INTERNATIONAL JOURNAL
OF
PHARMACEUTICAL SCIENCES
AND
RESEARCH

FORMULATION AND EVALUATION OF CLOPIDOGREL BISULFATE CAPSULE LOADED NANOPARTICLES

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ABSTRACT: The present study aims to formulate and evaluate clopidogrel bisulfate capsule-loaded nanoparticles. Clopidogrel bisulfate nanoparticles were produced by the nano-precipitation method and emulsification solvent evaporation method using ethylcellulose polymer at different ratios and characterized for particle size drug entrapment efficiency dissolution testing scanning electron microscopy imaging (SEM) and compatibility studies (Fourier transform infrared spectroscopy (FTIR) and differential scanning calorimetry (DSC). Optimized nanoparticles were filled into capsules with different concentrations of HPMC 4 KM and further evaluated. The prepared nanoparticles characterized and found to be the particle size was ranged from 100-2500 nm, and entrapment efficiency was ranged from 23.88 ± 0.20 % to 90.53 ± 0.31%. Amongst all formulations, F6 and F10 were considered for further optimization showed dissolution 53.20 ± 0.27 and 39.87 ± 0.12 % at the end of the first hour with entrapment efficiency 70% and 79%, respectively. SEM of clopidogrel bisulfate nanoparticles confirmed small particles without aggregation. The particle size of F6 and F10 obtained was 287.6 and 349.8 nm, respectively. Compatibility studies revealed that there is no chemical interaction between clopidogrel and excipients. The Poly-dispersity index narrowed down to 0.1-0.3. The Zeta potential of F6 and F10 were -40.4 mV and -37.8 mV, respectively. Nanoparticles containing capsules CF62 and CF102 showed floating time up to 12 h with drug release of 99.57 % and 97.052 %, respectively, and within the range of weight variation. Stability studies revealed prepared formulation is stable. The emulsification-solvent evaporation method was considered as an effective method for the preparation of nanoparticles due to its sustainability property and optimized in the form of capsules formulation.

INTRODUCTION: Nanotechnology is defined as design characterization, production and applications of structures, devices and systems by controlling shape and size at a nanometer scale. According

To International System of Units (SI) nanotechnology is typically measured on a nanometers scale of 1 billionth of a meter, referred to as "the tiny science. At this small size, molecules and atoms work differently, behave as a whole unit in terms of their properties, and transport provides a variety of advantages.

Nanoparticles (NPs) are defined as particulate dispersions or solid particles drug carriers that may or may not be bio-degradable. The drug is dissolved, entrapped, encapsulated, or attached to a nanoparticle matrix.

Different techniques like polymerization, preformed polymers, or ionic gelation have been used for the preparation of nano-particles [1]. Clopidogrel bisulfate, an antiplatelet agent structurally and pharmacologically similar to ticlopodine, is used to inhibit blood clots in various conditions such as peripheral vascular disease and coronary artery disease, and care bro vascular disease. Clopidogrel is sold under the name Plavix by sanofi and Bristol-myers Squibb. The drug is an irreversible inhibitor of the P2Y12 adenosine diphosphate receptor found on the membrane of platelet cells. The use of clopidogrel is associated with several serious adverse drug reactions such as severe neutropenia, various forms of hemorrhage, and cardiovascular edema [2]. In the present investigation, an attempt was made to develop clopidogrel bisulfate capsule-loaded nanoparticles to prolong the drug gastric retention. This helps to have improved bioavailability and therapeutic efficacy, which also reduces dosing frequency.

MATERIALS AND MATERIAL:
Materials: Clopidogrel bisulfate powder was obtained from Micro labs, Bangalore, Ethylcellulose was obtained from Otto Kemi. Tween 20, Ethanol, Chloroform were obtained from SD fine chemicals limited. Distilled water was obtained from in house source. HPMC K4M was obtained from Yarrow chem. Products, Mumbai.

Methods:
Preparation of Clopidogrel Nano-particles: Nano-particles are prepared by two methods: 1) Nano-precipitation 2) Emulsification followed by solvent evaporation.

Nanoprecipitation: Drug and polymer were dissolved in equal proportional of ethanol chloroeform mixture by using probe sonicator. This organic phase was added drop by drop (2 ml/min) in an external aqueous phase containing surfactant Tween 20 in a fixed concentration. During this mixing, the aqueous phase was stirred using a mechanical stirrer at 14,000 rpm for half an hour followed by magnetic stirring for 3 h and kept overnight. The formed nanoparticles suspension was filtered through what man filter paper and washed nanoparticles were dried.

Emulsification Followed by Solvent Evaporation: Drug and the polymer were accurately weighed and dissolved in equal proportional of ethanol and chloroform, respectively. The obtained solution was poured into the relevant amount of distilled water and containing a specified Tween [20]. An ultra-sonicator stirred the yielded mixture for 1 min to form a microemulsion.

The microemulsion was then continuously stirred on a magnetic stirrer for 4 h to allow the volatile solvent to evaporate, and the respective clopidogrel loaded ethyl cellulose nano-suspension containing nanoparticles were thus obtained, filtered through Whatman filter paper, washed nanoparticles were dried. Total 12 formulations (F1-F12) were prepared. Formulations F1-F6 prepared by the nano-precipitation method and F7-F12 were prepared by emulsification solvent evaporation method, where nanoparticles containing 98 mg of clopidogrel bisulfate were used equivalent to 75 mg of clopidogrel base. The formulation of nanoparticles is given in **Table 1**.

TABLE 1: FORMULATION OF NANOPARTICLES BY NANOPRECIPITATION METHOD

Ingredients	By nanoprecipitation method						By emulsification-solvent evaporation method					
	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
Clopidogrel bisulfate (mg)	98	98	98	98	98	98	98	98	98	98	98	98
Ethyl cellulose (mg)	37.5	75	150	225	300	375	37.5	75	150	225	300	375
Chloroform(ml)	10	10	10	10	10	10	10	10	10	10	10	10
Ethanol (ml)	10	10	10	10	10	10	10	10	10	10	10	10
Tween 20 (ml)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Water (ml)	100	100	100	100	100	100	100	100	100	100	100	100

Evaluation of Nanoparticles:
Percentage Yield: The prepared nanoparticles of all batches were accurately weighed. The weighed nanoparticle was divided by the total amount of all the excipients and drugs used to prepare the nanoparticles, which gives the total percentage yield of nanoparticles [3]. It was calculated by using the following equation,

Percentage yield = (Mass of nanoparticles recovered) / (Mass of drug and formulation excipients) × 100

Entrapment Efficiency: The freshly prepared nano-suspension was centrifuged at 20,000 rpm for 20 min at 50 °C temperature using a cool ultracentrifuge. The amount of unincorporated drug was measured by taking the absorbance of the appropriately diluted 100 ml of supernatant solution at 270 nm using UV spectrophotometer against blank [3]. EE was calculated by subtracting the amount of free drug in the supernatant from the initial amount of drug taken. The following equation could achieve the % entrapment efficiency (% EE).

% EE = (Total amount of drug-amount of drug detected in the supernatant) / (Total amount drug added) × 100

Compatibility Studies: The drug should be compatible with the polymers used to prepare nanoparticles. The compatibility of the drug with adjuvants can be determined by Fourier transform infrared spectroscopy (FT-IR) and differential scanning calorimetry (DSC) [4].

Fourier Transforms Infra-red Spectroscopy (FT-IR): Compatibility studies were carried out with polymers using the KBr pellet method. KBr is dried in an oven at 400 °C before analysis. Pure drug and excipients physical mixtures free from moisture content were used for analysis. The pure drug and excipients are triturated with KBr separately in a ratio of 1:100, and pellets are prepared by setting the pressure to 100 kg/cm 2 for 2 min. The obtained pellet is analyzed in FTIR 8400S, Shimadzu, Japan. KBr background is obtained initially before analysis of test samples. The same procedure was repeated for the analysis of different drug and drug-excipients physical mixtures.

Differential Scanning Calorimetry (DSC): The DSC analysis of pure drugs and the dried nanoparticles was carried out using the 2010 DSC module to evaluate any possible drug excipients interaction. Samples (4.12 mg) were weighed accurately using a single pan electronic balance and heated in a sealed aluminum pan at a rate of 5 °C / min from 25 to 450 °C temperature range under a 35 ml/min nitrogen flow.

***In-vitro* Dissolution Study:** *In-vitro* drug release studies were performed in United States Pharmacopeia (USP) apparatus II (paddle) at a speed of 50 rpm. Dissolution was carried out in 1000 ml of 0.1N HCl as the medium at 37.0 + 0.2 °C. 5 ml of sample was withdrawn periodically and replaced with an equal volume of fresh 0.1N HCl up to 8 h. Samples were diluted suitably and filtered through filter paper (0.22 μm, Whatman). The dialysate was then subject to UV analysis versus a blank (0.1NHCl) [5]. Percent release of drug was calculated based on the standard UV calibration curve at 270.2 nm [6].

Scanning Electron Microscopy (SEM): Particle morphology was observed by using scanning electron microscopy (S-4100, Hitachi, Shiga, Japan). The analysis by placing the sample on an SEM stub using double-sided adhesive tape and is made electrically conductive by coating with Au using sputter-coater at 20 mA for min [6, 7]. A scanning electron microscope with a secondary electron detector was used to obtain digital images of the samples at an accelerating voltage of 15 kV.

Particle Size, Polydispersity Index and Zeta Potential Analysis: The particle size analysis, polydispersity index, and zeta-potential measurement were analyzed by zeta sizer Nano ZS (Malvern Instruments, version 2.1 UK). For the analysis, the nanoparticle sample of the desired concentration was flushed through a folded capillary cell (DTS1060), and the measurement was carried out on the second filling; a sufficient sample volume was used to cover the electrodes of the cell completely. The sample was injected slowly, and analysis was carried out if there were no visible air bubble inclusions present. After inspection, the cell was placed into the zeta sizer and equilibrated for 2 min prior to the particle size measurements, of which there were six replicates.

Preparation of Capsules: Single-unit capsules were formulated with the help of different HPMCK4M polymers, which upon administration would attain a density of less than that of the gastric fluids and therefore would float. A weighed amount of clopidogrel nanoparticle equivalent to 98 mg was physically blended with polymers in a glass mortar and pestle and filled in a hard gelatin capsule # 0. The drug and polymer blend was transferred into the empty capsule shells manually [8]. The formulation of nanoparticles is given in **Table 2**.

TABLE 2: FORMULATION OF CAPSULES WITH NANOPARTICLES

Ingredients	Nanoprecipitation method		Emulsification- solvent evaporation method	
	$CF6_1$	$CF6_2$	$CF10_1$	$CF10_2$
Nanoparticles(mg)*	98	98	98	98
HPMCK4M(mg)*	24	49	24	49

*Note: Nanoparticles and HPMC K4M was taken in the ratio of 1:0.25 and 1:0.5

Evaluation of Capsule:

Appearance and Shape: The general appearance of the capsules includes morphological characteristics like size, shape, color, etc.

Weight Variation: 20 capsules were randomly selected, and the average weight was determined. Then individual capsules were weighed, and percent deviation from the average was calculated. The capsule was opened, and the contents were removed as completely as possible. The empty shells were weighed. The net weight of its contents was determined by subtracting the weight of the shells from the weight of the intact capsule. The procedure was repeated with other capsules [19]. The average net weight was determined from the sum of the individual net weights. The percentage deviation from the average net weight of each capsule was determined. The deviation of individual net weight should not exceed the limits given in **Table 3**.

TABLE 3: PERCENTAGE DEVIATION FOR THE CAPSULES

Pharmaceutical Form	Avg. Weight in mg	% Deviation
Capsules	Less than 300 mg	±10.0
	300 mg or more	±7.5

***In-vitro* Buoyancy Studies:** The capsules were immersed in 1000 ml of 0.1N HCl contained in a US Pharmacopeia (USP II) paddle-type apparatus where the speed of rotation was maintained at 50 rpm. The amount of time during which the capsules remained buoyant was the floating time. The ratio of polymer that showed the best floating behavior was used for in vitro release studies [8, 9, 10].

***In-vitro* Release Studies of Nanoparticles Filled In Capsule:** Based on the buoyancy period, various ratios of low-density polymer were used with 98.8 mg of clopidogrel nanoparticles.

Clopidogrel nanoparticles and HPMC K4M were used in 2 ratios (1:0.25 and 1:0.5) to optimize the formulation on the basis of release studies. *In -vitro* release studies of the capsules were performed in a USP paddle-type apparatus at 50 rpm using 1000 ml of 0.1N HCl. 5 ml samples were withdrawn at regular intervals and replaced with buffer. The samples were evaluated spectrophotometrically at 270.2 nm (λ_{max}).

Model Dependent Kinetics: Various models were tested for explaining the kinetics of drug release5.

To analyze the mechanism of the drug release rate kinetics of the dosage form, the obtained data were fitted into zero-order, first-order, Higuchi and Korsmeyer-Peppas release model as given in **Table 4**.

TABLE 4: APPLIED MATHEMATICAL MODELS TO THE DISSOLUTION DATA OF FORMULATIONS

Model	Equation	Plot of graph	Parameters
Zero order	$Q_t = Q_0 + K_0 t$	% drug release versus time	K_0– release rate constant
First order	$\ln Q_t = \ln Q_0 + K1t$	Log % drug release versus time	K_1 – release rate constant
Higuchi release	$Q_t = K_H t1/2$	% drug release versus square root of time	K_H–Higuchi constant
Korsmeyer-peppas	$Q_t/Q_8 = Kktn$	Log % drug release versus log time	n– release exponent

Regression coefficients (r^2) were calculated for all the formulations. Release component "n" was calculated from the Korsmeyer Peppas equation as given in **Table 5**. The kinetic release calculations were carried out using MS–office excels.

TABLE 5: INTERPRETATION OF DIFFUSION RELEASE MECHANISMS FROM "N" VALUES

Release Exponent (n)	Drug transport mechanism	Rate as a function of time
> 0.5	Fickian diffusion	t-0.5
0.5<n>1.0	Anomalous transport	tn-1
1.0	Case-II transport	Zero order release
Higher than 1.0	Super case-II transport	tn^{-1}

Performing Accelerated Stability Studies for the Optimized Formulations: Accelerated stability studies were conducted for the optimized formulation as per ICH guidelines.

The studies were carried out at different conditions of 40 °C / 75% RH for three weeks in a stability chamber. The samples were withdrawn every one week and evaluated for properties like appearance, dissolution, and drug content [5].

RESULTS AND DISCUSSION

Preparation of Clopidogrel Bisulfate Nanoparticles: Nanoparticles are prepared by different ratios of polymer using two different methods, *i.e.*, nano-precipitation and emulsification followed by solvent evaporation.

Preliminary trials were conducted by using various polymers to confirm the formation of nanoparticles. There was no formation of nanoparticles with Eudragit RL-100, Eudragit S-100, Eudragit RS-100, Eudragit L-100-55, and Chitosan as given in **Table 6**. A suspension of the solution was observed as the final product in which there was no nanoparticle formation. This may be due to partial solubility in the solvent taken, which could not properly coat the drug. Using different ratios of ethyl cellulose, nanoparticles were prepared as in **Table 7**. Nanoparticles were formed as polymer concen-tration increases, but at 1:6 ratio, there was no formation of nanoparticles due to an increase in viscosity of ethyl cellulose.

TABLE 6: PRELIMINARY TRIALS FORMATION OF NANOPARTICLES

Organic phase	Aqueous phase	Nanoparticles formation
Ethyl cellulose (1%)	Tween 20 (1%)	Formed
Eudragit RL-100 (1%)	Tween 20 (1%)	Not formed
Eudragit S-100 (1%)	Tween 20 (1%)	Not formed
Eudragit RS-100 (1%)	Tween 20 (1%)	Not formed
Eudragit L-100-5 (1%)	Tween 20 (1%)	Not formed
Chitosan (1%)	Tween 20 (1%)	Not formed

TABLE 7: FORMATION OF NANOPARTICLES USING DIFFERENT RATIO OF ETHYL CELLULOSE

Formulations	Drug: polymer ratio	Nanoparticles formation
	Nanoprecipitation method	
F1	1:0.5	Formed
F2	1:1	Formed
F3	1:2	Formed
F4	1:3	Formed
F5	1:4	Formed
F6	1:5	Formed
	Emulsification-solvent evaporation method	
F7	1:0.5	Formed
F8	1:1	Formed
F9	1:2	Formed
F10	1:3	Formed
F11	1:4	Formed
F12	1:5	Formed

Evaluation of Clopidogrel Bisulfate Nanoparticles: Evaluation of nanoparticles was done in terms of percentage yield, % entrapment efficiency, compatibility studies and *in-vitro* dissolution.

Percentage Yield and Entrapment Efficiency: For formulations, F1 to F12, the percentage yield, and % entrapment efficiency are in the range of 55 to 89% and 51 to 79%, respectively, as given in **Table 8** and shown in **Fig. 1**. The results clearly observed that percentage yield and entrapment efficiency are independent of each other.

An increase in polymer concentration showed an increase in entrapment efficiency, which is indent from the results.

Compatibility Studies: Fourier transforms infrared spectroscopy studies: To verify any interaction between the pure drug, polymer, and nanoparticles, FTIR spectroscopy studies were conducted. The results are interpreted and observed from **Fig. 2.**

From the IR spectra, peaks identified for the pure drug were observed with nanoparticles. From these

results, it was concluded that there was no intermolecular interaction between the drug and the polymers. Further characterization was done using DSC to determine the interaction.
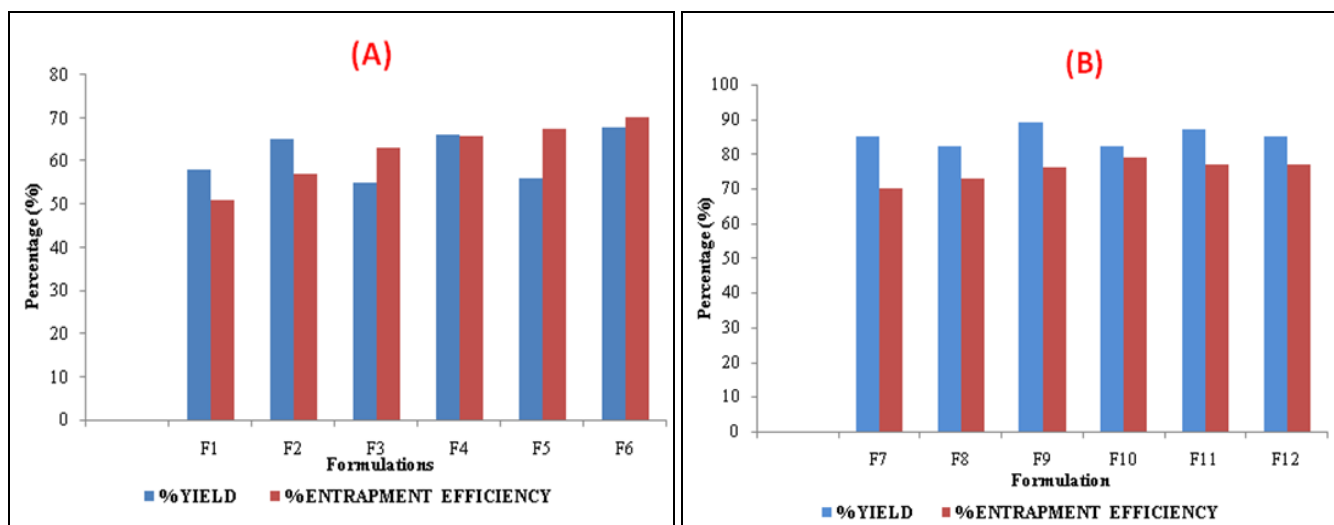

FIG. 1: (A) PERCENTAGE YIELD AND ENTRAPMENT EFFICIENCY OF NANOPARTICLES PREPARED BY NANO-PRECIPITATION (F1-F6) (B) percentage yield and entrapment efficiency of nanoparticles prepared by emulsification-solvent evaporation (f7-f12)

TABLE 8: PERCENTAGE YIELD AND ENTRAPMENT EFFICIENCY

Formulation*	Percentage yield (%)	Entrapment efficiency (%)
F1	58	51.1
F2	65	57.0
F3	55	63.0
F4	66	65.9
F5	56	67.4
F6	68	70.4
F7	85	70.0
F8	82	73.0
F9	89	76.0
F10	82	79.0
F11	87	77.0
F12	85	77.0

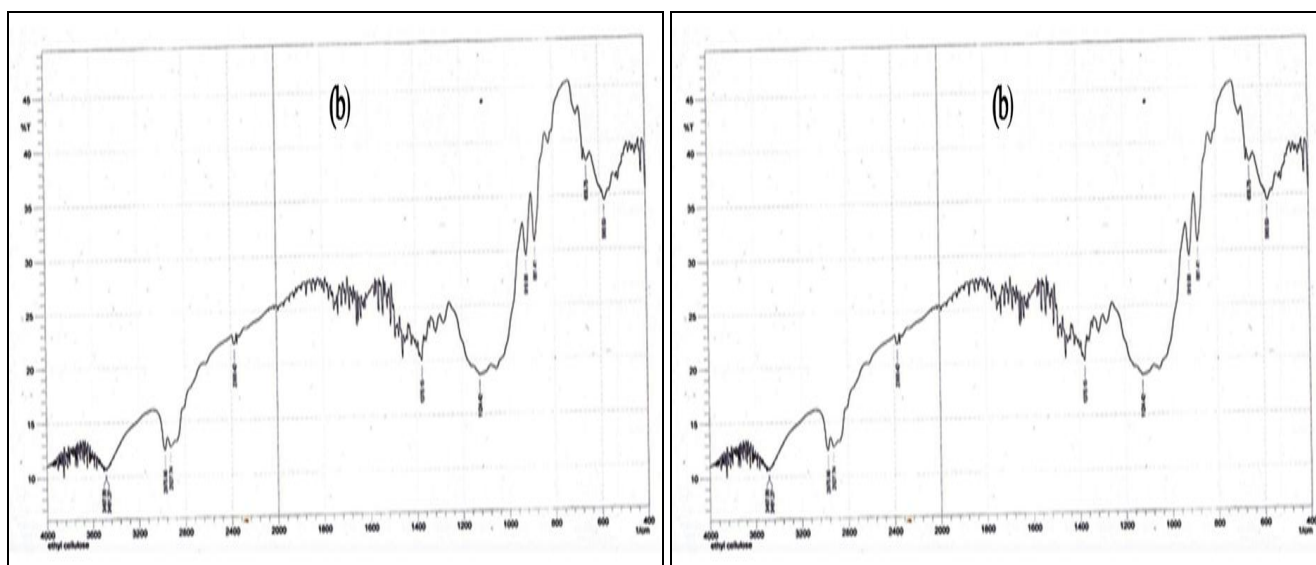*Note: F1-F6 - by nanoprecipitation method and F7-F12- by an emulsification-solvent evaporation method.
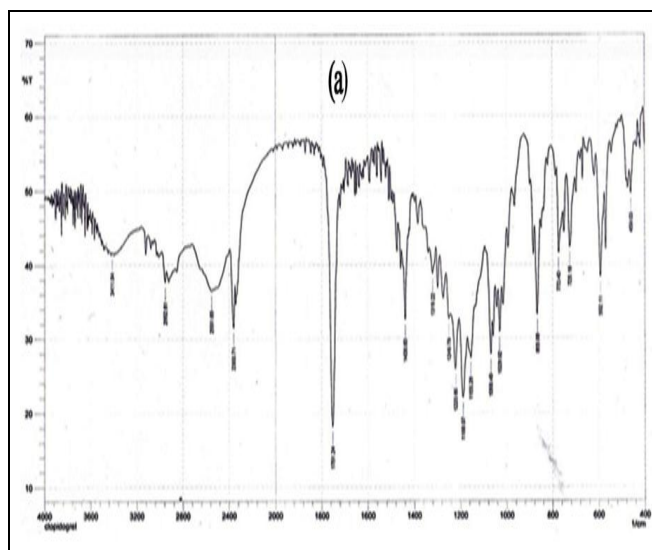
FIG. 2: FTIR SPECTRA OF (A) CLOPIDOGREL BISULFATE (B) ETHYL CELLULOSE (C) CLOPIDOGREL BISULFATE + ETHYL CELLULOSE

***In-vitro* Dissolution Studies:** The most important evaluation parameter that needs to be optimized is the drug release study by *in-vitro* dissolution. The *in-vitro* evaluation studies of clopidogrel bisulfate nanoparticles were performed using USP apparatus II (paddle) in 0.1N HCl. The drug analysis was done using a UV spectrophotometer at 270.2 nm, and the results were depicted in **Fig. 4**. From formulations, F1 to F6 cumulative percentage drug release was found to be 90.53 ± 0.31 to 53.20 ± 0.27 % in the first h. From F7 to F12, the cumulative percentage of drug release was 69.33 ± 0.58 to 23.88 ± 0.20 %, respectively, in the first hour. The release profile observed that the concentration of polymer influences the *in-vitro*

drug release pattern of different formulations, as shown in **Fig 4**. The results showed that formulation F1, F2, F3, F4, F5, F7, F8 and F9 could not show sustained release. Formulation F6 and F10 were considered for further optimization showing sustained drug release of 53.20 ± 0.27 and 39.87 ± 0.12 % in the first hour with entrapment efficiency 70% and 79%, respectively. Even though F6 does not obey the sustainability property, when mixed with HPMC K4M to form a plug-in capsule, it obeys the sustainability property. These optimized formulations were evaluated for particle size, polydispersity index, and zeta potential. These optimized formulations were taken for further filling with HPMC K_4M in a capsule.
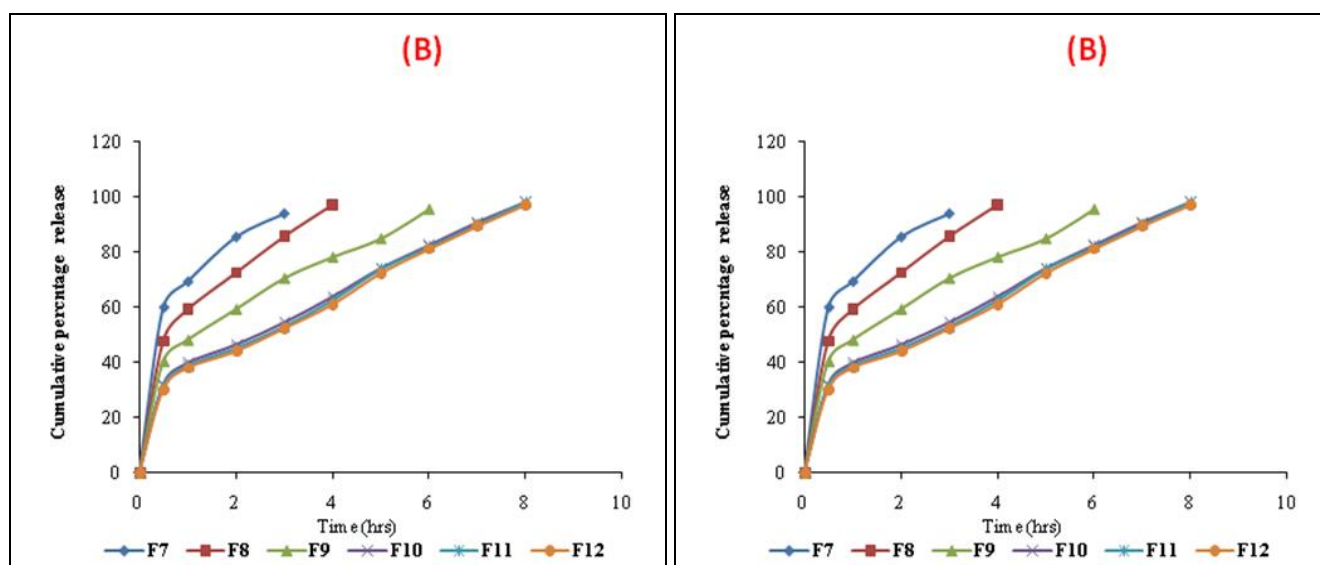

FIG. 4: (A) DISSOLUTION PROFILE OF PREPARED NANOPARTICLE (B) DISSOLUTION PROFILE OF PREPARED NANOPARTICLES

Scanning Electron Microscopy (SEM): Morphology of precipitated drug particles in the suspension after air drying followed by oven-drying is shown in **Fig. 5**.

The drug particles precipitated with the Tween 20 as stabilizer are spherical in shape. The particles are discrete and uniform in size.
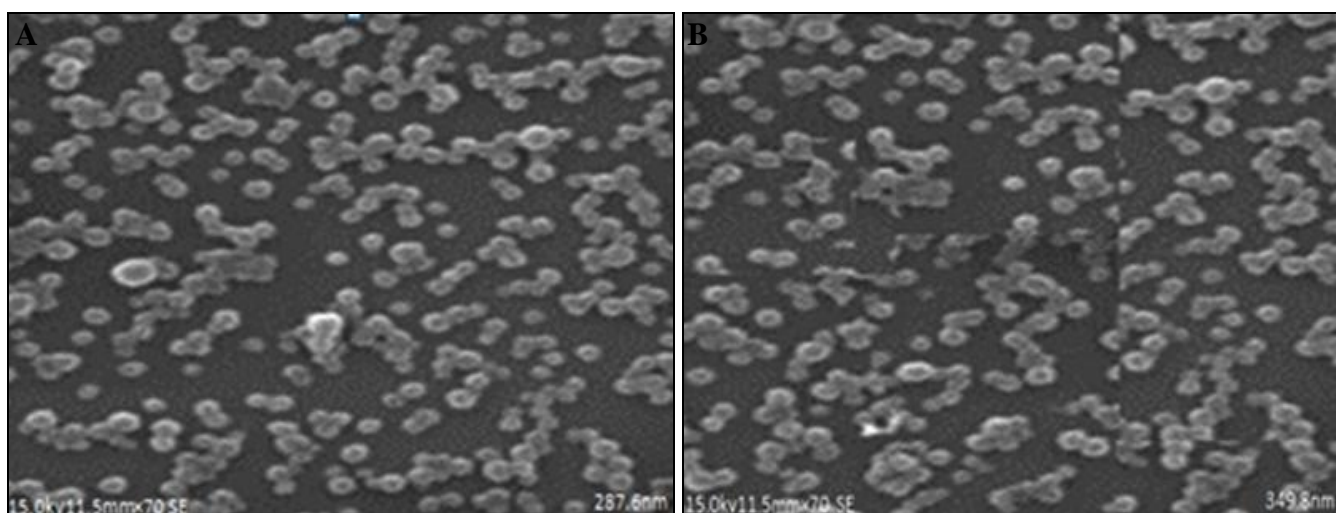

FIG. 5: SCANNING ELECTRON MICROSCOPY (SEM) OF (A) F6 CLOPIDOGREL BISULFATE NANOPARTICLES (B) F10 CLOPIDOGREL BISULFATE NANOPARTICLES
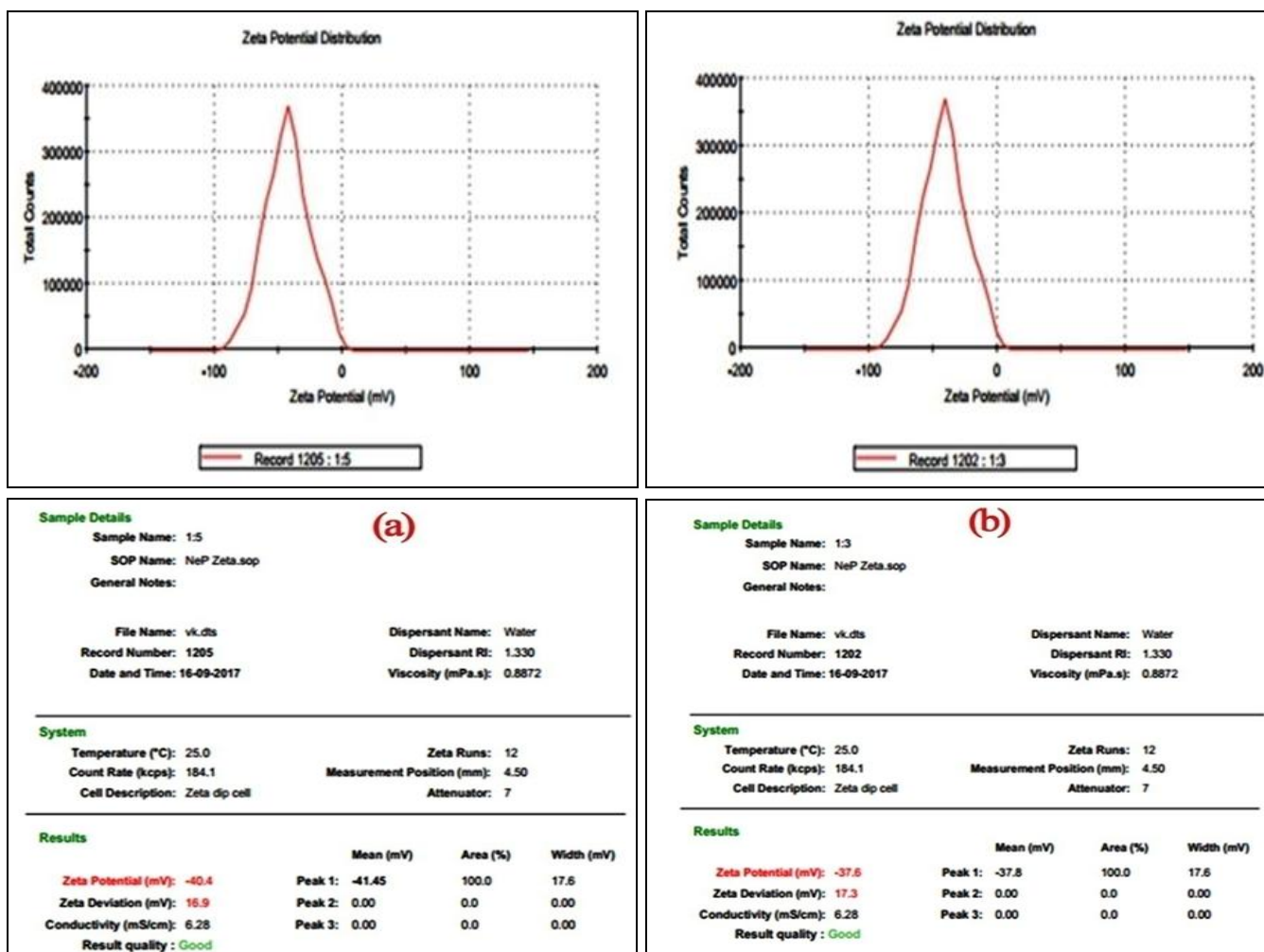

FIG. 7: ZETA POTENTIAL ANALYSIS OF (A) F6 CLOPIDOGREL BISULFATE NANOPARTICLE (B) F10 CLOPIDOGREL BISULFATE NANOPARTICLE

Particle size Poly Dispersity Index (PDI) and Zeta potential analysis: The particle size and the Poly Dispersity Index (PDI) of the prepared nanoparticles was measured by dynamic laser light scattering (Zetasizer Ver. 2.1 Malvern). The average particle size of clopidogrel nanoparticles was found to be in the range of nm, and the results have been shown in **Fig. 6**.

The formulations were homogeneous as indicated by the polydispersity index, and the results have been shown in **Fig. 6**. The average particle diameter was found to decrease further in formulations containing Tween 20 as a stabilizer at concentrations of 0.5%. The particle size distributions were found to be more uniform as the polydispersity index narrowed down to 0.1-0.3. The zeta potential of the nanoparticles was found to be negative, and the results are shown in **Fig. 7**. The zeta potential values of clopidogrel nanoparticles were in the negatives. Hence from these studies, formulation F6 (1:5 ratio) and F10 (1:3 ratio) (-40.4 mV) and (-37.8 mV) were considered as optimum. The results indicated that ethylcellulose nanoparticles possessed good stability.

Model Dependent Kinetics for Nanoparticles: The release kinetics of optimized nanoparticles was calculated using micro soft office excel 2010 version. The release data were analyzed by fitting all the formulation's drug release profiles into zero-order, first-order, Higuchi, and Korsmeyer- peppas models. The **Table 9** regression coefficients reported for the above release profile signified that nanoparticle formulations (F6) and (F10) followed zero-order and Higuchi release models. F6 showed anomalous Fickian transport having release component "n" values as 0.429, indicating diffusion mechanism, and F10 showed anomalous transport having release component "n" value as 0.509, indicating erosion and diffusion mechanism.

TABLE 9: MODEL DEPENDENT KINETICS

Code	Zero order R^2	First order R^2	Higuchi Kinetics R^2	Korsmeyer- Peppas R^2	n	Release mechanism
F1	0.964	0.952	0.996	0.990	0.429	Fickian diffusion
F2	0.928	0.906	0.996	0.990	0.429	Fickian diffusion
F3	0.951	0.923	0.996	0.990	0.429	Fickian diffusion
F4	0.946	0.935	0.996	0.990	0.429	Fickian diffusion
F5	0.975	0.936	0.996	0.990	0.429	Fickian diffusion
F6	0.983	0.939	0.996	0.990	0.429	Fickian diffusion
F7	0.974	0.957	0.995	0.935	0.308	Fickian diffusion
F8	0.990	0.961	0.997	0.976	0.414	Fickian diffusion
F9	0.989	0.955	0.994	0.975	0.429	Fickian diffusion
F10	0.997	0.971	0.974	0.960	0.509	Anomalous transport
F11	0.997	0.974	0.969	0.954	0.517	Anomalous transport
F12	0.997	0.972	0.969	0.957	0.528	Anomalous transport

Evaluation of Capsule:
Weight Variation: The average weight of capsules within each formulation was found to be uniform. This indicates uniform filling of powder blend during capsule filling.

Not more than two of the individual weights deviated from the average weight by more than 10%, and none deviated by more than twice that percentage, which provided good weight uniformity (**Table 10**).

In-vitro **Buoyancy Studies:** From the in vitro buoyancy studies, it was observed HPMC K4M–containing formulations showed good buoyancy, with floating up to 8 and 12 h on the dissolution medium (0.1 N HCl) **Table 10**. Sinkers (helix-like wire used to hold the capsules below the paddle during dissolution in the USP type II apparatus) were used for the preliminary *in-vitro* buoyancy studies, and the capsules floated after a period of 15 min, but the swelling of the capsules was hindered significantly. Therefore, we decided to carry out the study without the sinkers.

TABLE 10: *IN-VITRO* BUOYANCY TIME

Formulations	Total floating time (h)	Weight variation (mg)
$CF6_1$	8	258±0.11
$CF6_2$	12	292±0.23
$CF10_1$	8	258±0.12
$CF10_2$	12	292±0.25

Note: All values are expressed as mean ± SD, n=3

***In-vitro* Drug Release Studies of Nanoparticles Filled In Capsule:** *In-vitro* drug release studies revealed that the formulation containing clopidogrel nanoparticles and HPMCK4M (1:0.25, 1:0.5 and 1:1 ratio) showed a floating time of 8 and 12 hrs respectively. CF61 and CF101 showed floating time up to 8 h with drug release of 99.01 % and 97.02 %, respectively. CF62 and CF102 showed floating time up to 12 h with drug release of 99.57 % and 97.052 %, respectively **Fig. 8**. Therefore, CF62 and CF102 (1:0.5 ratio of clopidogrel nanoparticle, *i.e.*, two formulations) with HPMCK4M were used as optimized formulations because CF61 and CF101 release drugs within 8 h when compared to CF62 and CF102.
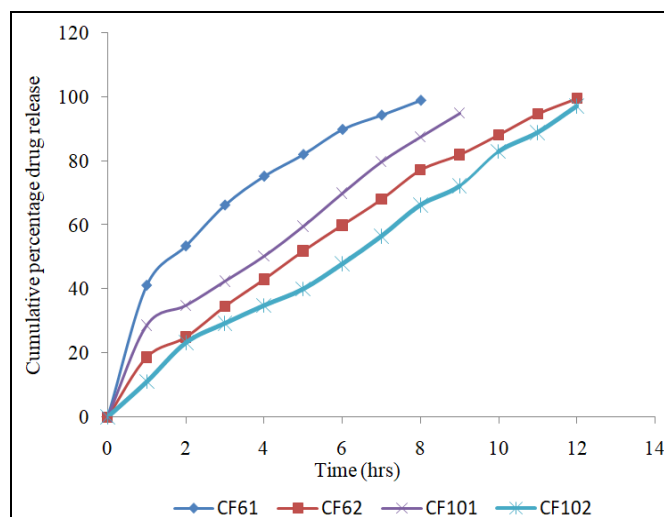
FIG. 8: DISSOLUTION PROFILE OF CAPSULE

TABLE 11: MODEL DEPENDENT KINETICS OF CF62 AND CF102 FORMULATION

Code	Zero-order	First-order	Higuchi kinetics	Korsmeyer- Peppas		Release mechanism
	R^2	R^2	R^2	R^2	n	
CF61	0.996	0.912	0.996	0.997	0.43	Fickian diffusion
$CF6_2$	0.993	0.922	0.988	0.990	0.71	Anomalous Transport
CF101	0.996	0.983	0.964	0.959	0.54	Anomalous Transport
$CF10_2$	0.995	0.913	0.965	0.990	0.84	Anomalous Transport

Model Dependent Kinetics Cf62 and Cf102 Formulations: The release kinetics of optimized nanoparticles was calculated using Microsoft Office Excel 2010 version. The release data were analysed by fitting the drug release profile of all the formulations into zero-order, first-order, Higuchi model, and Korsmeyer- Peppas model **Table 11**.

From the **Table 11** regression coefficients reported for the above release profile signified that nanoparticle formulations (CF6 and CF10) followed zero-order and Korsmeyer-Peppas release model showed anomalous transport having release component "n" value of 0.71 and 0.84, indicating erosion and diffusion mechanism.
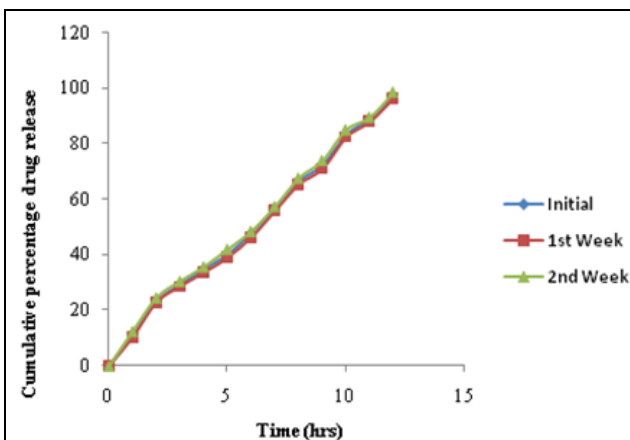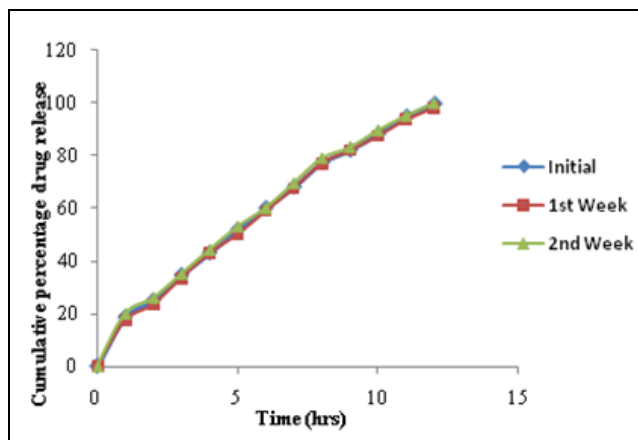

FIG. 9: DISSOLUTION PROFILE OF CAPSULE FILLED WITH NANOPARTICLES (A) CF62 (B) CF102 DURING STABILITY STUDIES

Stability Studies: Optimized formulation was subjected for stability studies, was evaluated for weight variation test **Table 12** and dissolution **Fig. 9** after subjecting to accelerating stability studies as per ICH guidelines. The aim of this study was to check for reproducibility. The studies were carried

out at 40 °C / 75% RH. Based on the results, it can be concluded that optimized formulations were stable during accelerated stability studies, with insignificant changes in weight variation and *in-vitro* drug release characteristics.

TABLE 12: WEIGHT VARIATION TEST

Formulations	Weight variation		
	Initial	1st week	2nd week
$CF6_2$	292 ± 0.23	292 ± 0.22	292 ± 0.21
$CF10_2$	292 ± 0.25	292 ± 0.24	292 ± 0.22

Note: Values are expressed as mean $\pm$ SD, n=3

CONCLUSION: Clopidogrel bisulfate capsule-loaded nanoparticles have been formulated to increase its efficacy of sustained release. Nanoparticles have been prepared by two methods, nano-precipitation and emulsification, followed by solvent evaporation. The emulsification-solvent evaporation method was considered an effective method for preparing nanoparticles due to its sustainability property. The prepared clopidogrel bisulfate nanoparticles were evaluated and optimized in the form of capsules formulation.

CONFLICTS OF INTEREST: The authors declare that there is no conflict of interest.

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