48		

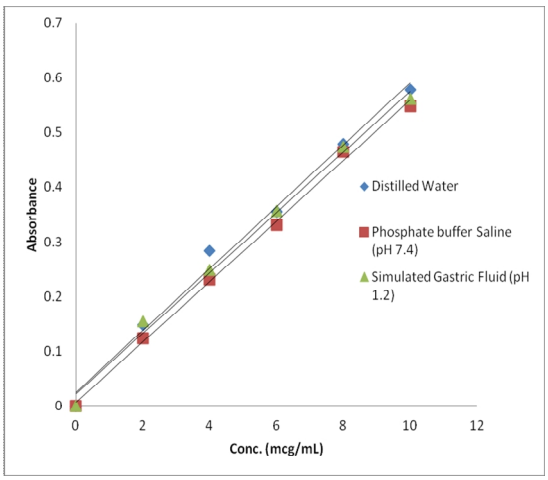

Fig. 1: Calibration curve of DTZ at λ_{max} 237nm with distilled water, PBS and SGF.

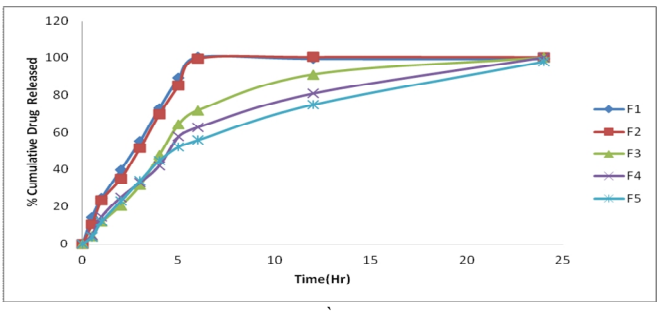

Fig. 2: Cumulative Percent Drug Released Vs Time Plots

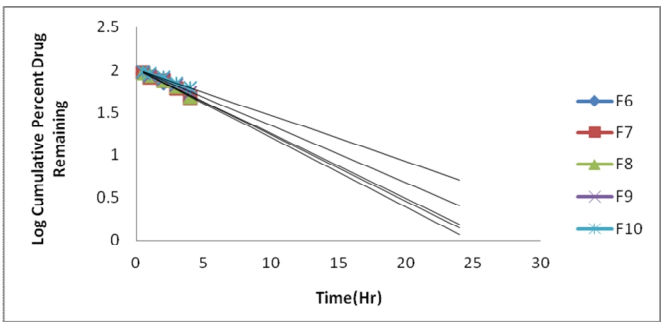

Fig. 3: Log cumulative percent drug remaining versus time. (first-order kinetic model)

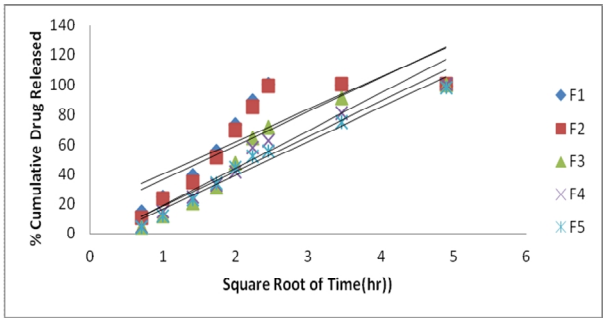

Fig. 4: Cumulative Percent Drug Released Vs Square Root of Time (Higuchi's Plots) of Formulations

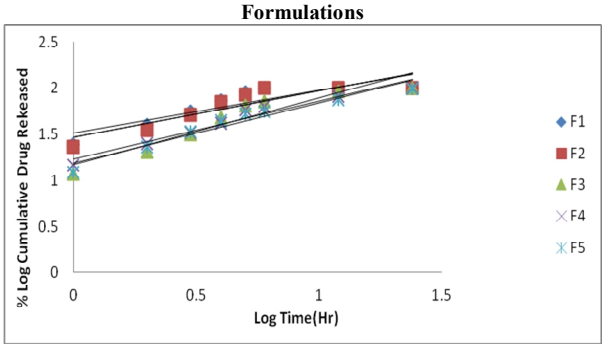

Fig. 5: Log Cumulative Percent Drug Released Vs Log Time (Peppas Plots) of Formulations

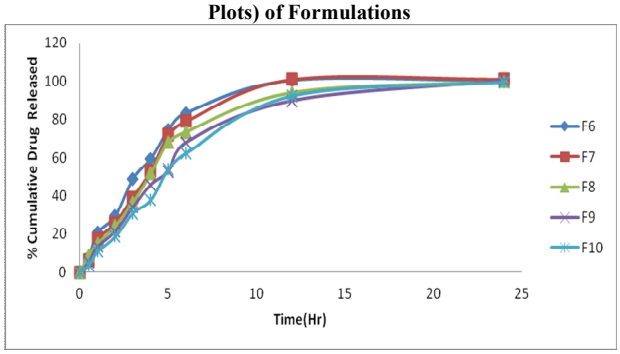

Fig. 6: Cumulative Percent Drug Released Vs Time Plots

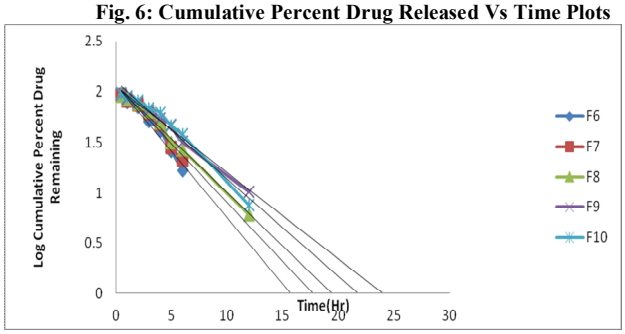

Fig.7: Log cumulative percent drug remaining versus time. (first-order kinetic model).

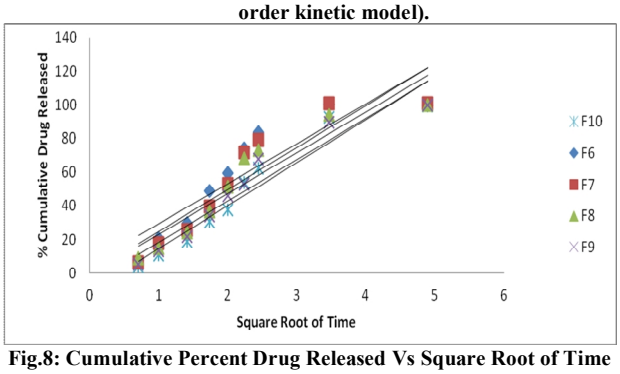

Fig.8: Cumulative Percent Drug Released Vs Square Root of Time (Higuchi's Plots) of Formulations

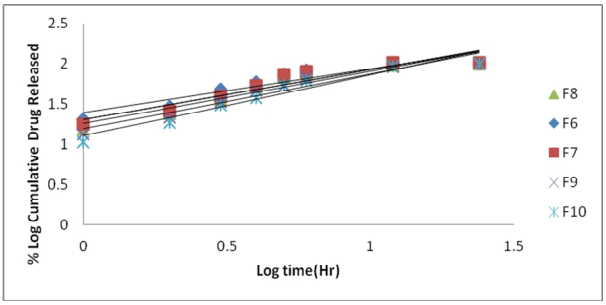

Fig. 9: Log Cumulative Percent Drug Released Vs Log Time (Peppas Plots) of Formulations

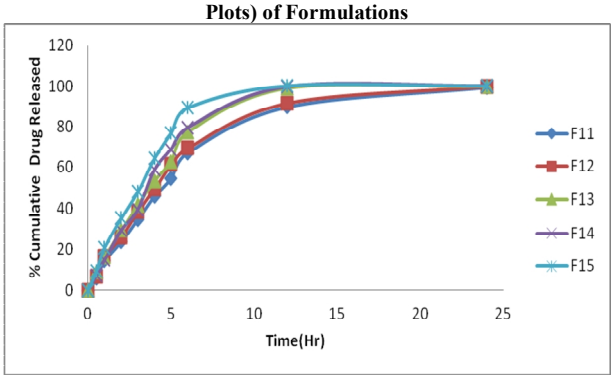

Fig. 10: Cumulative Percent Drug Released Vs Time Plots

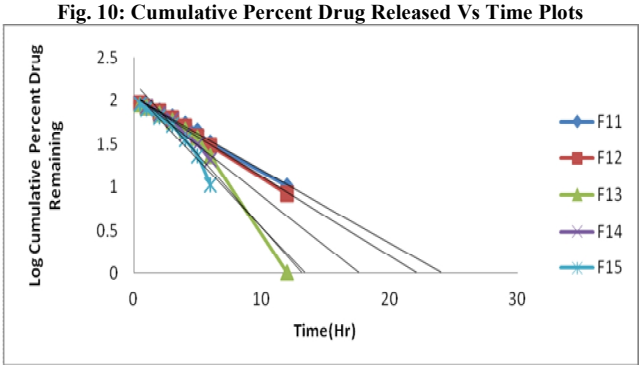

Fig. 11: Log cumulative percent drug remaining versus time. (first-order kinetic model).

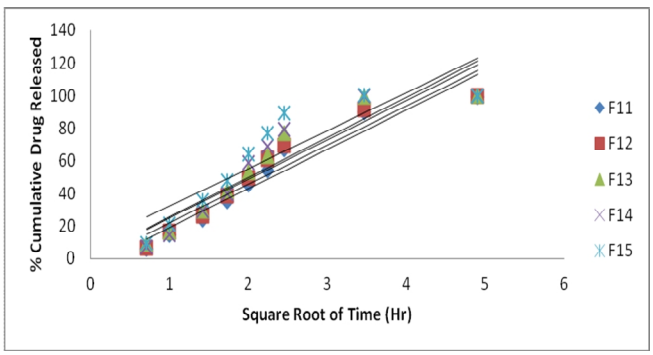

Fig. 12: Cumulative Percent Drug Released Vs Square Root of Time (Higuchi's Plots) of Formulations

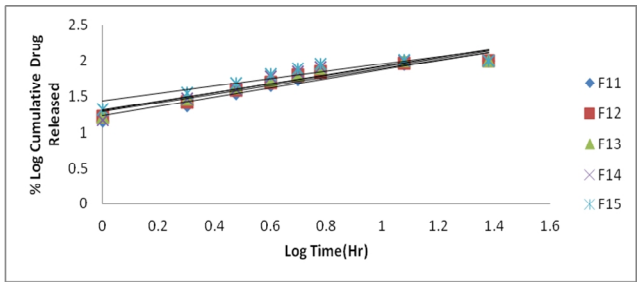

Fig. 13: Log Cumulative Percent Drug Released Vs Log Time (Peppas Plots) of Formulation

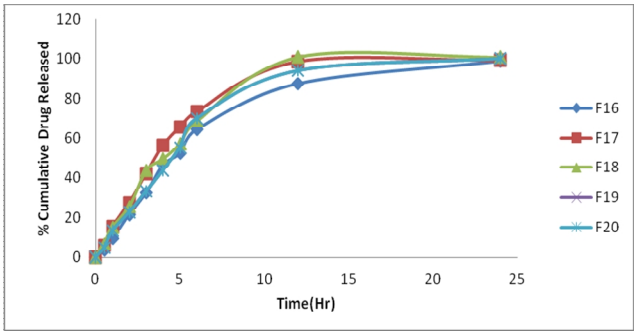

Fig. 14: Cumulative Percent Drug Released Vs Time Plots

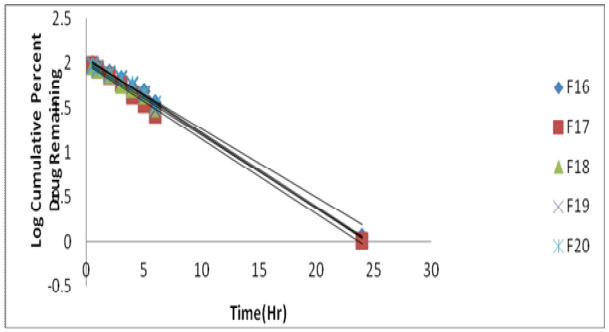

Fig.15: Log cumulative percent drug remaining versus time. (first-order kinetic model)

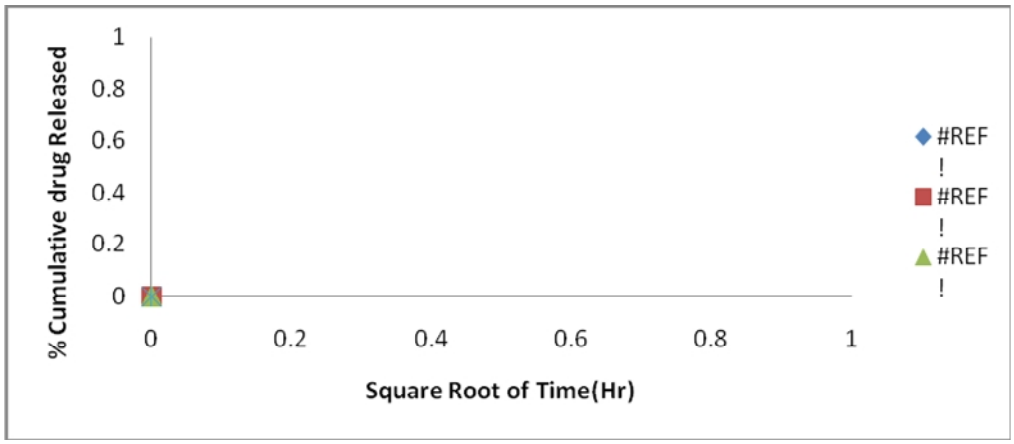

Fig. 16: Cumulative Percent Drug Released Vs Square Root of Time (Higuchi's Plots) Formulations

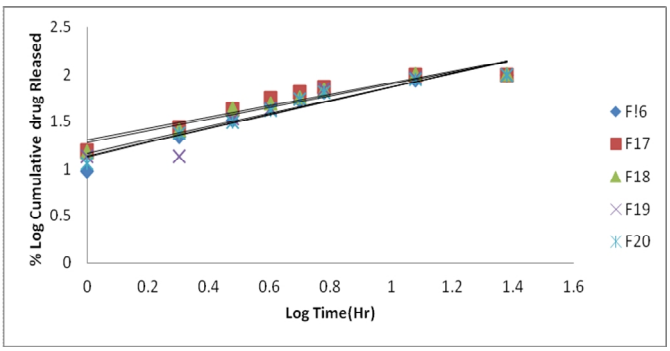

Fig. 17: Log Cumulative Percent Drug Released Vs Log Time (Peppas Plots) of Formulations

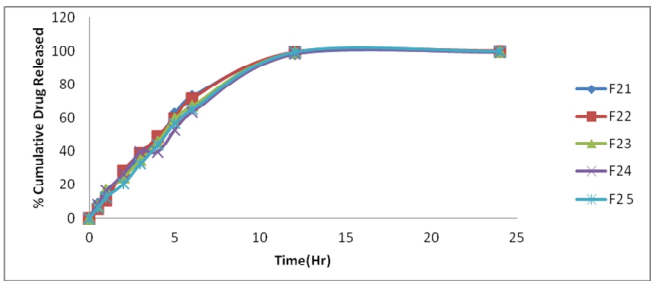

Fig. 18: Cumulative Percent Drug Released Vs Time Plot

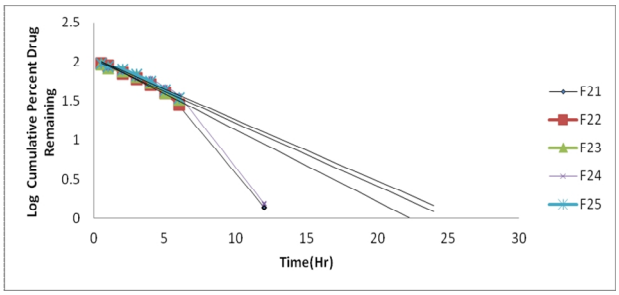

Fig.19: Log cumulative percent drug remaining versus time. (first-order kinetic model)

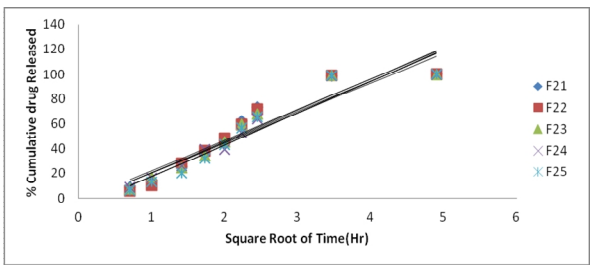

Fig. 20: Cumulative Percent Drug Released Vs Square Root of Time (Higuchi's Plots) of Formulations

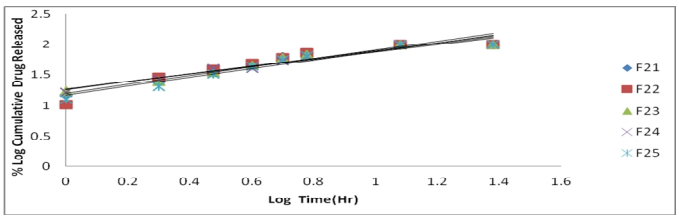

Fig. 21: Log Cumulative Percent Drug Released Vs Log Time (Peppas Plots) of Formulation

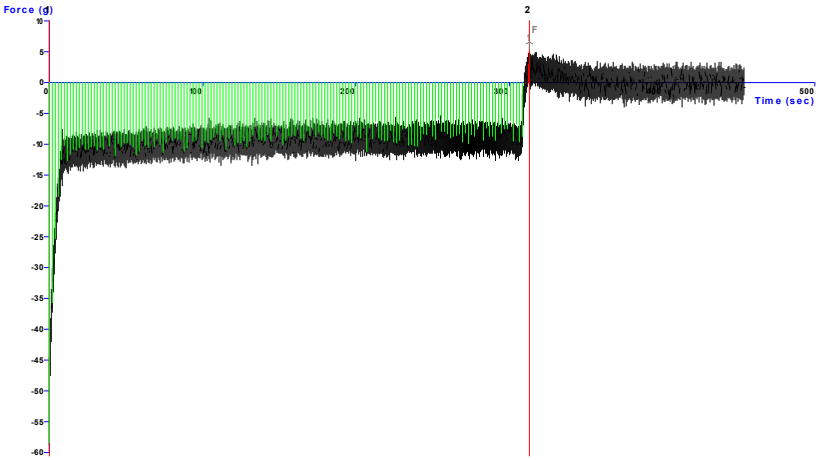

Fig. 22: Graphical representation of determination of Mucoadhesion strength

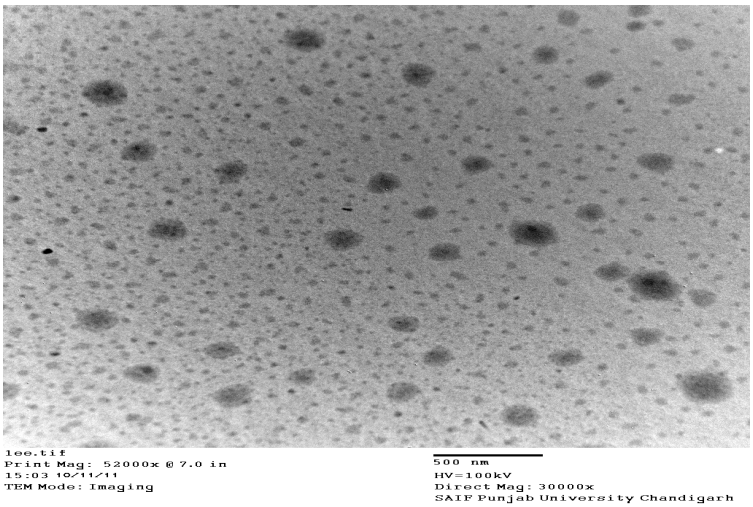

Fig. 23: TEM of an Optimized Formulation F24

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