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FORMULATION AND EVALUATION OF ORODISPERSIBLE DOSAGE FORMS INCORPORATING DRUG NANOPARTICLES CONTAINING CLOPIDOGREL BISULPHATE

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ABSTRACT: Clopidogrel bisulphate (CB) is the rate of dissolution often controls a sparingly soluble orally administered drug and the rate of absorption. Reports suggest that the drug has poor water solubility which may be challenging for developing liquid dosage forms of Clopidogrel bisulphate. Hence in the present study, we sought to develop orodispersible nanoparticles to enhance the solubility by an anti-solvent evaporation technique using stabilizers as PVPK-30, Poloxamer-188, PVA, L-arginine and tween-80. We found that the particle size was ranging between 154.7 nm to 844.2 nm and % Drug entrapment efficiency was 42.4% to 97.1% respectively. Among all the formulations, F3 stabilized with L-arginine and PVA has been found to show 92.62 ± 0.81% drug release at the end of 10 min in both 0.1N HCl and phosphate buffer (pH6.8). Through SEM studies we found that the particles were small with no aggregation. Further, the particles (F3) were compressed into tablets F3(c) through direct compression method and characterized based on some selected parameters in comparison with the marketed tablet. We have also observed a satisfactory clopidogrel excipient compatibility through FT-IR investigation. DSC and XRD results have illustrated that the crystallinity of drug was lost in lyophilized powder and tablet converted to an amorphous form. Hence, we concluded that the optimized formulation of Clopidogrel bisulphate could improve the rate of absorption controlled by the rate of dissolution.

INTRODUCTION: Oral route has been one of the most popular routes of drug delivery due to its ease of administration, patient's compliance, least sterility constraints and flexible design in dosage forms. For many days treatment of an acute disease or chronic illness has mostly accomplished but the delivery of drugs to patients using conventional drug delivery system, even today these conventional drug delivery systems are the primary pharmaceutical products commonly seen in the prescription.

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Cardiovascular diseases are a major cause of illness and death worldwide; it is also estimated by scientists that the mortality rate of disease and death will be more in the future. Five major cardiovascular diseases are a heart attack, stroke, hypertension, inflammatory heart disease, rheumatic heart disease, and these diseases cause 16 million deaths per year all over the world.

According to the World Health Organization, cardiovascular disease prevalence and incidence vary according to gender also and a few females getting affected is more than the number of males. Different types of cardiovascular drugs are used for the treatment of these diseases [1].

Drug absorption is defined as the process of movement of unchanged drug from the site of administration to the systemic circulation.

The rating process includes

- Dissolution of the drug in an aqueous environment.
- Absorption across cell membranes into systemic circulation [2].

Clopidogrel, a thienopyridine derivative, binds specifically and irreversibly to the platelet P2RY12 purinergic receptor, inhibiting ADP-mediated platelet activation and aggregation. After oral administration, clopidogrel is rapidly absorbed. Owing to its extensive metabolism, clopidogrel is not detected in human plasma. Clopidogrel is a prodrug that is absorbed in the intestine and activated in the liver. Drug-drug interactions of clopidogrel were reported with atorvastatin, the calcium-channel antagonist verapamil, and the proton-pump inhibitor omeprazole. The clinical implications of these findings are still under investigation. Several clinical studies did not support the finding that atorvastatin can interfere with the effect of clopidogrel [3].

These observations lead us to the conclusion that nanosuspension seem to be a promising drug delivery system, which can provide an effective and practical solution to the problem of with low aqueous solubility and poor systemic bioavailability. The present work is to formulate orodispersible nanoparticulated dosage forms to enhance the dissolution rate of poorly water-soluble drugs like Clopidogrel bisulphate by using antisolvent precipitation method [4].

MATERIALS AND METHODS: Active Pharmaceutical Ingredient (API) working standards of Clopidogrel bisulphate (CB) was obtained as a gift sample from Aurobindo Pharmaceuticals, Hyderabad. The stabilizers used in the study such as L-arginine hydrochloride and Poloxamer-188 are from Bright Labs, Hyderabad. Tween 80 is obtained from Merc specialties Pvt. Ltd., Mumbai, India. Polyvinyl pyrrolidine K-30 (PVPK- 30) is procured from Dr. Reddy's. Polyvinyl alcohol was obtained from Bright Labs, Hyderabad. Sodium starch glycolate was obtained from Ascot Pharmachem Pvt. Ltd., Gujarat. Crospovidone was obtained from Merc specialties Pvt. Ltd., Mumbai. Microcrystalline cellulose was obtained from Vijlak Pharma, Mumbai. Spray dried Mannitol was obtained from KP Lalwuai & Co, India. Cross Povidone was obtained from Nan hand industries. Magnesium stearate was obtained from Amishi Drugs and chemicals, Hyderabad.

METHODS:

Preformulation Study of Clopidogrel Bisulphate (CB) Nanoparticles:

Formulation Optimisation of Clopidogrel Bisulphate (CB) Nanoparticles: The Clopidogrel bisulphate (CB) nanoparticles were prepared by using the anti-solvent evaporation method. The following design of Clopidogrel bisulphate (CB) nanoparticles were shown in **Table 1**.

Preparation of Clopidogrel Bisulphate (CB) Nanoparticles by an Anti-Solvent Evaporation Method: Clopidogrel bisulphate (CB)nanoparticles were prepared by the anti-solvent precipitation technique method. Clopidogrel bisulphate was dissolved in a methanol (3 ml) at room temperature, this was poured into 10 ml of water containing different types of surfactants (alone and in combination) maintained at a temperature of 50 ºC and subsequently stirred at agitation speed of 250 revolution per minute (rpm) on magnetic stirrer for 1 h to allow the volatile solvent to evaporate. Addition of organic solvents by means of a syringe drop by drop positioned with the needle directly into surfactant-containing water [5]. Total 15 formulations (F1-F15) were prepared by this technique demonstrated in **Table 1** with their composition.

TABLE 1: FORMULATION DESIGNOF CLOPIDOGREL BISULPHATE (CB) NANOPARTICLE

Materials (mg)	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12	F13	F14	F15
CB	95	95	95	95	95	95	95	95	95	95	95	95	95	95	95
L-Arginine	95	95	95	95	-	-	-	-	-	-	190	-	-	-	-
Poloxamer 188	95	-	-	-	95	95	95	-	-	-	-	190	-	-	-
PVPK -30	-	95	-	-	95	-	-	95	95	-	-	-	190	-	-
PVA	-	-	95	-	-	95	-	95	-	95	-	-	-	190	-
Tween 80	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Methanol	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
Water	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10

Particle Size Analysis, Polydispersity Index (PI), Zeta Potential and Drug Entrapment Efficiency: Particle size determination of the prepared formulae (F1-F15) was done by using ABT-9000 Nano-laser particle size analyzer at scattering angle 90º. The average particle size analyzer which is also called volume moment mean reflects the size of those particles which constitute the bulk of the sample volume was measured after experimenting triplicates. The polydispersity index (PDI) of each formula was also determined as a measurement for the width of the size distribution; it is a parameter to define the particle size distribution of nanoparticles obtained from a particle analyzer. PDI is an index of width or spread or variation within the particle size distribution.

The analyzer also determines the specific surface area for each sample [6]. The freshly prepared liquid nanoparticles were centrifuged at 20,000 rpm for 20 min using ultracentrifuge. The amount of unincorporated drug was measured by taking the absorbance of the appropriately diluted 25 ml of the supernatant solution at 268 nm using UV spectrophotometer. DEE was calculated by subtracting the amount of free drug in the supernatant from the initial amount of drug taken. The experiment was performed in triplicate [7].

Drug entrapment efficiency (DEE %) could be achieved by the following equation:

Entrapment efficiency % = W (Initial drug) – W (Free drug) × 10 / W (Initial drug)

***In-vitro* Release Profile of Nanoparticulate Crystals of Clopidogrel Bisulphate (CB) Nanoparticles:** *In-vitro* dissolution study was performed using Electro lab - TDT 08L (paddle assembly). The dissolution was performed using dialysis membrane -60 in 900 ml in 0.1N HCl for 60 min and phosphate buffer (pH 6.8) for 120mins as dissolution medium maintained at 37 ± 0.5 ºC and 50 rpm for Clopidogrel bisulphate nanoparticles formulae. The freshly prepared Clopidogrel bisulphate nanoparticles (10 ml) added to a dialysis bag and fitted to the paddle, samples (5 ml) were withdrawn at regular intervals of 10 minutes for 120 min and replaced with fresh dissolution medium to maintain sink condition. Samples were filtered through ashless filter paper and assayed spectrophotometer ion UV-Visible spectrophotometer at 270 nm wavelength in 0.1N HCl and 269 nm wavelength in phosphate buffer (pH 6.8) [8]. The release of the selected formula was compared with the pure drug in both media of 0.1N HCl and phosphate buffer pH 6.8.

Freeze-Drying of Liquid Nanoparticles (Lyophilization): After the evaluation of the prepared Formulae (F1-F15), the ideal formula was lyophilized using vacuum freeze dryer at a controlled temperature of -44 ºC and the pump operating at a pressure of 2.5 × 10 pascals over a period of 48-72 hour (Advantage, Freeze- Dryer, Virtis ES-53). The yielded powder was used for further studies and it is used to prepare the tablets [9].

Drug-Excipient Compatibility Studies by Using FTIR: The Fourier transform infrared spectroscopy (FT-IR) spectrum were studied to detect any sign of interaction or complexation may occur between lyophilized nanoparticle drug and super-disintegrants used in the preparation and with the excipients used in the preparation of tablets. The spectrum was obtained using FT-IR Shimadzu 8300. Samples which are lyophilized nanoparticle drug, physical mixtures of lyophilized nanoparticle drug and Cross Povidone (CP), lyophilized nanoparticle drug and sodium starch glycolate (SSG), lyophilized nanoparticle drug and Microcrystalline cellulose (MCC) respectively. All these samples were grounded and mixed thoroughly with potassium bromide at 1:5 (sample: Potassium bromide) weight ratio [10]. The spectrum obtained was in between the wave number of 4000-400 cm^{-1}.

Formulation Optimisation of Clopidogrel Bisulphate (CB) Nanoparticles of F3 Batch:

Differential Scanning Calorimetry (DSC): DSC can be used to determine the compatibility between the drug and excipients and used to evaluate the nanoparticles. Thermal characteristics of the same materials that examined in FTIR study were determined by an automatic thermal analyzer system (Shimadzu, DSC– 60, Japan) [11].

Accurately weighed samples were placed in non-hermetically aluminum pans and heated at the rate of 10 ºC/min against an empty aluminum pan as a reference covering a temperature range of 40 ºC to 300 ºC.

TABLE 2: FORMULATION DESIGN OF CLOPIDOGREL BISULPHATE (CB) NANOPARTICLE TABLETS

Materials	Quantity per Tablet (mg)					
	F3(a)	F3(b)	F3(c)	F3(d)	F3(e)	F3(f)
Nanoparticles equivalent to 75mg of drug	95	95	95	95	95	95
Spray dried Mannitol	95	92	90	95	92	90
Micro crystalline cellulose	14	14	14	14	14	14
SSG	4	7	9	-	-	-
Crospovidone	-	-	-	4	7	9
Aspartame	1	1	1	1	1	1
Talc	4	4	4	4	4	4
Magnesium stearate	2	2	2	2	2	2
Tablet weight	215	215	215	215	215	215

Scanning Electron microscopy (SEM): The morphology of raw drug and Clopidogrel bisulphate (CB) nanoparticles F3 were examined by scanning electron microscope operated with a secondary detector at an acceleration voltage of 10kv and 10kv and 100x magnification for raw drug and 15kv and 10kx for F3. The morphology of raw drug was done by direct deposition of powder on double-sided carbon tape and coated with gold was performed by STEREOSCAN-90 [12].

Transmission Electron Microscopy (TEM): While for the liquid F3 sample was prepared by the droplet evaporation technique. A droplet of liquid was deposited on double-sided carbon tape and dried at room temperature for the evaporation of water and then coated with gold. The morphological evaluation was performed by transmission electron microscopy (TEM, EM-1200EX, Japan) [13].

Powder X-Ray Diffraction: X-ray diffraction is used to study the atomic and molecular structure of crystalline substances such as drugs and excipients. X-rays diffraction patterns (diffractograms) can be used to confirm the crystalline nature of a sample. Therefore, this information is used to verify whether the substances are crystalline or amorphous. PXRD diffractograms of the pure drug, lyophilized powder of F3 and the F3(c) tablet were recorded using Shimadzu diffractometer 6000 (Shimadzu, Japan) with the input voltage at 220V/ 50Hz and the measurement condition was at voltage 40 kV and current 30 mA, the axis of 2 thetas ranged from 5-50 degree [14].

Precompression Evaluation Studies:

Angle of Repose (θ): The frictional force in a loose powder can be measured by the angle of repose. It is defined as the maximum angle possible between the surface of the pile of the powder and the horizontal plane. If more powder is added to the pile, it slides down the sides of the pile until the mutual friction of the particles producing a surface angle is in equilibrium with the gravitational force. A funnel was secured with its tip at a given height (h), above a graph paper that is placed on a flat horizontal surface [15]. The blend was carefully poured through the funnel until the apex of the conical pile just touches the tip of the funnel. The radius (r) of the base of the conical pile was measured. The angle of repose calculated using the following formula:

$$\tan \theta = h / r$$

Tan θ = Angle of repose
h = height of the cone
r = radius of the cone base

TABLE 3: RANGE VALUES OF ANGLE OF REPOSE

S. no.	Angle of repose	Nature of flow
1	<25	Excellent
2	25-30	Good
3	30-40	Possible
4	>40	Very poor

Loose Bulk Density (LBD) (g/cm^3): Loose bulk density (LBD) was measured using the formula:

LBD = Weight of the powder / Volume of the packing

Tapped Bulk Density (TBD) (g/cm^3): Tapped bulk density (TBD) were measured using the formula

TBD = Weight of the powder / Tapped volume of the packing

Compressibility Index (%): Compressibility index of the granules was determined by using the formula [16].

$$CI (\%) = (TBD - LBD) / TBD$$

Formulation Development of Orodispersible Tablet of F3 by Direct Compression Method: Clopidogrel bisulphate tablets were prepared by direct compression method after freeze-drying that showed the best *in-vitro* dissolution profile in 10 min in comparison with other nanoparticle formulae and pure drug. The amount of lyophilized powder taken was 95 mg equivalent to 75 mg of Clopidogrel bisulphate, different Clopidogrel bisulphate (CB) nanoparticle tablets were prepared using Crospovidone (CP) microcrystalline cellulose (MCC) and sodium starch glycolate (SSG) as a diluent, binder, lubricant, and disintegrants at different concentration were passed in #40. All the above ingredients were properly mixed together in a poly bag [17]. Talc and magnesium were passed through sieve #80 and blended with the initial mixture in a polybag. The powder blend was compressed into tablets on a ten-station rotary punch – tableting machine (Reek Mini Press -1) using 7mm concave punch set. The optimum formula that shows the accepted hardness and the best *in-vitro* dissolution profile [18].

Post-Compression Studies:

Hardness (kg/cm^2): The crushing strength of the tablets was measured using a Monsanto hardness tester. Three tablets from each formulation batch were tested randomly, and the average reading noted. The test was carried out in triplicate (n=3) [19].

Friability (%): Ten tablets were weighed and placed in a Roche friabilator, and the equipment was rotated at 25 rpm for 4 min. The tablets were taken out, dedusted and reweighed. The percentage friability of the tablets was measured as per the following formula; the test was carried out in triplicate (n=3) [20].

Percentage friability = Initial weight – Final weight × 100 / Initial weight

Drug Content (%): Ten tablets were weighed and powdered was dissolved in 100 ml of pH 6.8 phosphate buffer, filtered, diluted suitably and analyzed for drug content at 269 nm using UV-Visible spectrophotometer (UV 160 Shimadzu, Japan). The test was carried out in triplicate (n=3) [21].

Weight Variation (mg): Randomly, twenty tablets were selected after compression, and the mean weight was determined [22]. The test was carried out in triplicate (n=3)

Wetting Time (Sec) and Water Absorption Ratio: Twice folded tissue paper was placed in a petri dish having an internal diameter of 6.5 cm to that 10 ml of purified water containing an eosin dye solution (0.05% w/v) was added to the petri dish. A tablet was carefully placed on the surface of the tissue paper in the Petri dish. The time required for the dye to reach the upper surface of the tablet and to completely wet noted as the wetting time. Water absorption ratio (R) was then determined [23]. The test was carried out in triplicate (n=3). According to the following equation

$$R = (Wa - Wb) / Wb \times 100$$

Where Wa and Wb are tablet weight after and before water absorption, respectively.

***In-vitro* Disintegration Time:** *In-vitro* disintegration time was determined using a modified disintegration method (n=3) by using disintegration tester (Lab India, DS 1400, India) at 37 ± 0.5 °C in distilled water. The tablet was carefully kept in a basket. The time taken for the tablet to disintegrate completely into smaller particles was noted [24]. The test was carried out in triplicate (n=3)

***In-vitro* Release Profile of Nanoparticulate Orodispersible Tablets:** *In-vitro* dissolution studies for orally disintegrating tablets carried using (Electro lab – TDT 08L) paddle method at 50 rpm in 900 ml of 0.1N HCl for 60 min and phosphate buffer (pH 6.8) for 120 min as dissolution media, maintained at 37 ± 0.5 ºC. Five ml aliquots were withdrawn at the specified time intervals, filtered and analyzed spectro-photometrically against blank at 270 nm in 0.1N HCl and 269 in phosphate buffer (pH 6.8) in a UV spectrophotometer. An equal volume of fresh medium, which was prewarmed at 37 ºC, is replaced into the dissolution medium after each sampling to maintain the constant volume throughout the test [25]. Dissolution studies were performed in triplicate. Comparison between the marketed drug and the lyophilized ideal dosage form in 0.1 HCl and pH 6.8. Each value represents the mean ± SD (n=3).

Accelerated Stability Studies: Stability testing of optimized formulation batch F3(c) was carried out to determine the stability of drug and carrier and also to determine the physical stability of formulation under accelerated storage condition. The prepared tablets were placed in borosilicate screw-capped glass containers. The samples were kept at the condition of 45 ºC/75% RH and were analyzed at 0^{th}, 30^{th}, 60^{th} and 90^{th} days [26].

Statistical Analysis: The results of the experiments are given as a mean sample ± standard deviation (SD) and were analysed according to t-test and one-way analysis of variance (ANOVA) using Sigma Plot 11 software at which significant results were of ($p<0.05$), highly significant of ($p<0.01$) and non-significant ($p>0.05$) [27].

RESULTS AND DISCUSSION:

Particle Size Analysis, Polydispersity Index (PI), Zeta Potential and Drug Entrapment Efficiency:

TABLE 4: PARTICLE SIZE, PI, ZETA POTENTIAL AND DEE OF CLOPIDOGREL BISULPHATE (CB) NANOPARTICLES

S. no.	Batch number	Particle size diameter (nm)	Polydispersity Index (PDI)	Zeta Potential (MV)	% DEE
1	F1	402.1	2.439	-11.3	85.1
2	F2	547.5	3.998	-0.1	68.6
3	F3	844.2	0.912	-20.7	97.1
4	F4	154.7	0.050	-0.9	52.5
5	F5	345.2	0.912	-3.1	92.4
6	F6	402.9	2.439	-3.0	42.4
7	F7	507.3	0.269	-9.3	46.7
8	F8	485.6	2.851	-8.2	67.4
9	F9	458.2	2.397	-0.1	82.6
10	F10	447.7	0.659	-19.1	88.4
11	F11	256.2	0.843	-9.7	84.3
12	F12	614.8	0.819	-19.6	94.7
13	F13	347.5	0.525	-12.38	89.5
14	F14	430.0	0.438	-5.9	85.1
15	F15	632.6	0.526	-6.9	75.4

The particle size and the polydispersity index (PDI) of the prepared nanoparticles were measured by ABT-9000 Nanolaser particle size analyzer. Particle size was expressed by the volume moment mean diameter, as this mean is quite sensitive to the presence of large particles and therefore considered to the most suitable for comparing different nanoparticle formulations. The average particle size of Clopidogrel nanoparticles from all the formulae was found to be in the range of 154.7 nm to 844.2 nm that is summarized in **Table 4**.

For the effective size reduction of the drug particles, water-soluble polymers and surfactants have been used as stabilizers to inhibit the particles agglomeration and improve the physicochemical properties of the drug. The formulae (F11-F15) that contain one stabilizer yield particles sizes ranged from 256.2 nm to 632.6 nm, using Poloxamer-188, L-arginine and tween 80 as primary stabilizers, PVPK-30, PVA are polymeric non-ionic stabilizers for nanosuspensions they stabilize the system by steric stabilization which is achieved by adsorbing polymers onto the drug particles surface through an anchor segment that strongly interacts with the dispersed particles, while the other well-solvated tail segment extends into the bulk medium while arginine HCl (cationic amino acid) and tween 80 (non-ionic surfactant) are electrostatic stabilizers stabilized the nanoparticle by countered Vander Waals attractions between particles, additionally promoted wetting and dispersion of the drug particles, which is usually very hydrophobic.

The formulae (F1- F10) using a combination of two stabilizers yield particle sizes ranged from 154.7 - 844.2 nm. The most significant effect ($p<0.01$) of the combination was shown in the formulae that contain L-Arginine, PVP K-30 with another stabilizer, in F3 at ratio 1:2 when L-Arginine, PVA used as primary stabilizers the particle size was 844.2 nm which is the larger particle size ($p<0.01$) indicate poor stabilization and their combination was not appropriate for Clopidogrel bisulphate (CB) nanoparticles.

Drug Entrapment Efficiency (DEE): Drug entrapment efficiency of the formulations showed in the range of 42.4% to 97.1%. The results have been shown in **Table 4**.

In-vitro Dissolution Profile of Clopidogrel Bisulphate (CB) Nanoparticles by Using 0.1N HCl and Phosphate Buffer (pH 6.8)

TABLE 5: *IN-VITRO* DISSOLUTION PROFILE OF F1 TO F5 IN 0.1N HCl

Time (Min)	Cumulative % drug release				
	F1 ± SD	F2 ± SD	F3 ± SD	F4 ± SD	F5 ± SD
0	0	0	0	0	0
10	67.2 ± 0.12	74.2 ± 0.73	89.2 ± 0.16	69.4 ± 0.52	62.5 ± 0.11
20	71.4 ± 0.72	76.1 ± 0.13	91.1 ± 0.65	74.3 ± 0.27	73.5 ± 0.35
30	80.1 ± 0.13	82.3 ± 0.41	93.5 ± 0.36	78.9 ± 0.03	76.4 ± 0.84
40	83.2 ± 0.34	85.4 ± 0.94	95.4 ± 0.58	82.1 ± 0.26	81.3 ± 0.36
50	91.5 ± 0.85	91.4 ± 0.48	97.4 ± 0.28	87.9 ± 0.35	86.7 ± 0.77
60	93.1 ± 0.35	92.3 ± 0.14	99.0 ± 0.85	91.6 ± 0.63	93.3 ± 0.25

SD = Standard deviation (n=3). The difference in mean of cumulative % drug release between batch series 'F' was significant ($p<0.05$)

TABLE 6: *IN-VITRO* DISSOLUTION PROFILE OF F6 TO F10 IN 0.1N HCl

Time (Min)	Cumulative % drug release				
	F6 ± SD	F7 ± SD	F8 ± SD	F9 ± SD	F10 ± SD
0	0	0	0	0	0
10	77.2 ± 0.25	64.2 ± 0.63	69.2 ± 0.52	71.4 ± 0.36	72.5 ± 0.63
20	81.4 ± 0.63	75.1 ± 0.25	71.1 ± 0.26	74.3 ± 0.84	75.5 ± 0.26
30	84.1 ± 0.74	81.3 ± 0.47	73.5 ± 0.14	78.9 ± 0.27	86.4 ± 0.39
40	88.2 ± 0.95	89.4 ± 0.27	85.4 ± 0.11	85.1 ± 0.27	91.3 ± 0.13
50	92.5 ± 0.37	90.4 ± 0.28	87.4 ± 0.27	88.9 ± 0.73	93.7 ± 0.26
60	94.1 ± 0.27	91.3 ± 0.83	89.4 ± 0.63	92.6 ± 0.80	95.3 ± 0.84

SD = Standard deviation (n=3). The difference in mean of cumulative % drug release between batch series 'F' was significant ($p<0.05$)

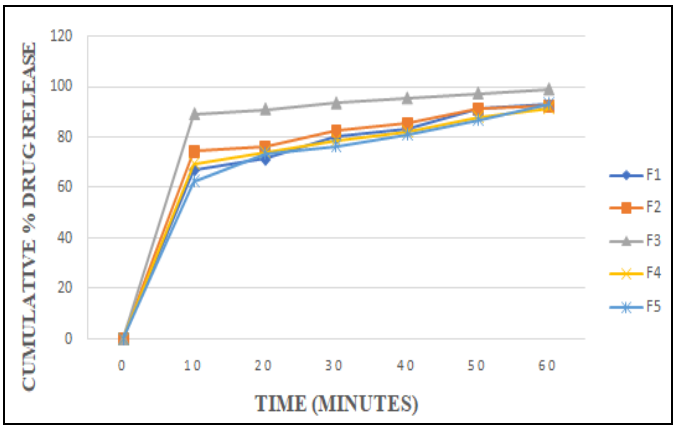


FIG. 1: CUMULATIVE % DRUG RELEASE OF F1 TO F5 IN 0.1N HCl

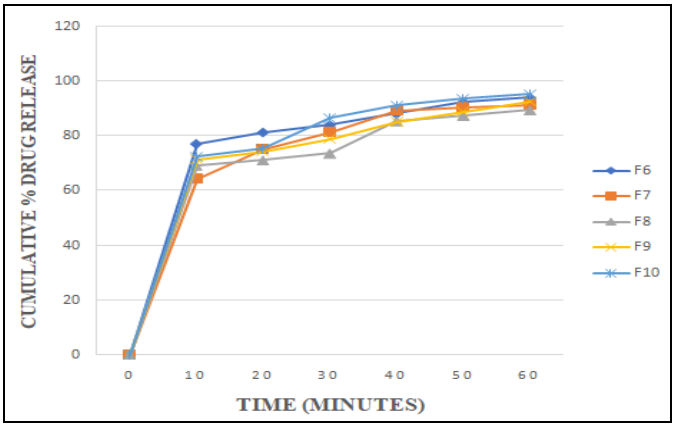


FIG. 2: CUMULATIVE % DRUG RELEASE OF F6 TO F10 IN 0.1N HCl

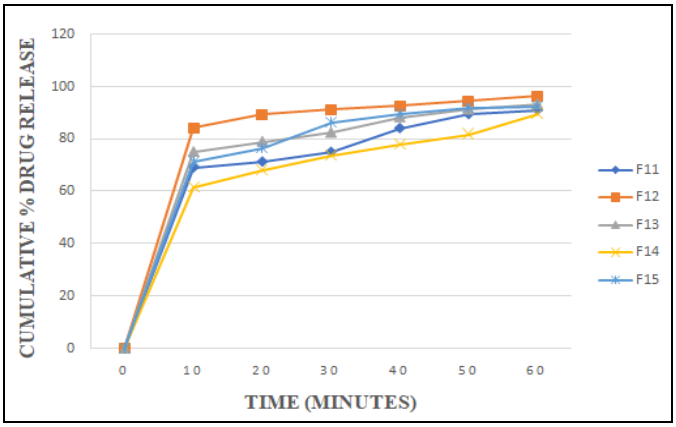


FIG. 3: CUMULATIVE % DRUG RELEASE OF F11 TO F15 IN 0.1N HCl

TABLE 7: *IN-VITRO* DISSOLUTION PROFILE OF F11 TO F15 IN 0.1N HCl

Time (Min)	Cumulative % drug release				
	F11 ± SD	F12 ± SD	F13 ± SD	F14 ± SD	F15 ± SD
0	0	0	0	0	0
10	69.2 ± 0.25	84.2 ± 0.25	75.2 ± 0.16	61.4 ± 0.25	71.5 ± 0.36
20	71.4 ± 0.53	89.1 ± 0.15	79.1 ± 0.52	68.3 ± 0.27	76.5 ± 0.27
30	75.1 ± 0.84	91.3 ± 0.05	82.5 ± 0.84	73.9 ± 0.11	86.3 ± 0.15
40	84.2 ± 0.26	92.4 ± 0.74	88.4 ± 0.53	78.1 ± 0.81	89.3 ± 0.30
50	89.5 ± 0.22	94.4 ± 0.35	91.4 ± 0.23	81.9 ± 0.18	91.7 ± 0.48
60	91.1 ± 0.15	96.3 ± 0.42	93.4 ± 0.42	89.6 ± 0.73	92.3 ± 0.94

SD = Standard deviation (n=3). The difference in mean of cumulative % drug release between batch series 'F' was significant ($p < 0.05$)

TABLE 8: *IN-VITRO* DISSOLUTION PROFILE OF F1-F5 IN PHOSPHATE BUFFER (pH 6.8)

Time (Min)	Cumulative % drug release				
	F1 ± SD	F1 ± SD	F1 ± SD	F1 ± SD	F1 ± SD
0	0	0	0	0	0
10	81.3 ± 0.32	77.7 ± 0.53	92.2 ± 0.11	72.2 ± 0.21	88.1 ± 0.43
20	82.1 ± 1.53	78.3 ± 0.62	93.3 ± 0.44	73.1 ± 0.51	80.1 ± 0.34
30	84.3 ± 0.54	79.1 ± 0.21	94.1 ± 0.54	78.3 ± 0.31	86.9 ± 0.32
40	85.2 ± 0.59	80.9 ± 0.72	94.1 ± 0.52	79.1 ± 0.35	91.1 ± 0.73
50	87.1 ± 1.98	74.1 ± 0.34	94.8 ± 1.87	79.6 ± 0.62	92.3 ± 0.64
60	87.8 ± 0.33	78.9 ± 0.91	96.4 ± 0.64	81.1 ± 1.94	93.1 ± 0.54
70	89.6 ± 0.22	79.8 ± 0.42	97.5 ± 0.43	81.6 ± 1.63	93.3 ± 0.43
80	92.9 ± 0.77	82.1 ± 0.74	97.6 ± 0.32	82.1 ± 0.54	94.9 ± 0.32
90	92.3 ± 0.61	84.2 ± 0.21	98.4 ± 0.21	84.3 ± 0.63	95.1 ± 0.62
100	93.8 ± 0.88	86.8 ± 0.71	98.3 ± 0.52	87.1 ± 0.42	95.4 ± 0.21
110	95.1 ± 0.21	89.1 ± 0.99	99.9 ± 0.42	92.1 ± 0.12	96.1 ± 0.32
120	96.8 ± 0.82	90.1 ± 0.33	99.3 ± 0.82	95.8 ± 0.74	96.9 ± 0.73

SD = Standard deviation (n=3). The difference in mean of cumulative % drug release between batch series 'F' was significant ($p < 0.05$)

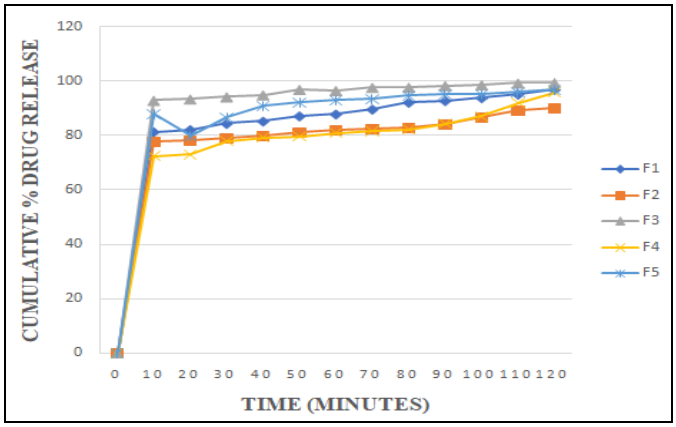


FIG. 4: CUMULATIVE % DRUG RELEASE OF F1 TO F5 IN PHOSPHATE BUFFER (pH 6.8)

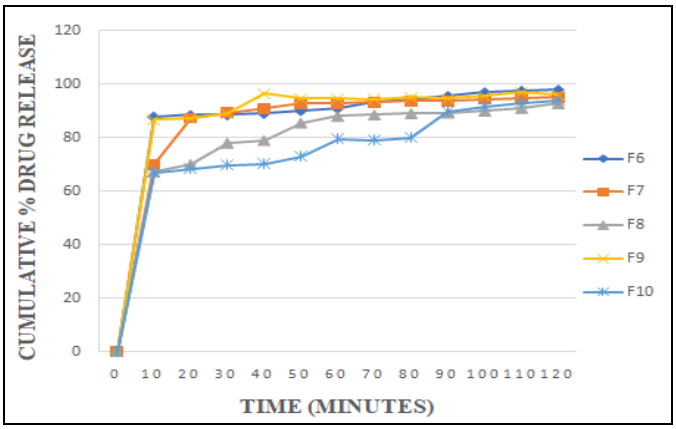


FIG. 5: CUMULATIVE % DRUG RELEASE OF F6 TO F10 IN PHOSPHATE BUFFER (pH 6.8)

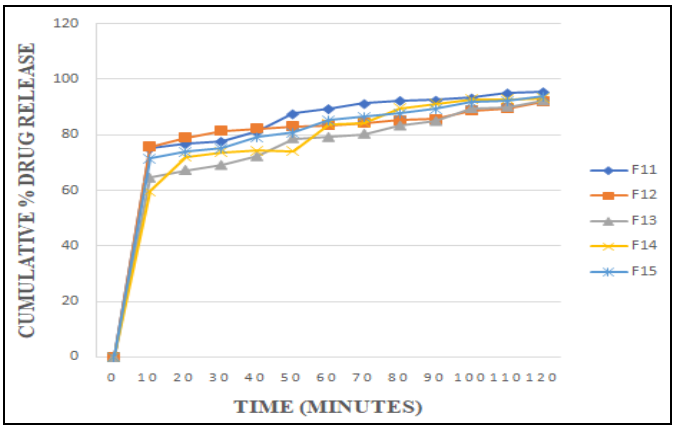


FIG. 6: CUMULATIVE % DRUG RELEASE OF F11 TO F15 IN PHOSPHATE BUFFER (pH 6.8)

TABLE 9: *IN-VITRO* DISSOLUTION PROFILE OF F6-F10 IN PHOSPHATE BUFFER (pH 6.8)

Time (Min)	Cumulative % drug release				
	F6 ± SD	F7 ± SD	F8 ± SD	F9 ± SD	F10 ± SD
0	0	0	0	0	0
10	89.2 ± 0.43	70.1 ± 0.65	67.2 ± 0.53	86.5 ± 1.44	67.1 ± 0.99
20	88.1 ± 1.76	87.4 ± 0.23	70.1 ± 1.44	87.2 ± 1.88	68.1 ± 1.87
30	88.3 ± 0.64	89.3 ± 1.89	78.1 ± 0.22	89.1 ± 0.43	69.5 ± 0.22
40	89.1 ± 0.24	91.1 ± 0.86	79.1 ± 0.33	96.2 ± 0.81	70.1 ± 0.11
50	90.1 ± 0.52	92.6 ± 0.43	85.6 ± 0.78	94.6 ± 0.31	72.1 ± 0.23
60	91.2 ± 0.62	89.3 ± 1.87	88.1 ± 1.42	94.5 ± 0.75	72.3 ± 1.87
70	93.1 ± 0.91	88.1 ± 1.89	88.6 ± 1.97	94.2 ± 0.31	79.1 ± 0.12
80	94.2 ± 0.74	91.6 ± 1.77	89.1 ± 0.45	95.1 ± 1.90	80.1 ± 1.87
90	95.1 ± 1.62	93.7 ± 1.86	89.3 ± 0.35	94.3 ± 0.31	89.6 ± 0.66
100	96.6 ± 0.76	92.4 ± 0.53	90.1 ± 0.21	95.3 ± 0.88	91.6 ± 1.76
110	97.4 ± 1.99	94.1 ± 0.43	91.1 ± 0.30	96.8 ± 1.43	92.9 ± 0.88
120	97.9 ± 0.11	93.8 ± 0.11	92.8 ± 1.65	95.9 ± 0.87	93.9 ± 1.65

SD = Standard deviation (n=3). The difference in mean of cumulative % drug release between batch series 'F' was significant ($p<0.05$)

TABLE 10: *IN-VITRO* DISSOLUTION PROFILE OF F11-F15 IN PHOSPHATE BUFFER (pH 6.8)

Time (Min)	Cumulative % drug release				
	F11 ± SD	F12 ± SD	F13 ± SD	F14 ± SD	F15 ± SD
0	0	0	0	0	0
10	74.2 ± 1.99	75.5 ± 0.32	64.5 ± 0.87	59.2 ± 1.65	71.3 ± 1.23
20	76.1 ± 0.54	78.9 ± 1.87	67.3 ± 0.65	72.1 ± 0.55	74.1 ± 0.77
30	77.3 ± 0.76	81.5 ± 0.65	69.1 ± 1.65	73.1 ± 0.21	75.3 ± 0.34
40	81.1 ± 0.54	82.1 ± 1.87	72.2 ± 1.98	74.2 ± 0.98	79.2 ± 0.23
50	87.4 ± 0.82	83.1 ± 1.98	78.7 ± 0.44	73.9 ± 1.33	80.7 ± 0.22
60	89.2 ± 0.31	83.4 ± 0.33	79.1 ± 0.56	83.4 ± 0.43	85.1 ± 0.11
70	91.1 ± 0.64	84.1 ± 0.22	80.3 ± 0.34	84.1 ± 0.41	86.6 ± 0.56
80	92.2 ± 0.19	85.2 ± 0.54	83.2 ± 0.23	89.4 ± 0.87	87.9 ± 0.33
90	92.3 ± 0.47	85.6 ± 1.98	84.1 ± 0.22	91.1 ± 0.24	89.3 ± 1.87
100	93.1 ± 0.15	88.6 ± 0.86	89.5 ± 1.87	92.6 ± 1.99	91.8 ± 0.33
110	94.9 ± 0.18	89.4 ± 1.76	90.1 ± 0.43	92.2 ± 0.43	92.1 ± 1.98
120	95.3 ± 0.24	91.9 ± 0.23	92.3 ± 0.32	93.1 ± 0.54	93.8 ± 031

SD = Standard deviation (n=3). The difference in mean of cumulative % drug release between batch series 'F' was significant ($p<0.05$)

TABLE 11: DISSOLUTION PROFILE OF PURE DRUG AND F3 IN 0.1N HCl

Time (Min)	Cumulative % drug release	
	F3 ± SD	Pure drug(CB) ± SD
0	0	0
10	92.6 ± 0.81	19.7 ± 1.22
20	93.1 ± 0.32	43.3 ± 0.34
30	94.4 ± 1.23	58.1 ± 0.15
40	96.6 ± 0.43	64.7 ± 0.73
50	98.8 ± 0.51	85.6 ± 0.32
60	99.4 ± 0.62	93.48 ± 0.73

SD = Standard deviation (n=3). The difference in mean of cumulative % drug release between batch series 'F' was significant ($p<0.05$)

TABLE 12: DISSOLUTION PROFILE OF PURE DRUG AND F3 IN PHOSPHATE BUFFER (pH 6.8)

Time (Min)	Cumulative % drug release	
	F3 ± SD	Pure drug(CB) ± SD
0	0	0
10	92.2 ± 0.11	12.34 ± 0.08
20	98.34 ± 1.45	18.5 ± 1.93
30	97.15 ± 0.32	29.5 ± 0.52
40	95.45 ± 0.67	40.6 ± 0.31
50	95.11 ± 1.64	48.9 ± 0.53
60	97.59 ± 1.74	74.5 ± 0.42
70	97.52 ± 0.53	81.9 ± 1.87
80	97.61 ± 0.43	84.5 ± 0.64
90	99.32 ± 1.54	87.6 ± 0.63
100	98.76 ± 0.21	92.4 ± 0.64
110	98.32 ± 0.33	94.5 ± 1.43
120	99.32 ± 0.82	95.8 ± 0.21

SD = Standard deviation (n=3). The difference in mean of cumulative % drug release between batch series 'F' was significant ($p<0.05$)

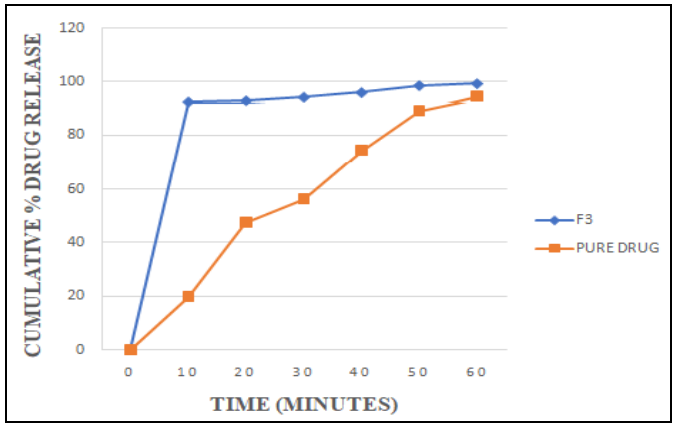


FIG. 7: CUMULATIVE % DRUG RELEASE OF PURE DRUG AND F3 IN 0.1N HCl

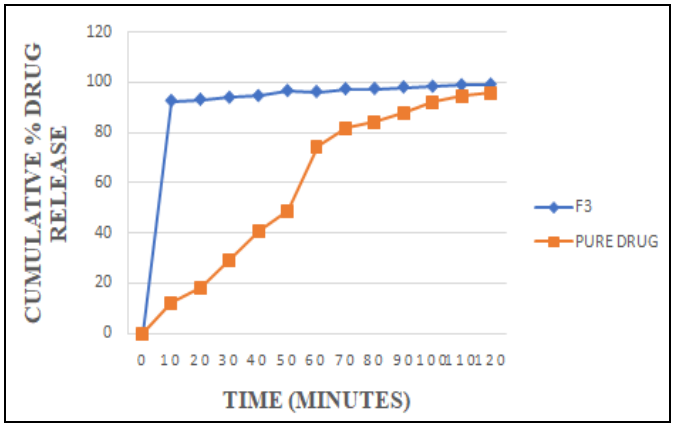


FIG. 8: CUMULATIVE % DRUG RELEASE OF PURE DRUG AND F3 IN PHOSPHATE BUFFER (pH 6.8)

The dissolution studies were performed for the fifteen formulae of prepared Clopidogrel nanoparticles in comparison with the formulae in both the media as represented. F3 is showing the maximum drug release of 99.47 ± 0.62% compared to all the formulations by using 0.1N HCl in 60 min and the dissolution profile by using the phosphate buffer (pH 6.8) the maximum drug release is F3 formulation at 99.32 ± 0.82% with a comparison of the all the formulations in 120 min. In 0.1N HCl, the cumulative percentage drug release of F3 was 92.62 ± 0.81% within 10 min, whereas the pure drug having a release of 19.7 ± 1.22% in 10 min as represented. In pH 6.8 the pure drug having a release of 12.34 ± 0.08% in 10 min whereas the F3(c) having the drug release of 99.16 ± 1.87% as represented. This may be attributed to the fact that the reduction of drug particle size caused the surface area to increase and consequently to enhance the contact between nanoparticles and the dissolution medium.

The obtained results are in good accordance with the Noyes-Whitney equation which states that the increase in saturation and the decrease in particle size lead to an increased dissolution rate. F3 containing L-arginine and PVA as stabilizers were selected to formulate Clopidogrel nanoparticles as a tablet dosage form. F3 was considered as the selected formula because it has a higher dissolution rate in comparison with another formula.

Formulation Optimisation of Clopidogrel Bisulphate Nanoparticles of Tablets:

Freeze-Drying of Liquid Nanoparticles (Lyophilisation): After the evaluation of the prepared formulae (F1-F15), the selected formula was lyophilized using vacuum freeze dryer at a controlled temperature of -44 ºC and the pump operating at a pressure of 2.5 × 10 pascals over a period of 48-72 h. The yielded powder was used for further studies and it is used to prepare the tablets.

Drug-Excipient Compatibility Studies by Using FTIR:

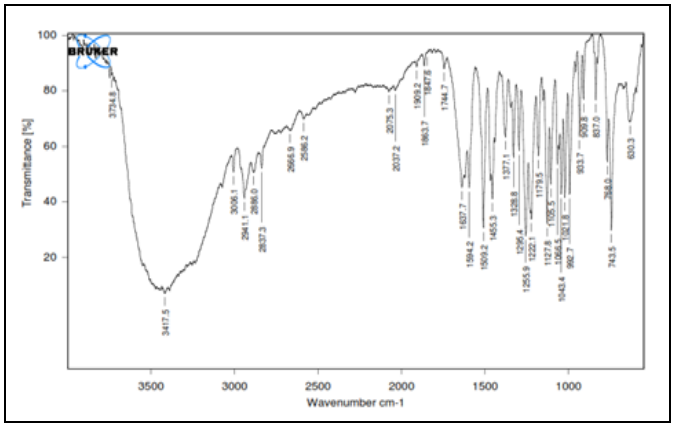


FIG. 9: FTIR SPECTRUM OF CLOPIDOGREL BISULPHATE (CB) NANOPARTICLE

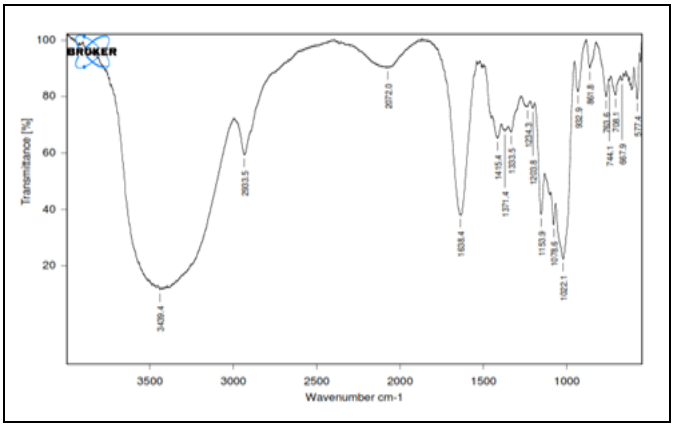


FIG. 10: FTIR SPECTRUM (CB) NANOPARTICLE + CROSS POVIDONE (CP)

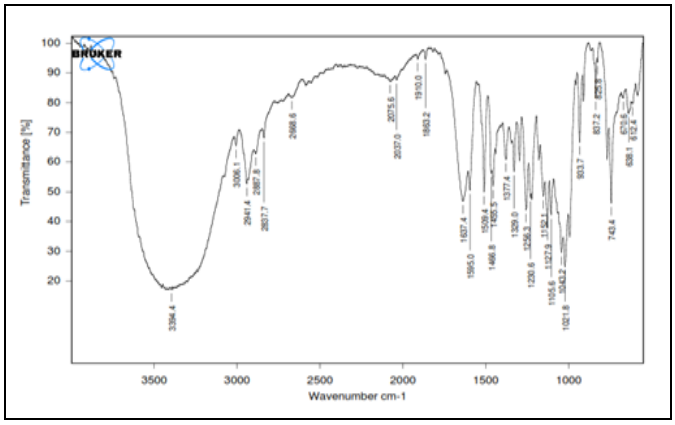


FIG. 11: FTIR SPECTRUM OF (CB) NANOPARTICLE + SODIUM STARCH GLYCOLATE (SSG)

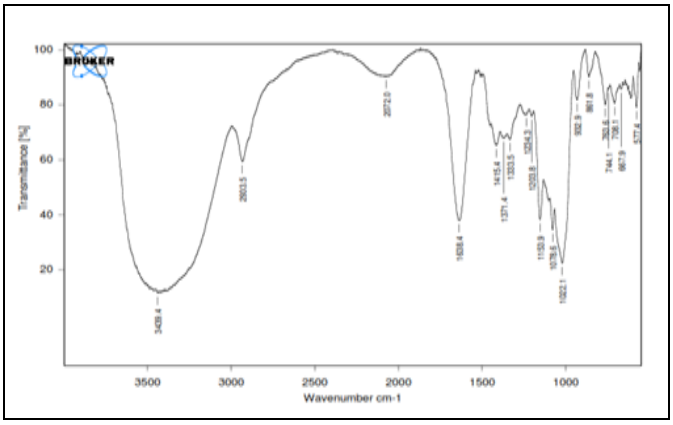


FIG. 12: FTIR SPECTRUM OF (CB) NANOPARTICLE + MCC

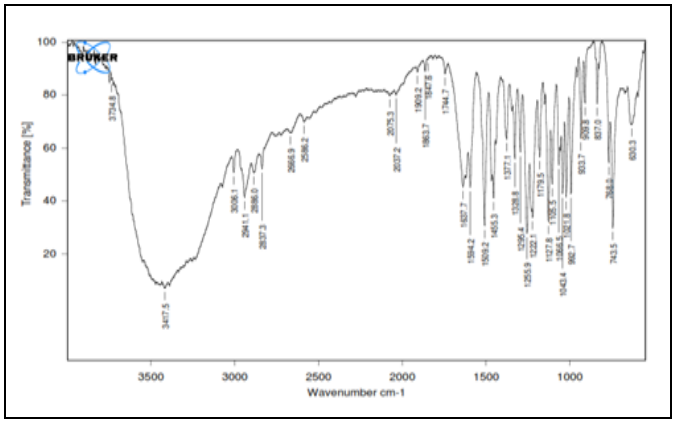


FIG. 13: FTIR SPECTRUM OF (CB) NANOPARTICLE + CP + SSG + MCC

TABLE 13: FTIR INTERPRETATION OF CLOPIDOGREL BISULPHATE NANOPARTICLE TABLET

S. no.	Materials	FTIR Spectrum	Groups	Stretching/ Bending
1	(CB) nanoparticle	3417.5	C=O	Stretching
		2075.3	C=C	Stretching
		1744.4	C=C	Bending
2	(CB) nanoparticle + CP	3439.4	N-H	Stretching
		2933.5	C-H	Stretching
		1638.4	C-H	Stretching
3	(CB) nanoparticle + SSG	3394.4	C-H	Stretching
		3006.1	O-H	Stretching
		1637.4	C=C	Stretching
4	(CB) nanoparticle + MCC	3417.5	C=O	Stretching
		2941.1	C-F	Stretching
		1455.3	C-H	Bending
5	(CB) nanoparticle + CP + SSG + MCC	3006.1	O-H	Stretching
		1637.4	C=C	Stretching
		2933.5	C-H	Stretching

The FT-IR spectra of the Clopidogrel bisulphate nanoparticles, MCC, Cross povidone and sodium starch glycollate, the drug-polymer mixture was recorded to check interaction between drug and polymers. The characteristic peaks of Clopidogrel bisulphate (CB) appeared in all the spectra and values were shifted due to the formation of the complex. The results showed that the characteristic peak of lyophilized clopidogrel nanoparticle was 3417.5 cm^{-1} which is due to C=O stretching of the carbonyl as a functional group present in all the spectrum. This indicated that there was no chemical interaction between lyophilized clopidogrel nanoparticle and other excipients.

Differential Scanning Calorimetry (DSC):

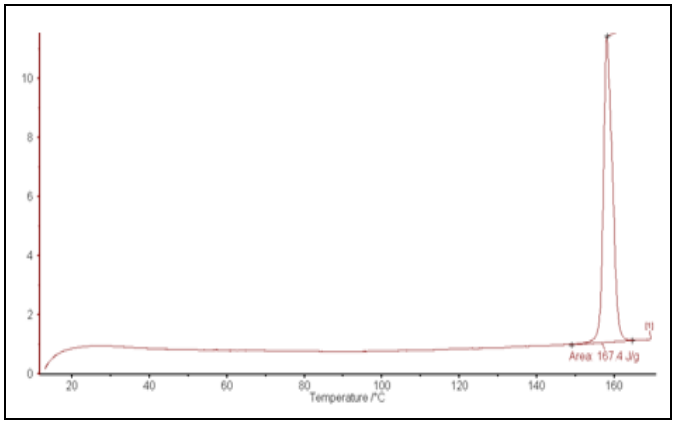


FIG. 14: DIFFERENTIAL SCANNING CALORIMETRY OF LYOPHILISED CLOPIDOGREL BISULPHATE (CB) NANOPARTICLE

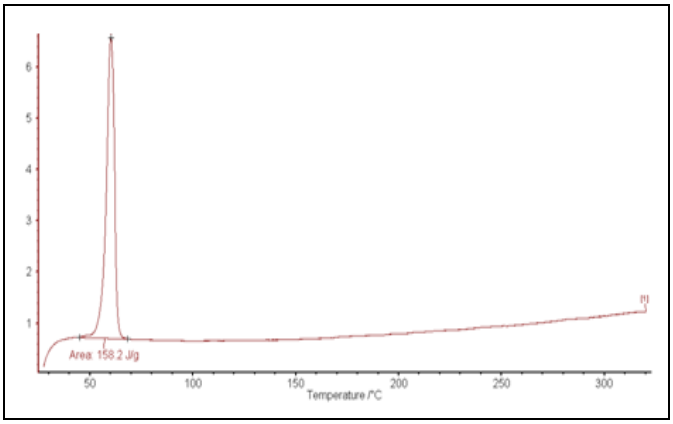


FIG. 15: DIFFERENTIAL SCANNING CALORIMETRY (CB) NANOPARTICLES PREPARED BY USING CROSS POVIDONE (CP)

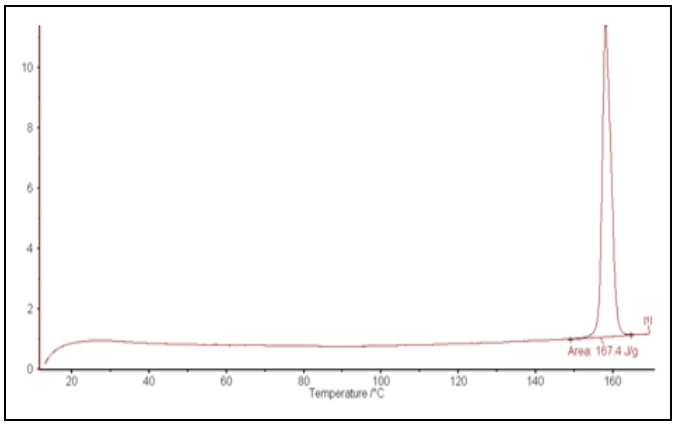


FIG. 16: DIFFERENTIAL SCANNING CALORIMETRY (CB) NANOPARTICLES PREPARED BY USING MICRO CRYSTALLINE CELLULOSE (MCC)

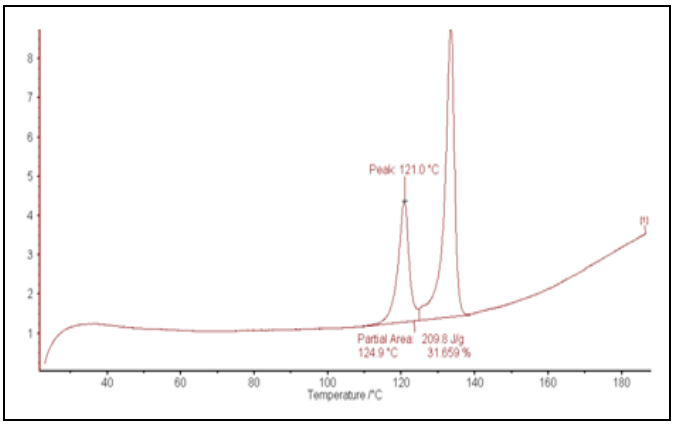


FIG. 17: DIFFERENTIAL SCANNING CALORIMETRY (CB) NANOPARTICLES PREPARED BY USING SODIUM STARCH GLYCOLATE (SSG)

While in the lyophilized powder the melting point of Clopidogrel bisulphate nanoparticle having the exothermic peak at 158.2 ºC, 135.4 ºC and 133.6 ºC which corresponds to its melting point this means that the drug loses the crystalline state and converted to an amorphous form. This may be due to the conditions used to prepare the clopidogrel nanoparticles to lead to the cause of complete drug amorphization. The melting point of the clopidogrel nanoparticles was estimated and found agrees well with the DSC data.

Scanning Electron Microscopy (SEM):

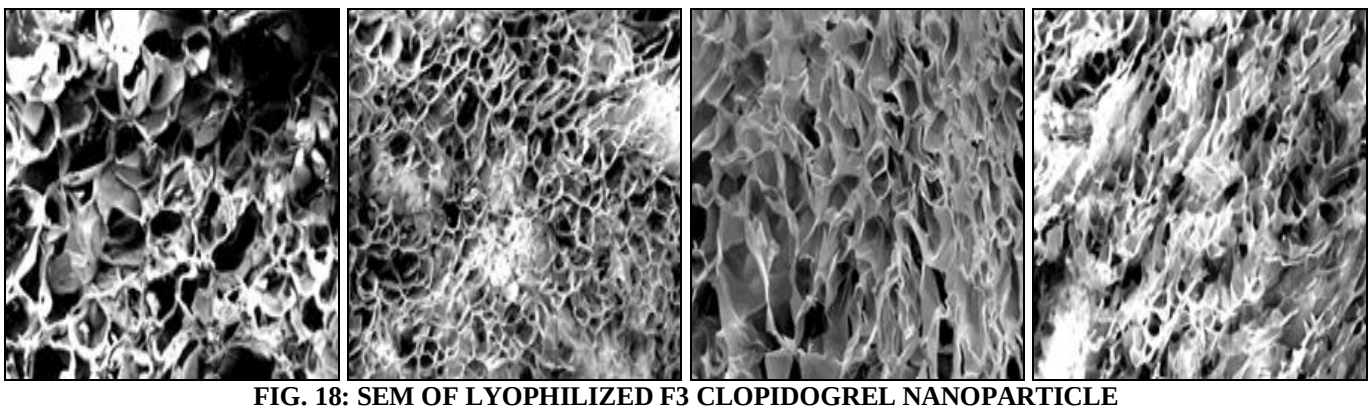


FIG. 18: SEM OF LYOPHILIZED F3 CLOPIDOGREL NANOPARTICLE

SEM of lyophilized F3 Clopidogrel nanoparticle shown in **Fig. 18**. The raw drug particles have a rough surface with large particle size while the SEM of F3 liquid deposit showed small particle. It was seen that stabilizers were adsorbed onto the drug particle surface inhibiting particle growth.

Transmission Electron Microscopy (TEM):

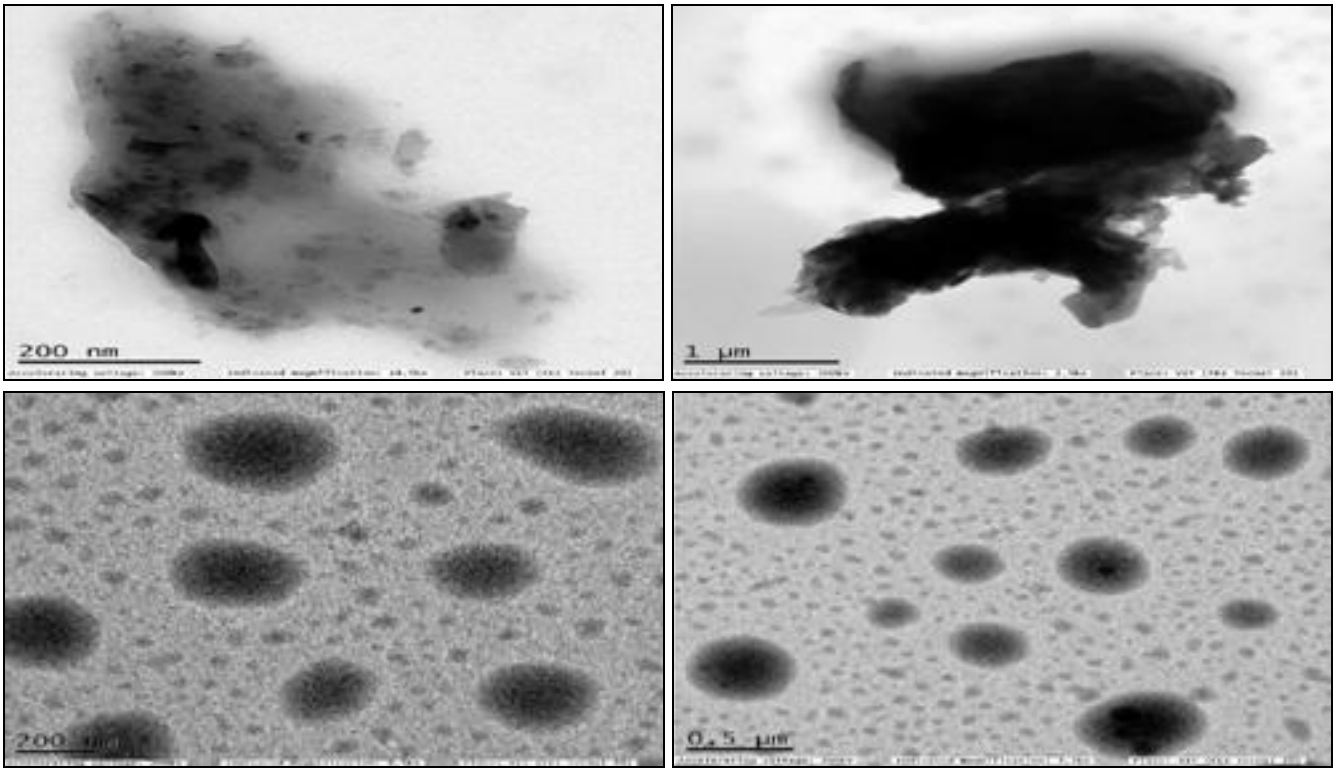
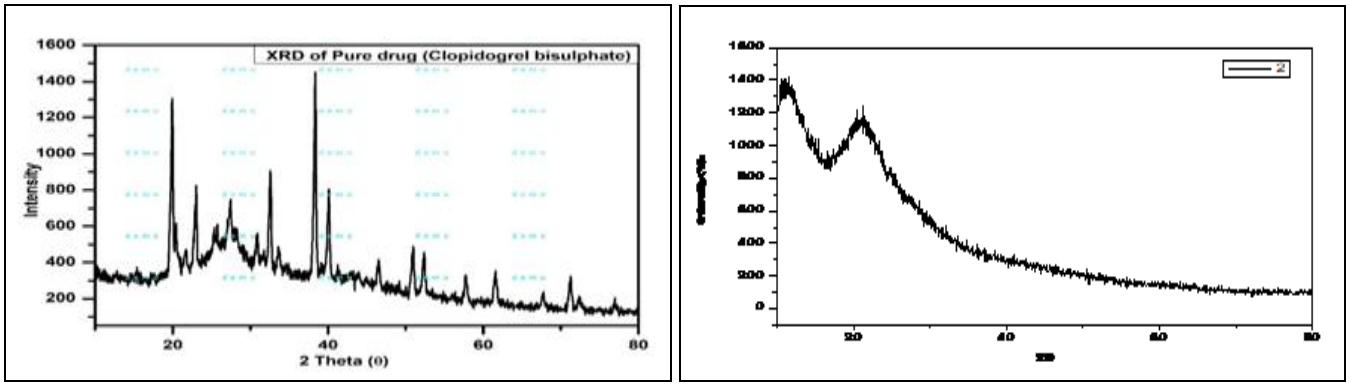


FIG. 19: TEM OF LYOPHILIZED F3 CLOPIDOGREL NANOPARTICLE

TEM of lyophilized F3 Clopidogrel nanoparticle shown in **Fig. 19**. In this report, the size and shape provide important control over many of the properties of physical, chemical and catalytic properties of nanomaterials. When nanomaterials interact with biological systems, their properties are changed significantly, it may also be desirable characters in sometimes. While in contact with a biological fluid they may become coated with proteins and other bio-molecules. Capping agents assisted synthesis methods usually produce spherical particles due to the low surface energy associated with particles.

Powder X-Ray Diffraction:



(A) XRD OF PURE DRUG (CB) **B) XRD OF LYOPHILISED CLOPIDOGREL NANOPARTICLE F3 POWDER**

FIG. 20: POWDER X-RAY DIFFRACTION OF DRUG LYOPHILIZED CLOPIDOGREL NANOPARTICLE OF F3

The results obtained from DSC reasonably agreed with the results obtained by PXRD. The change in the crystalline state of the dried Clopidogrel bisulphate nanoparticles was further confirmed by X-ray diffraction. The X-ray patterns of the pure drug displayed the presence of numerous narrow and symmetrical characteristic diffraction peaks, the strongest 3 peaks are 19º, 28º and 39º at 2θ and with high intensity this indicated the crystalline structure of the drug, while XRD for lyophilized Clopidogrel nanoparticle F3 having no sharp peaks when compared to that of raw drug indicating that the crystalline structure of Clopidogrel was lost this means that the drug loses the crystalline state and converted to an amorphous form.

Pre-compression Evaluation Studies:

TABLE 14: PRE-COMPRESSION STUDIES OF CLOPIDOGREL NANOPARTICLE TABLET

Parameters	F3(a) ± SD	F3(b) ± SD	F3(c) ± SD	F3(d) ± SD	F3(e) ± SD	F3(f) ± SD
Angle of repose*(θ)	25.22 ± 0.13	27.71 ± 0.36	30.17 ± 0.15	23.14 ± 0.12	28..58 ± 0.29	21.22 ± 0.40
Loose bulk density (LBD) (g/cm^3)	0.57 ± 0.12	0.54 ± 0.75	0.62 ± 0.48	0.59 ± 0.15	0.51 ± 0.44	0.60 ± 0.17
Tapped bulk density (TBD) (g/cm^3)	0.71 ± 0.21	0.67 ± 0.18	0.73 ± 0.43	0.69 ± 0.61	0.71 ± 0.68	0.70 ± 0.34
Compressibility Index (%)	15.49 ± 0.31	13.11 ± 0.16	17.80 ± 0.74	14.50 ± 0.25	14.25 ± 0.35	13.43 ± 0.23

SD = Standard deviation (n=3). The difference in mean of cumulative % drug release between batch series 'F' was significant ($p<0.05$)

The results of the angle of repose and compressibility index (%) of the powder of the formulae (F3a-F3f) ranged from (21.22 ± 0.40 to 30.17 ± 0.15) and (13.11 ± 0.16 to 17.80 ± 0.74), respectively. The results of loose bulk density and tapped bulk density ranged from (0.51 ± 0.44 to 0.62 ± 0.48) and (0.67 ± 0.18 to 0.73 ± 0.43), respectively. The results of the angle of repose (<30) indicate good flow properties of granules. This was further supported by lower compressibility index values. The lowest compressibility index is 5-15% which indicates excellent flow properties. All experiments were carried out in triplicate (n=3).

Formulation Development of Orodispersible Tablet of Clopidogrel Bisulphate of F3(c): Tablets containing 75 mg of lyophilized Clopidogrel bisulphate were prepared by direct compression method and the various formulae used in the study are shown in **Table 2**. The drug, diluents superdisintegrants were passed in #40. All the above ingredients were properly mixed (in a poly-bag). Talc and magnesium stearate were passed through sieve # 80 and blended with the initial mixture in a Polybag. The powder blend was compressed into tablets on a ten- station rotary punch – tableting machine (Rimek Mini Press -1) using 7 mm concave punch set.

Post-Compression Studies:

TABLE 15: POST- COMPRESSION EVALUATION OF CLOPIDOGREL BISULPHATE NANOPARTICLE TABLETS

Parameters	F3(a) ± SD	F3(b) ± SD	F3(c) ± SD	F3(d) ± SD	F3(e) ± SD	F3(f) ± SD
Hardness (kg/cm^2)	3.24 ± 0.12	3.70 ± 0.17	3.87 ± 0.21	3.66 ± 0.34	2.96 ± 0.29	3.81 ± 0.13
Friability (%)	0.210 ± 0.01	0.134 ± 0.14	0.349 ± 0.11	0.473 ± 0.03	0.135 ± 0.13	0.276 ± 0.03
Drug content (%)	97.90 ± 1.48	98.51 ± 0.18	99.78 ± 0.41	97.6 ± 0.14	98.4 ± 0.54	98.0 ± 0.31
Weight Variation (mg)	213.70 ± 1.43	211.25 ± 1.22	214.65 ± 1.41	213.16 ± 1.64	210.35 ± 5.03	212.41 ± 1.11
Wetting time (Sec)	18.00 ± 0.67	24.67 ± 0.36	23.67 ± 0.55	28.33 ± 0.59	25.33 ± 0.59	26.00 ± 1.01
Water absorption ratio (%)	19.84 ± 0.663	18.45 ± 2.135	30.89 ± 1.637	37.89 ± 1.345	28.08 ± 1.428	34.09 ± 1.936
In- vitro disintegration time (Sec)	18.33 ± 0.567	19.30 ± 0.556	9.33 ± 1.1	16.33 ± 0.5	11.67 ± 2.0	19.33 ± 0.5

SD = Standard deviation (n=3). The difference in mean of cumulative % drug release between batch series 'F' was significant ($p<0.05$)

The prepared tablets in all the formulations possessed good mechanical strength with significant hardness in the range of 2.96 ± 0.29 to 3.87 ± 0.29 sq. cm. Friability values below 1% were an indication of the good mechanical resistance of the tablets. Formulations prepared by direct compression method were found to be more friable. All the tablets from each formulation passed weight variation was within the pharmacopeia limits of ± 7.5% of the weight. The weight variation in all six formulations was found to be 0.134 ± 0.14 to 214.65 ± 1.41, which was in pharmacopeia limits of ± 7.5% of the average weight. The percentage drug content of all the tablets was found to be between 97.6 ± 0.14 to 99.78 ± 0.41 of Clopidogrel bisulphate (CB) tablets

which were within the acceptable limits. The wetting time of all the six formulations was performed in triplicate. The values lie between 18.00 ± 0.67 to 28.33 ± 0.59 sec. Tablets were prepared with sodium starch glycolate (SSG) and F3(a) to F3(c) and with Cross Povidone F3(d) to F3(f). The wetting time of the tablets was also considerably reduced in tablets containing crospovidone which may be attributed due to the wicking type of disintegrants (Sodium starch glycollate) formed thus facilitating the disintegrants to bring about faster disintegration. The results of water absorption ratio (%) and *in-vitro* disintegrating time (sec) ranged from 18.45 ± 2.1 to 37.89 ± 1.3 and 9.3 ± 1.14 to 19.33 ± 0.5, respectively. In the quantitative effect that each factor had on the responses is expressed as "Effect" and the level of significance of the quantitative effect is represented by a *p*-value, where p<0.05 is considered statistically significant.

In-vitro Release Profile of Orodispersible Tablets of Clopidogrel Bisulphate (CB) Nanoparticles:

TABLE 16: *IN-VITRO* DISSOLUTION PROFILE OF CLOPIDOGREL BISULPHATE TABLET BY USING 0.1N HCl

Time (Min)	Cumulative % drug release					
	F3(a) ± SD	F3(b) ± SD	F3(c) ± SD	F3(d) ± SD	F3(e) ± SD	F3(f) ± SD
0	0	0	0	0	0	0
10	78.2 ± 0.87	84.2 ± 0.12	91.2 ± 0.40	59.4 ± 0.14	52.5 ± 0.23	61.5 ± 0.61
20	81.4 ± 0.25	89.1 ± 0.13	93.1 ± 0.48	64.3 ± 0.38	63.5 ± 0.36	64.6 ± 0.45
30	83.1 ± 0.65	86.3 ± 0.36	95.7 ± 0.63	68.9 ± 0.67	66.4 ± 0.74	69.7 ± 0.39
40	88.2 ± 0.58	88.4 ± 0.75	97.4 ± 0.55	72.1 ± 0.46	71.3 ± 0.45	75.4 ± 0.87
50	85.0 ± 0.37	94.4 ± 0.64	98.4 ± 0.83	77.9 ± 0.85	86.7 ± 0.84	83.2 ± 0.54
60	91.1 ± 0.57	96.3 ± 0.83	99.1 ± 0.31	85.6 ± 0.63	89.3 ± 0.03	89.4 ± 0.92

SD = Standard deviation (n=3). The difference in mean of cumulative % drug release between batch series 'F' was significant (p<0.05)

TABLE 17: *IN-VITRO* DISSOLUTION PROFILE OF CLOPIDOGREL BISULPHATE TABLET WITH PHOSPHATE BUFFER (pH 6.8)

Time (Min)	Cumulative % drug release					
	F3(a) ± SD	F3(b) ± SD	F3(c) ± SD	F3(d) ± SD	F3(e) ± SD	F3(f) ± SD
0	0	0	0	0	0	0
10	76.2 ± 0.35	67.2 ± 0.35	94.2 ± 0.48	62.4 ± 0.76	68.5 ± 0.45	71.5 ± 0.46
20	82.4 ± 0.47	74.4 ± 0.57	95.4 ± 0.18	74.3 ± 0.47	71.5 ± 0.85	74.6 ± 0.28
30	85.1 ± 0.37	79.1 ± 0.37	95.1 ± 0.85	73.9 ± 0.46	76.4 ± 0.73	79.7 ± 0.38
40	87.2 ± 0.27	81.2 ± 0.17	96.2 ± 0.73	79.1 ± 0.35	81.3 ± 0.37	81.4 ± 0.82
50	89.5 ± 0.28	85.5 ± 0.84	97.5 ± 0.52	71.9 ± 0.32	86.7 ± 0.28	86.2 ± 0.28
60	91.1 ± 0.71	87.1 ± 0.38	97.1 ± 0.41	85.6 ± 0.82	88.3 ± 0.72	89.7 ± 0.18
70	92.7 ± 0.48	89.7 ± 0.28	97.7 ± 0.86	88.1 ± 0.37	89.2 ± 0.27	91.3 ± 0.57
80	93.3 ± 0.28	92.3 ± 0.29	98.3 ± 0.65	89.1 ± 0.27	91.4 ± 0.31	91.4 ± 0.38
90	94.4 ± 0.17	93.4 ± 0.21	98.4 ± 0.38	89.9 ± 0.86	92.3 ± 0.76	92.4 ± 0.27
100	94.5 ± 0.37	94.5 ± 0.38	98.5 ± 0.18	91.9 ± 0.39	94.5 ± 0.27	93.2 ± 0.27
110	96.5 ± 0.27	96.5 ± 0.17	99.2 ± 0.38	92.1 ± 0.21	95.4 ± 0.21	94.3 ± 0.83
120	97.4 ± 0.91	98.4 ± 0.29	99.5 ± 0.73	93.2 ± 0.48	96.3 ± 0.81	95.3 ± 0.47

SD = Standard deviation (n=3). The difference in mean of cumulative % drug release between batch series 'F' was significant (p<0.05)

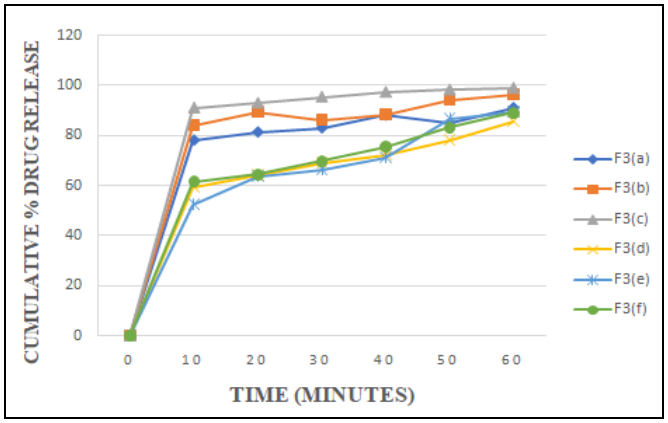


FIG. 21: CUMULATIVE % DRUG RELEASE OF CLOPIDOGREL BISULPHATE TABLET BY USING 0.1N HCl

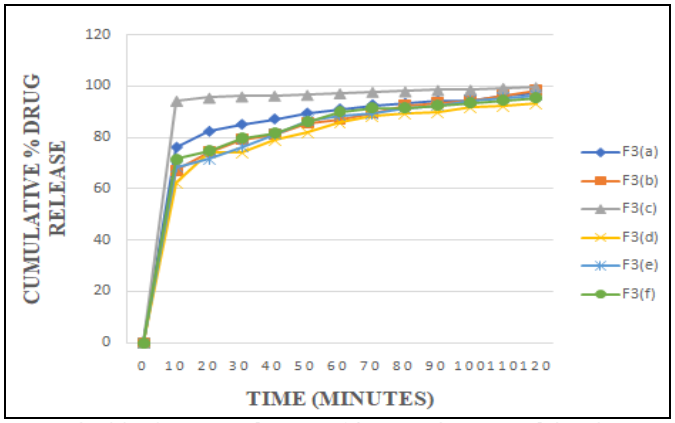


FIG. 22: CUMULATIVE % DRUG RELEASE OF CLOPIDOGREL BISULPHATE TABLET BY USING PHOSPHATE BUFFER (pH 6.8)

TABLE 18: *IN-VITRO* DISSOLUTION PROFILE OF THE LYOPHILIZED DOSAGE FORM OF F3(C) WITH THE MARKETING DRUG LOPIN IN THE 0.1N HCl

Time (Min)	Cumulative % drug release	
	F3(c) ± SD	Plavix ± SD
0	0	0
10	91.2 ± 0.40	19.7 ± 0.54
20	93.1 ± 0.48	32.8 ± 0.38
30	95.7 ± 0.63	64.5 ± 0.28
40	97.4 ± 0.55	75.6 ± 0.18
50	98.4 ± 0.83	81.5 ± 0.53
60	99.1 ± 0.31	90.2 ± 0.27

SD = Standard deviation (n=3). The difference in mean of cumulative % drug release between batch series 'F' was significant ($p<0.05$)

TABLE 19: *IN-VITRO* DISSOLUTION PROFILE OF THE LYOPHILISED DOSAGE FORM OF F3(C) WITH THE MARKETING DRUG LOPIN IN PHOSPHATE BUFFER (pH 6.8)

Time (Min)	Cumulative % drug release	
	F3(c) ± SD	Plavix ± SD
0	0	0
10	94.2 ± 0.48	15.7 ± 0.32
20	95.4 ± 0.18	28.8 ± 0.18
30	95.1 ± 0.85	39.5 ± 0.37
40	96.2 ± 0.73	45.6 ± 0.29
50	97.5 ± 0.52	69.5 ± 0.28
60	97.1 ± 0.41	71.2 ± 0.84
70	97.7 ± 0.86	79.4 ± 0.74
80	98.3 ± 0.65	84.3 ± 0.21
90	98.4 ± 0.38	90.1 ± 0.31
100	98.5 ± 0.18	92.4 ± 0.82
110	99.2 ± 0.38	94.3 ± 0.74
120	99.5 ± 0.73	96.8 ± 0.16

SD = Standard deviation (n=3). The difference in mean of cumulative % drug release between batch series 'F' was significant ($p<0.05$)

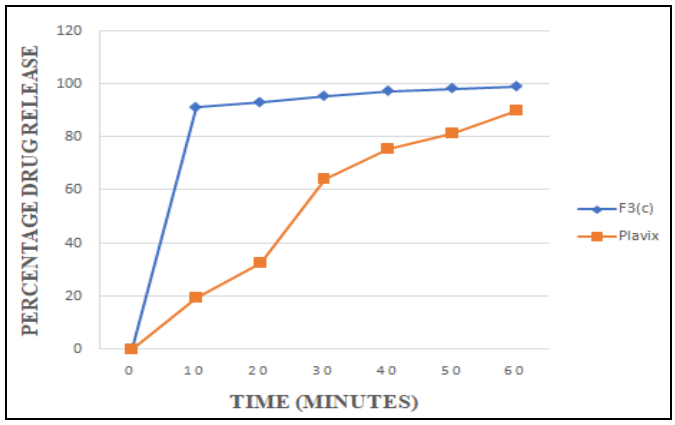


FIG. 23: CUMULATIVE % DRUG RELEASE OF THE LYOPHILISED DOSAGE FORM OF F3(C) WITH THE MARKETING DRUG PLAVIX IN 0.1N HCl

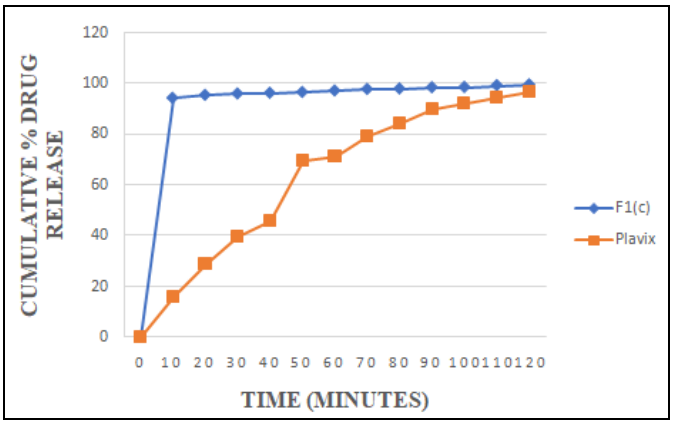


FIG. 24: CUMULATIVE % DRUG RELEASE OF THE LYOPHILISED DOSAGE FORM OF F3(C) WITH THE MARKETING DRUG PLAVIX IN PHOSPHATE BUFFER (pH 6.8)

The dissolution studies were performed for the six formulae of prepared Clopidogrel bisulphate (CB) tablet in comparison with the formulae. The *in-vitro* dissolution profile indicated faster and maximum drug release at 99.1 ± 0.31% in formulation F3(c) using 0.1N HCl, and *in-vitro* dissolution profile indicated faster and maximum drug release at 99.5 ± 0.73% in formulation F3(c) using phosphate buffer (pH 6.8). In Comparison with the ideal formulation F3(c) with the marketed product (Plavix) by using the 0.1N HCl for the time interval of 10 min the cumulative drug release of the F3(c) 91.2 ± 0.40, whereas the marketed drug (Plavix) the cumulative drug release in 10 min is 19.7 ± 0.54. In pH 6.8 the marketed drug (Plavix) having a release of 15.7 ± 0.32% in 10 min,

whereas the ideal formulation F3(c) the cumulative drug release in 10 min is 94.2 ± 0.48%. F3(c) had a dissolution rate compared with other formula and marketed tablet (Plavix). Microcrystalline cellulose (MCC) and Sodium starch glycolate (SSG) enhances dissolution by speeding tablet disintegration and utilizes dual disintegration mechanisms of wicking and swelling for more disintegration so that MCC act as dissolution enhancer. F3(c) was considered the selected formula to form a tablet containing clopidogrel bisulphate nanoparticles.

Accelerated Stability Studies:

TABLE 20: ACCELERATED STABILITY STUDIES OF THE IDEAL BATCH F3 (C)

Parameter / Days	Hardness (kg/cm^2) Mean ± SD	Drug content (%) Mean ± SD	*In-vitro* disintegration time (sec) Mean ± SD	*In-vitro* drug release study Mean ± SD (10 min)
0	3.93 ± 0.11	99.71 ± 1.22	9 ± 1.16	99.02 ± 0.42
30	3.64 ± 1.8	96.2 ± 0.61	11.4 ± 0.12	94.07 ± 0.61
60	3.32 ± 2.1	93.9 ± 0.72	22.1 ± 0.16	92.32 ± 0.12
90	3.12 ± 1.2	91.1 ± 0.12	45.3 ± 0.12	91.02 ± 0.37

An optimized batch F3(c) was carried for stability study to its higher release, lower disintegration time, uniform content uniformity. So, accelerated stability studies (AST) was carried for optimized batch F3(c) exposing it to 45 ºC/75% RH for 0, 30, 60 and 90 days. The sample was analyzed for hardness, drug content *in-vitro* disintegration time and *in-vitro* dissolution studies in 10 min using phosphate buffer (pH 6.8).

CONCLUSION: The study conclusively demonstrated significant results for Lyophilised clopidogrel bisulphate orodispersible nano-particulate dosage form tablet. Anti-solvent precipitation method can be used as an effective tool for preparation of Nanosized formulations. clopidogrel bisulphate nanoparticles prepared by this method showed a significant improvement in aqueous solubility as well as dissolution characteristics which may significantly improve its oral bioavailability.

Hence, at the end of this investigation, it can be concluded that orodispersible nanoparticulate dosage form of a tablet of clopidogrel bisulphate was successfully prepared by direct compression method and anti-solvent evaporation method be a useful approach to produce nanoparticles of poorly soluble drugs.

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CONFLICT OF INTEREST: The authors declare there are no conflicts of interests regarding this study.

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