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Research Article

# FORMULATION AND EVALUATION OF CLOPIDOGREL TABLET INCORPORATING DRUG NANOPARTICLES

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**ABSTRACT**

Objective: The present study aims to enhance the dissolution rate of a poorly water-soluble drug, clopidogrel bisulfate by the preparation of the nanoparticles.

Methods: Nanoparticles of clopidogrel were produced by antisolvent-precepitation method using different stabilizers at drug :stabilizer ratio 1:2 alone and in combination and characterized for particle size, drug entrapment efficiency (DEE), dissolution testing, scanning electron microscopy imaging (SEM) and atomic force microscopy (AFM). Lyophilized nanoparticles were compressed into tablets by direct compression method and evaluated by different methods. Compatibility studies (Fourier transform infrared spectroscopy (FTIR) and differential scanning calorimetry (DSC) ), and powder x-ray diffraction (PXRD) were also done.

Results: Through this study, the particle size was ranged from 5.29 to 480 nm and %DEE was ranged from 83% to 97%. Amongst all formulations F13,stabilized with PVPK-30 and PVA, showed complete dissolution (100%) at the end of 10 minutes in both media 0.1N HCL (pH 1.2) and PBS (pH 6.8).SEM of clopidogrel nanoparticles confirmed small particle size without aggregation. Particle size of F13 obtained by AFM was 250 nm. F13a tablet characterized by short disintegration time, high hardness, low friability and produced higher dissolution rate in comparison with the marketed tablet. FTIR study revealed that there is no chemical interaction between clopidogrel and the excipients. DSC and XRD illustrated that the crystallinity of clopidogrel was lost in lyophilized powder and tablet and converted to an amorphous form.

Conclusion: Antisolvent precipitation method was a promising method to produce clopidogrel nanoparticles with markedly enhanced dissolution rate.

**Keywords:** Clopidogrel, Nanoparticles, Anti-solvent precipitation, Tablet, PVPK-30, PVA.

## INTRODUCTION

Solubility enhancement of poorly water-soluble drugs is a crucial issue to improve solubility and bioavailability. Numerous attempts to improve the dissolution behavior have been made by using solid dispersions of drugs with polymers, inclusion complexes with cyclodextrins, liposomes, emulsions, and microemulsions [1].

Recently, nanoparticle engineering processes have been developed and reported for pharmaceutical applications to increase the dissolution rate of low-soluble drugs which in turn may leads to substantial increases in bioavailability [2]. Nanoparticle engineering enables poorly soluble drugs to be formulated as particles alone, or with a combination of pharmaceutical excipients. By decreasing the particle size from a micron to a nanometer scale, there is a significant increase in the surface area and related dissolution rate [3].

Current techniques used to obtain drug nanoparticles can be divided into two categories : bottom up and top down technologies. In the bottom up technologies the low water soluble drugs are dissolved in a solvent and then precipitated in different ways in a surfactant solution. The top down technologies are based on particle fragmentation to submicron units and include ball milling and high-pressure homogenization [4].

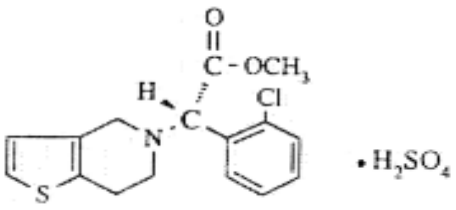



**Fig. 1: Chemical structure of clopidogrel bisulfate**

Clopidogrel is a potent anti-platelet drug, with the brand name Plavix® selectively inhibits the binding of adenosine diphosphate (ADP) to its platelet receptor [5]. It is indicated for the prevention of vascular thrombotic events in patients at risk [6]. Clopidogrel bisulfate is a weak base known chemically as Methyl (S)- α-(2chlorophenyl)-6,7-dihydrothieno[3,2-c]pyridine-5(4H)-acetate sulfate (1:1) [figure (1)].It is practically insoluble in water at neutral pH, freely soluble in aqueous buffer at pH 1, and in methanol, sparingly soluble in methylene chloride, and practically insoluble in ethyl ether [7].

According to the biopharmaceutics classification system (BCS), Clopidogrel is categorized as a class II agent (poorly water soluble and highly permeable) [8]. Oral bioavailability of clopidogrel is very low (less than 50%), due to poor water solubility [9]. The aim of this work is to formulate the clopidogrel nanoparticles by antisolvent precipitation method and find out the effect of stabilizer (alone and in combination) on the formulation, when all parameters of operation are kept constant.To overcome the particles growth, lyophilization was carried out in order to assess the feasibility of transferring nanosuspensions to a dry powder which was further incorporated as a tablet [10].

## MATERIALS AND METHODS

### Materials

Clopidogrel bisulfate powder was obtained from ZhejiangMenovo Pharmaceutical Co.,LTD,China,the stabilizer used are Poloxamer 188 (pluronic F68) obtained from HiMedia (Mumbai, India), Tween 80 from Scharlau S.L Spain, Polyvinyl pyrollidone K-30 (PVPK-30) from china Polyvinyl alcohol from Barcelona Espana, L-arginine hydrochloride from Riedel-deHaen AG. Sodium lauryl sulfate obtained from Sd Fine-Chem limited MUMBAI. All other chemicals used were of analytical grade.

### Methods

### Preparation of clopidogrel nanoparticles

Clopidogrel nanoparticles were prepared by the precipitation technique which is also called antisolvent precepitation method. Clopidogrel 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°C and subsequently stirred at agitation speed of 250 revolution per minute (rpm) on magnetic stirrer for 1 hour 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. The ratio of drug to surfactant used was 1:2 [11].

15 formulas (F1-F15) were prepared by this technique demonstrated in table 1 with their composition. 98 mg of clopidogrel bisulfate was used which is equivalent to 75 mg of clopidogrel base

**Table 1**: **Formulation of clopidogrel nanosuspension using different stabilizer at ratio 1:2 of drug : stabilizer**

| Material (mg) | F1 | F2 | F3 | F4 | F5 | F6 | F7 | F8 | F9 | F10 | F11 | F12 | F13 | F14 | F15 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Clopidogrel | 98 | 98 | 98 | 98 | 98 | 98 | 98 | 98 | 98 | 98 | 98 | 98 | 98 | 98 | 98 |
| L-arginin HCL | 196 | | | | | 98 | 98 | 98 | 98 | | | | | | |
| Poloxamer 188 | | 196 | | | | 98 | | | | 98 | 98 | 98 | | | |
| PVPK-30 | | | 196 | | | | 98 | | | 98 | | | 98 | 98 | |
| Polyvinyl alcohol | | | | 196 | | | | 98 | | | 98 | | 98 | | 98 |
| Tween 80 (ml) | | | | | 0.2 | | | | 0.1 | | | 0.1 | | 0.1 | 0.1 |
| Methanol (ml) | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| Water (ml) | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |

**Characterization of Nanoparticles**

**Particle size analysis**

Particle size determination of the prepared formulas (F1-F15) was done by using ABT-9000 nano laser particle size analyzer at scattering angle 90º. The average particle size (D[4,3]) which is also called volume moment mean (De Brouckere Mean Diameter) reflects the size of those particles which constitute the bulk of the sample volume was measured after performing the experiment in triplicates. The polydispersity index (PDI) of each formula was also determined as a measurment 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 determine the specific surface area for each sample.

**Determination of drug entrapment efficiency of nanoparticles [12]**

The freshly prepared nanoparticles were centrifuged at 20,000 rpm for 20 minute using ultracentrifuge. The amount of unincorporated drug was measured by taking the absorbance of the appropriately diluted 25 ml of supernatant solution at 269 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.

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

$$\text{Entrapment efficiency } (\%) = \frac{W_{\text{initial drug}} - W_{\text{free drug}}}{W_{\text{initial drug}}} \times 100 \quad \textbf{eq (1)}$$

***In vitro* dissolution study**

In vitro dissolution study was performed using USP dissolution test apparatus-II (paddle assembly). The dissolution was performed using dialysis membrane-60 (HIMEDIA) in 900 ml of phosphate buffer solution (PBS) of pH 6.8 as dissolution mediums containing 1% SLS maintained at 37 ± 0.5°C and 50 rpm for clopidogrel nanoparticles formulas. The freshly prepared clopidogrel nanoparticles (10 ml) add to dialysis bag and fitted to the paddle, samples (5ml) were withdrawn at regular intervals of 10 minutes for 120 minutes and replaced with fresh dissolution medium to maintain sink condition. Samples were filtered through ASHLESS filter paper and assayed spectrophotometrically on UV-VISIBLE spectrophotometer at 269.0 nm wavelength [4, 12, 13]. The release of selected formula was compared with the pure drug (98 mg) in both media of 0.1N HCL and phosphate buffer pH 6.8. The release in 0.1 N HCL was done for 60 minute [14].

**Freeze drying of liquid nanoparticles**

After the evaluation of the prepared formulas (F1-F15), the selected formula was lyophilized using vacuum freeze dryer at a controlled temperature of −44 ◦C and the pump operating at a pressure of 2.5 × 10 pascal over a period of 48–72 hour. The yielded powder was used for further studies and also it is used to prepare the tablets.

**Preparation of clopidogrel bisulfate nanoparticle tablet [15, 16]**

Clopidogrel bisulfate tablets were prepared by direct compression method after freeze drying of formula (F13) that gave the best *in vitro* dissolution profile in 10 minute in comparison with other nanoparticle formulas and pure drug. The amount of lyophilized powder taken was 294 mg equivalent to 98 mg of clopidogrel bisulfate, different clopidogrel nanoparticle tablets were prepared using microcrystalline cellulose MCC (Avicel)® PH 102, PVPK30, polyethylene glycol PEG 6000 and sodium starch glycolate SSG (Explotab)® as a diluent, binder, lubricant and disintegrant at different concentration and tested to obtain the optimum formula that show the accepted hardness and the best *in vitro* dissolution profile. The composition of nanoparticle tablets indicated in table 2.

**Precompression studies of the prepared nanoparticle powder**

The flowability of a powder is of critical importance in the production of pharmaceutical dosage forms in order to get a uniform feed as well as reproducible filling of tablet dies otherwise high dose variations will occur. The powder flowability of prepared clopidogrel tablets were characterized by angle of repose and carr's index [17].

**Evaluation of Clopidogrel Nanoparticle Tablets**

Tablets were evaluated for hardness test, friability test,in vitro disintegration time, content uniformity testand weight variation tests [18], and dissolution study.

**Table 2: Composition of clopidogrel nanoparticle tablets**

| Materials | Quantity per tablet (mg) | | | |
|---|---|---|---|---|
| | F13a | F13b | F13c | F13d |
| Lyophilized powder | 294 | 294 | 294 | 294 |
| MCC (Avicel)® | 100 | 80 | 50 | 92 |
| PVPK 30 | -------- | 23 | 50 | -------- |
| SSG(Explotab)® | -------- | --------- | --------- | 8 |
| PEG 6000 | 6 | 3 | 6 | 6 |
| Tablet weight(mg) | 400 | 400 | 400 | 400 |

***In vitro* dissolution profile of clopidogrel tablet**

In vitro dissolution study was performed using USP dissolution test apparatus-II (paddle assembly). The dissolution was performed using 900 ml of 0.1 N HCL solution of pH 1.2 and phosphate buffer solution (PBS) of pH 6.8 (containing 1% SLS) as dissolution mediums maintained at 37 ± 0.5°C and 50 rpm for tablet formulas (F13a-F13d) in comparison with marketed clopidogrel tablet (Plavix)®. Samples (5ml) were withdrawn at regular intervals of 10 minutes for 60 minutes in 0.1N HCL and 120 minutes in PBS and replaced with fresh dissolution medium to keep sink condition.

Samples were filtered through filter paper and assayed spectrophotometrically on UV-VISIBLE spectrophotometer at 270 nm wavelength for 0.1 N HCL and at 269 nm for PBS pH 6.8. For each formulation the experiment was repeated in triplicate [15, 19].

**Fourier transform infrared spectroscopy (FT-IR)**

The Fourier transform infrared spectroscopy (FT-IR) spectrum were studied to detect any sign of interaction or complexation may occur between clopidogrel and stabilizers used in the preparation of the nanoparticles and with the excipients used in the preparation of tablets. the spectrum was obtained using FT-IR Shimadzu 8300 Japan. Samples which studied are :Pure drug, physical mixtures of (1:1:1) clopidogrel,PVPK-30 and PVA respectively, lyophilized powder of selected formula (F13) and F13a tablet. All these samples were grounded and mixed thoroughly with potassium bromide, at 1:5 (sample : potassium bromide) weight ratio. The spectrum obtained was in between the wave number of 4000-400 $cm^{-1}$[20, 21].

**Differential scanning calorimetry (DSC) study**

DSC can be used to determine the compatibility between the drug and excipients and also used to evaluate the crystalline state of drug especially when converted to nanoparticles.Thermal characteristics of the same materials that examined in FTIR study were determined by an automatic thermal analyzer system (Shimadzu, DSC– 60, Japan). Accurately weighed samples (5mg) were placed in nonhermetically aluminium pans and heated at the rate of 10 ºC/minute against an empty aluminium pan as a reference covering a temperature range of 40 ºC to 300 ºC [22, 23].

**Powder X-ray diffraction analysis (PXRD)**

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 F13 and the F13a tablet were recorded using Shimadzu diffractometer 6000 (Shimadzu, Japan) with input voltage at 220V/50Hz and the measurement condition was at voltage 40 kV and current 30 mA, axis of 2 theta ranged from 5-50 degree [24].

**Surface Morphology Studies**

**Scanning electron microscopy (SEM)**

The morphology of raw drug and clopidogrel nanoparticles (F13) were examined by scanning electron microscope (VEGA3 Tescan Czech republic) operated with a secondary detector at an acceleration voltage of 10 kv and at 100x magnification for raw drug and 15 kv and 10 kx for F13. The morphology of raw drug was done by direct deposition of powder on double-sided carbon tape and coated with gold. While for liquid F13 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 [25].

**Atomic force microscopy (AFM)**

AFM is capable of scanning the surfaces in controlled environmental conditions and is complementary to SEM imaging and also can measure the particle size of the nanoparticles accurately. Droplets of selected formula F13 were deposited on freshly cleaved mica and dried 15 minutes in oven. Particle size,histogram of particle size distribution and 3D surface morphology of clopidogrel nanoparticles were obtained [25].

**Statistical Analysis**

The results of the experiments are given as a mean samples ± standard deviation (SD) and were analyzed 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 ($p>0.05$).

**RESULT AND DISCUSSION**

**Particle size analysis**

**Effects of the stabilizers types**

The particle size and the polydispersity Index (PI) of the prepared nanoparticles were measured by ABT-9000 nano laser particle size analyzer. Particle sizes were expressed by the volume moment mean diameter, D[4,3] as this mean is quite sensitive to the presence of large particles and, therefore, considered to be most suitable for comparing different nanoparticle formulations. The average particle size of clopidogrel nanoparticles from all the formulas was found to be in the range of 5.29 to 482 nm that summarized in table 3.

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.

**Using One Stabilizer**

The formulas (F1-F5) that contain one stabilizer yield particle sizes ranged from 5.29 to 349 nm, using PVPK-30, poloxamer 188, ARG, PVA and tween 80 as primary stabilizers, PVPK-30, poloxamer and PVA are polymeric non ionic stabilizers for nanosuspensions they stabilize the system by steric stabilization which is achieved by adsorbing polymers onto the drug particle surface through an anchor segment that strongly interacts with the dispersed particles [26], while the other well-solvated tail segment extends into the bulk medium while arginine HCL (cationic amino acid ) and tween 80 (nonionic surfactant) are electrostatic stabilizers stabilized the nanoparticle by countered Vander Waals attractions between particles,additionally promotes wetting and dispersion of the drug particles, which are usually very hydrophobic.

The particle size of F2 stabilized with poloxamer 188 is significantly ($p<0.01$) greater than that stabilized with other stabilizers, Poloxamer 188 (pluronic F68) is a block co-polymer, responsible for the hydrophobic interaction with the drug molecule,the crystal growth inhibition is mainly due to the hydrophobic polypropylene oxide group (PPO) in the Pluronic polymer, while the hydrophilic polyethylene oxide (PEO) chains provide steric hindrance against aggregation [27]. Although of this mechanism of poloxamer 188, but it gave larger particle size this may attributed to the insufficient affinity of poloxamer to drug molecule. However, if there is no affinity between the particle surface and the polymer, the attractive forces between two particles become dominant due to depletion of polymer from the gap of two particles (depletion force) [28].

**Using Two Stabilizers**

The formulas (F6-F15) using combination of two stabilizers yield particle sizes ranged from 5.29 to 482 nm. The most significant effect ($p<0.01$) of combination was shown in the formulas that contain poloxamer with other stabilizer,in F2 at ratio 1:2 when poloxamer used as primary stabilizer the particle size was 349 nm, but in F6,F10,F11,F12 polxamer used with ARG, PVP K-30, PVA and tween 80 respectively the particle sizes were 164,6.82,13 and 11.8 nm respectively,that mean the combination have good surface affinity and could form a substantial mechanical and thermodynamic barrier at the interface of dug molecule [29].

Arginine HCL as secondary stabilizer is not always give small particle size, the particle size of F6 is still have large particle size, in addition the particle size of F8 containing ARG and PVA as stabilizers is 482 nm which is the larger particle size ($p<0.01$) indicate poor stabilization and their combination was not appropriate for

clopidogrel nanoparticles. PVA contains a number of OH groups, which might have formed hydrogen bonds with the solvent resulting in an increased viscosity of the dispersion, in addition PVA may form a connected network with the ARG at the interface which may lead to increase the particle size [30].

Electrostatic and steric stabilization are generated to provide energetic barrier hindering agglomeration, it is quite important whether the stabilizers could cover the particle surface immediately and completely. The stabilization ability of the stabilizers is a critical parameter determining the minimal achievable size and subsequent physical stability [31]. The formulations were homogeneous as indicated by polydispersity index that range from 0 - 0.247. Monodisperse samples have a lower PDI value, whereas higher values of PDI indicate a wider particle size distribution and the polydisperse nature of the sample. The usual range of PDI values is; 0-0.05 (monodisperse standard), 0.05-0.08 (nearly monodisperse), 0.08-0.7 (mid range polydispersity), and >0.7 (very polydisperse) [32], the results show that PDI was monodisperse with narrow size distribution. A narrow size distribution is essential to prevent particle growth, due to ostwald ripening phenomenon that is caused by different saturation solubility in the vicinity of differently sized particles. Specific surface area of each samples are also obtained that range from 4.61-419.58 $m^2/kg$ which are inversely proportional to the particle size.

**Drug entrapment efficiency**

Drug entrapment efficiency of the formulations showed in the range of 83% to 97 %. The results have been shown in table 3.

**Table 3: Particle size, polydispersity index and specific surface area of formulas of ratio 1:2 (drug : stabilizer)**

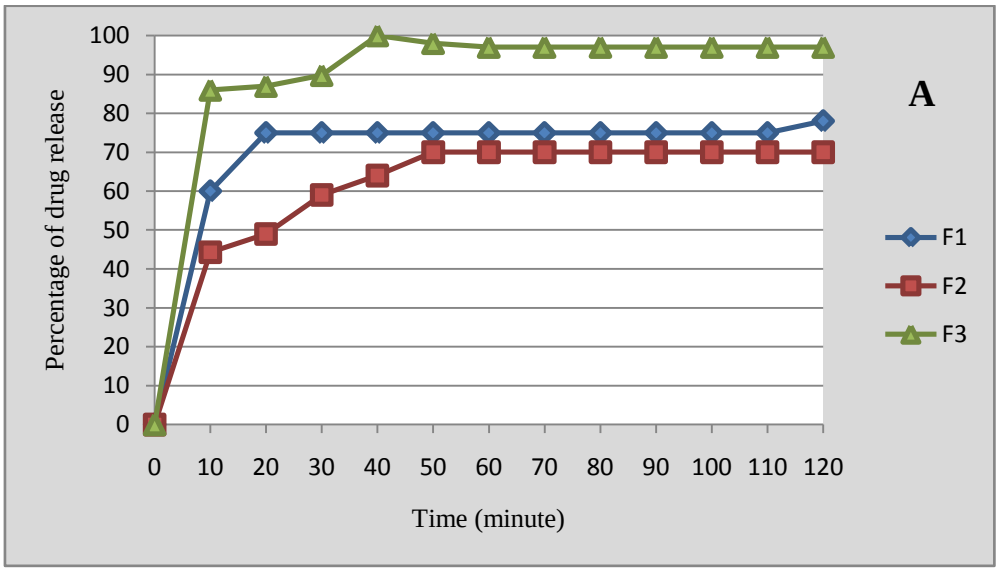
| Formulas | Stabilizers | D[4,3] nm | PDI | SSA ($m^2/g$) | % DEE |
|---|---|---|---|---|---|
| F1 | ARG | 5.29±0 | 0.0 | 419.58 | 95 |
| F2 | Poloxamer 188 | 349±0 | 0.119 | 6.97 | 91 |
| F3 | PVPK-30 | 5.88±0 | 0.015 | 379.19 | 87 |
| F4 | PVA | 16±0 | 0.01 | 139.2 | 87 |
| F5 | Tween 80 | 6.38±0 | 0.014 | 349.77 | 88 |
| F6 | ARG + Poloxamer 188 | 164±0 | 0.247 | 16.55 | 92 |
| F7 | ARG + PVPK-30 | 15.1±0 | 0.008 | 147.51 | 96 |
| F8 | ARG + PVA | 482±0 | 0.01 | 4.61 | 91 |
| F9 | ARG + Tween 80 | 8.67±0 | 0.041 | 261.41 | 88 |
| F10 | PVPK-30+Poloxamer | 6.82±0 | 0.226 | 366.09 | 83 |
| F11 | Poloxamer + PVA | 13±0 | 0.034 | 174.35 | 92 |
| F12 | Poloxamer+Tween 80 | 11.8±0 | 0.016 | 188.01 | 91 |
| F13 | PVPK-30+ PVA | 13.5±0 | 0.007 | 164.62 | 97 |
| F14 | PVPK-30 + Tween 80 | 8.19±0 | 0.017 | 272.88 | 86 |
| F15 | PVA + Tween 80 | 5.29±0 | 0.0 | 419.58 | 95 |

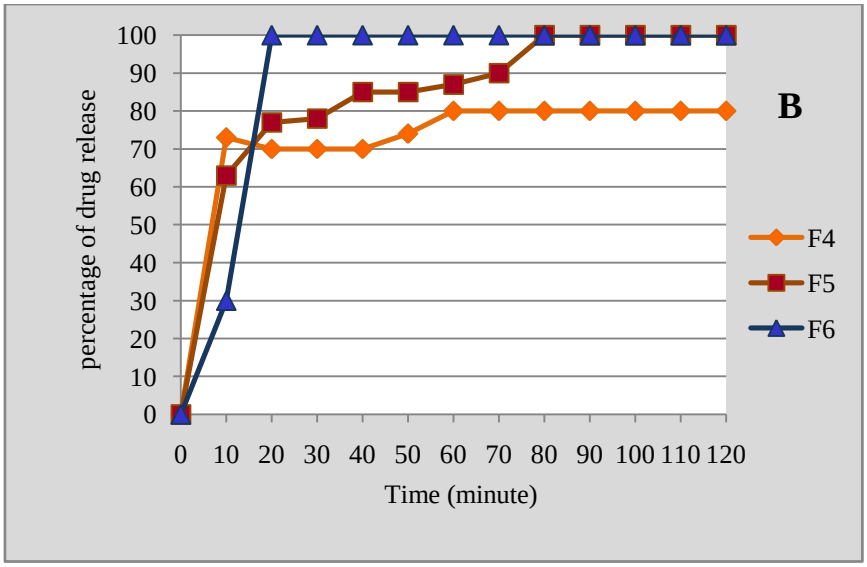
**In Vitro Dissolution Study**

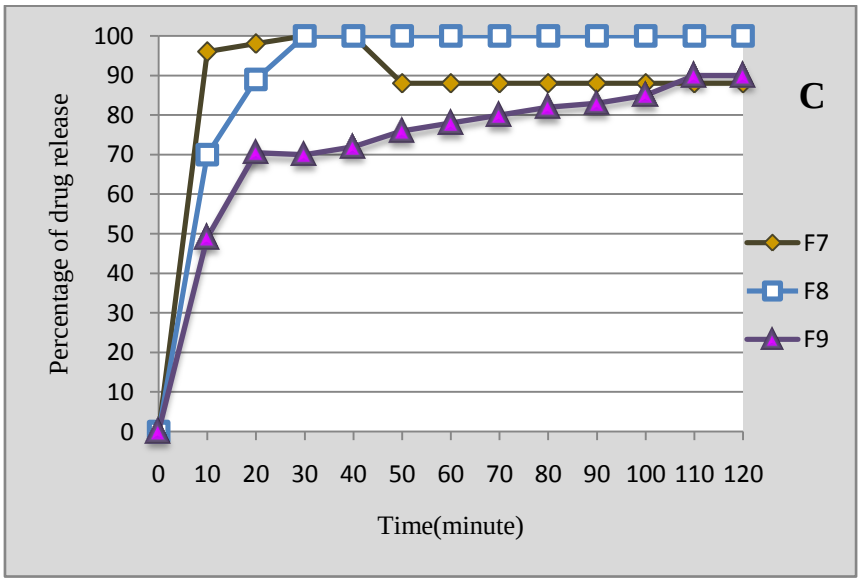
**Effect of stabilizers on the *In vitro* dissolution profile**

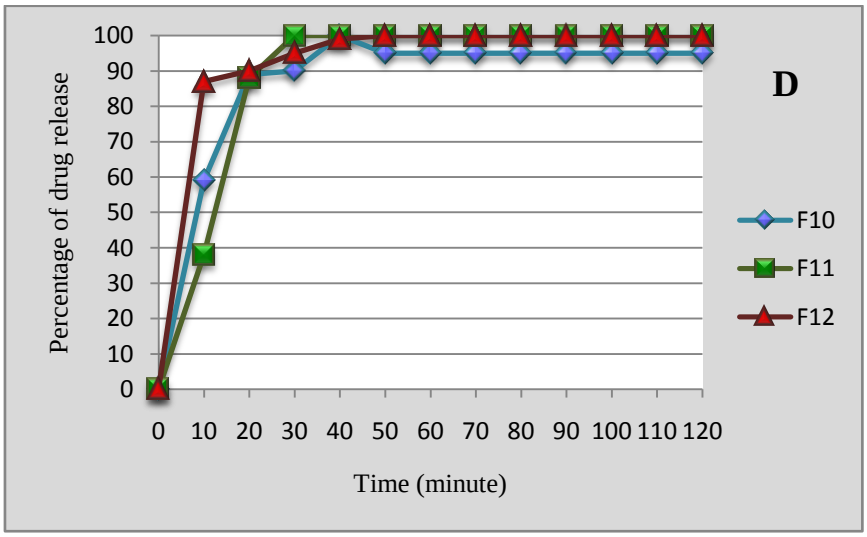
The dissolution profile of all the formulas (F1-F15) were firstly studied in PBS of pH 6.8 with 1% SLS to determine the best formula that give the best release in first 10 minutes. The release of F1-F15 in PBS of pH 6.8 presented in figure (2) [A-E].

From the study the results show that the formula F13 that contain PVP-PVA as stabilizer gave the best release in 10 minutes in comparison with other formula and the formula shows a maximum cumulative percentage drug release of 100 % within 10 minutes, F13 was considered as the selected formula because it had the higher dissolution rate in comparison with other formula.




B
percentage of drug release
Time (minute)
F4
F5
F6


C
Percentage of drug release
Time(minute)
F7
F8
F9


D
Percentage of drug release
Time (minute)
F10
F11
F12

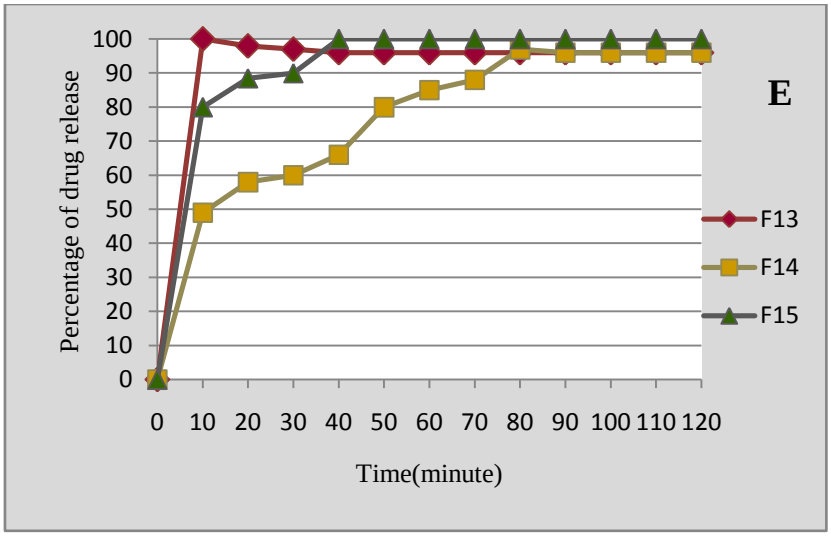



**Fig. 2: Dissolution profile of formulas F1-F15 [A-E] in PBS of pH 6.8. (n=3)**

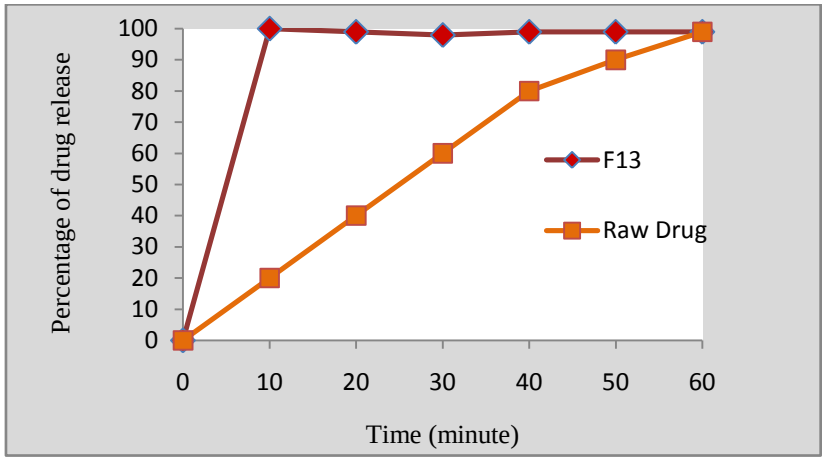



**Fig. 3: Dissolution profile of pure powder and F13 in 0.1N HCL (n=3)**

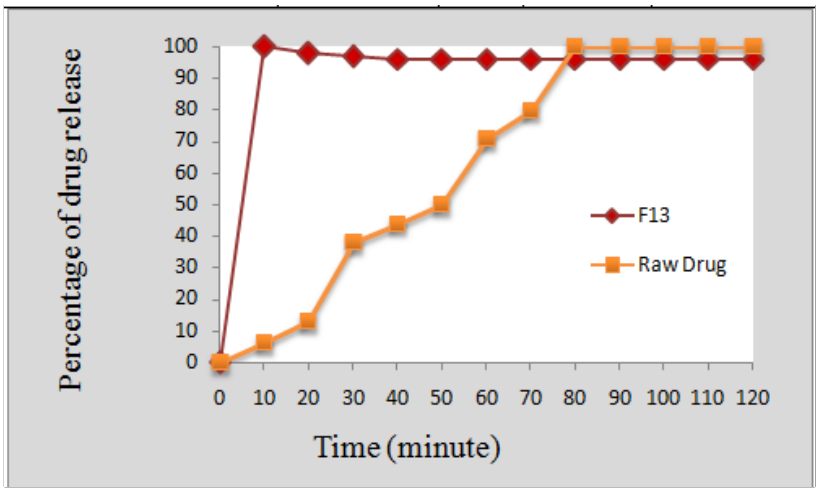



**Fig. 4: Dissolution profile of pure powder and F13 in PBS of pH 6.8 + 1% SLS. (n=3)**

The release of F13 was compared with the pure drug in both media of 0.1N HCL and PBS as in figures (3) and (4) respectively. In 0.1N HCL the maximum cumulative percentage drug release of F13 was 100 % within 10 minute, where as the pure drug having a release of 20 % in 10 minutes and maximum cumulative percentage release reach 100 % in 60 minutes. In PBS of pH 6.8 the pure drug having a release of 6.4 % in 10 minutes and maximum cumulative percentage release reach 100 % in 80 minutes in PBS of pH 6.8 with 1% SLS. 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 dissolution medium. The obtained results are in good accordance with Noyes–Whitney

equation which states that; the increase in saturation solubility and the decrease in particle size lead to an increased dissolution rate.

F13, containing PVP-PVA as stabilizers, was selected to formulate clopidogrel nanoparticles as tablet dosage form.

**Evaluation of clopidogrel nanoparticles powder and tablet**

**Powder flowability:** Angle of repose and compressibility index of the powder of the formulas (F13a-F13d) were reported in table 4.

**Table 4: Flow properties of the prepared formulas**

| Formula | Angle of repose | Carr's index | Physical properties [33] | |
|---|---|---|---|---|
| | | | Angle of repose | Carr's index |
| F13a | 29±0.58 | 7±0.9 | Good | Excellent |
| F13b | 26±3.5 | 25±1.5 | Good | Poor |
| F13c | 46±3.05 | 25±1.2 | Very poor | poor |
| F13d | 25±1.5 | 16± 0.8 | good | good |

**Physical Properties of Tablet**

Physical Properties of clopidogrel nanoparticle tablet were demonstrated in table 5.

From the results F13a had good flowability, compressibility and highest hardness. The high hardness may attributed to the hydrogen bonds formed among the hydroxyl groups of the adjacent cellulose particles of MCC (Avicel)®, which are brought closely together by plastic deformation during compression. F13a formula disintegrate in 8 minutes may be due to the mechanism of action that MCC (Avicel)® is an insoluble swellable material with good disintegrating properties, attributed to either capillary action or swelling action [34].

All tablets obtained were of uniform weight with acceptable variation. The content uniformity was within the acceptable limit which is 85 to 115 % of the average content. Drug content was found between 98-100%.

**Table 5: Physical properties of clopidogrel nanoparticle tablet**

| Formula | Physical Properties | | | |
|---|---|---|---|---|
| | Hardness (kg/cm²) | Friability % | In vitro DT (min) | Weight variation (mg) |
| F13a | 8 ± 0.58 | 0.5 | 8 ± 1.5 | 399.1 ± 1.11 |
| F13b | 7 ± 0.57 | 0.8 | 17 ± 0.7 | 399.2 ± 2.1 |
| F13c | 6.5±0.0 | 0.7 | 20 ± 1.0 | 400.2 ± 0.24 |
| F13d | 6±0.0 | 0.9 | 8 ± 0.9 | 399.9 ± 2.67 |

**Dissolution profile of prepared clopidogrel tablets**

The dissolution studies were done for the 4 formulas of prepared clopidogrel tablet in comparison with the marketed tablet (plavix)®,the dissolution profiles in 0.1 N HCL and PBS pH 6.8 were shown in figures (5) and (6) respectively.

The incorporation of PVPK-30 in F13b and F13c in different concentration as a binder (5.75%) and a carrier (12.5%) respectively, the addition of PVP K-30 at low concentration 5.75% was significantly ($p<0.05$) affect on the release of clopidogrel nanoparticles from F13b, while at high concentration 12.5% the release was ($p<0.1$) very slow. This may due to the effect of binder films that can form viscous gels on the granule surface and will retard dissolutionin. SSG (Explotab)® was used as a disintegrant at 2% in F13d gave good disintegration time and good dissolution behavior in comparison with F13b and F13c, but slower than F13a that mean SSG (Explotab)® has less influence than MCC PH 102 toward dissolution rate of the tablet[35].

F13a had higher dissolution rate compared with other formula and marketed tablet (Plavix)®. MCC (Avicel)® enhances drug dissolution by speeding tablet disintegration, and utilizes dual disintegration mechanisms of wicking and swelling for more rapid disintegration so that MCC act as dissolution enhancer [36]. F13a was considered the selected formula to form a tablet containing clopidogrel nanoparticles.

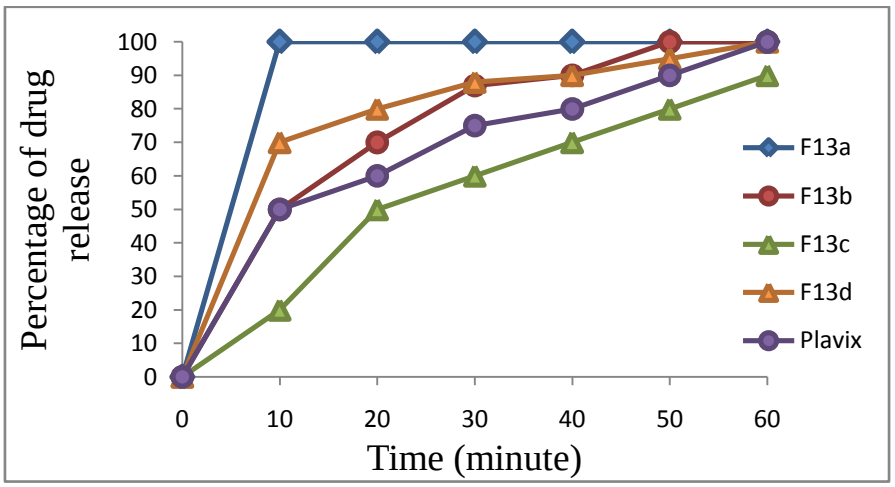



**Fig. 5: Dissolution profile of prepared tablet with marketed tablet in 0.1N HCL. Each value represents the mean ±S.D. (n=3)**

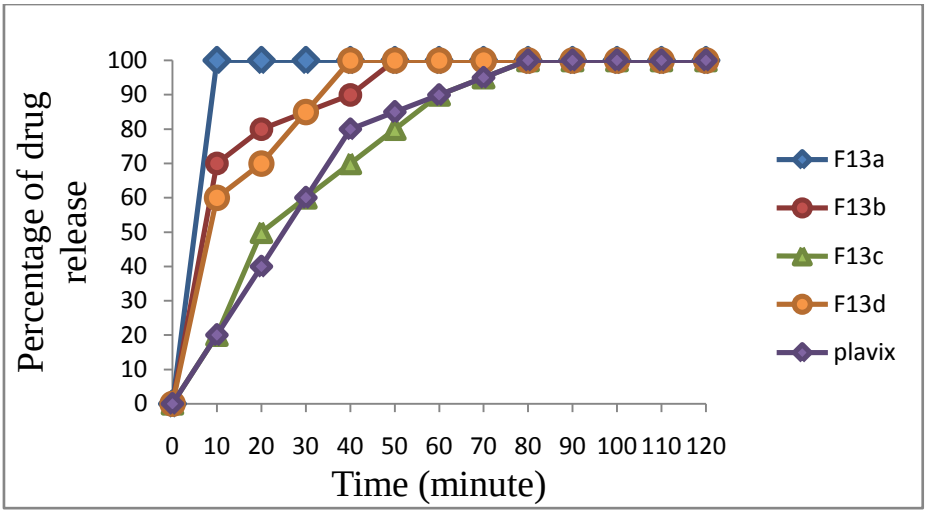
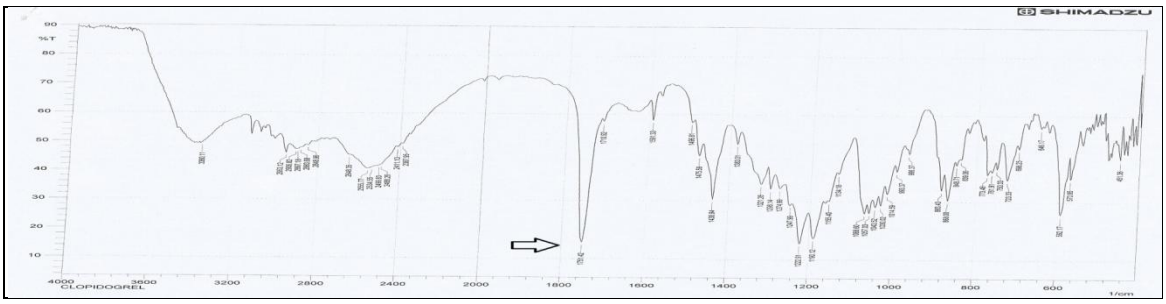



**Fig. 6: Dissolution profile of prepared tablet with marketed tablet in PBS pH 6.8 with 1% SLS. Each value represents the mean ±S.D. (n=3)**

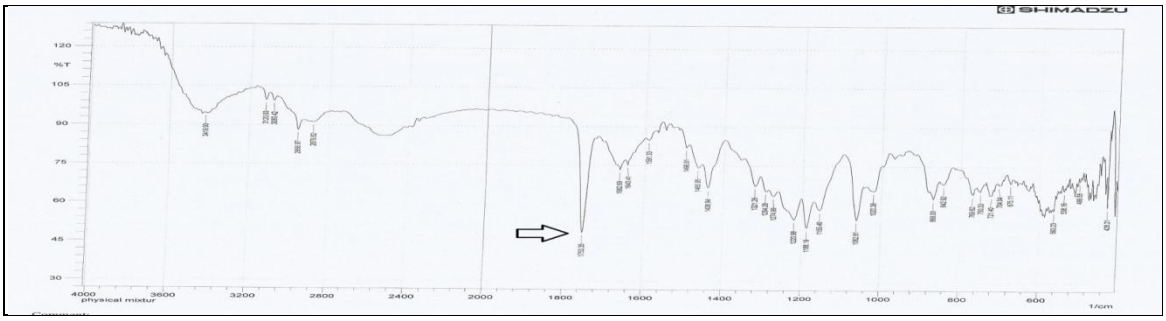
**Fourier Transform Infrared Spectroscopy (FT-IR)**

The FT-IR spectrum of pure clopidogrel bisulfate, physical mixtures of (1:1:1) clopidogrel, PVP and PVA respectively, lyophilized powder of selected formula (F13) and F13a tablet are given in figure (7:A-E).

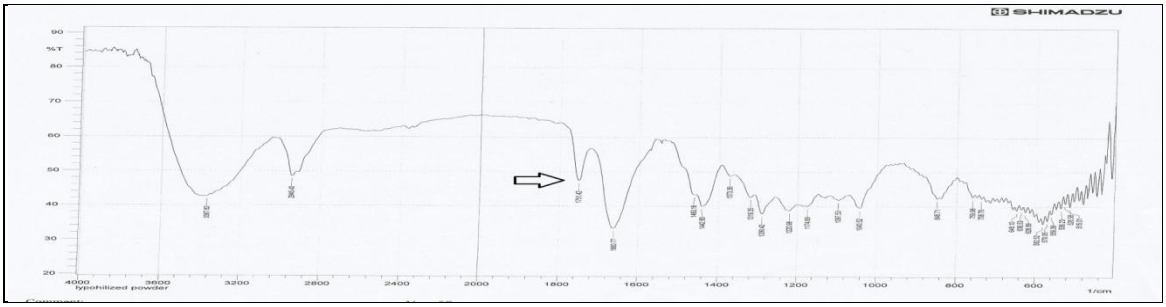
The results showed that the characteristic peak of clopidogrel bisulfate was 1751 $cm^{-1}$ which is due to C=0 stretching of the ester as a functional group present in all the spectrum indicating that there is no chemical interaction between the clopidogrel (pure and lyophilized powder ) and the other excipients [37].



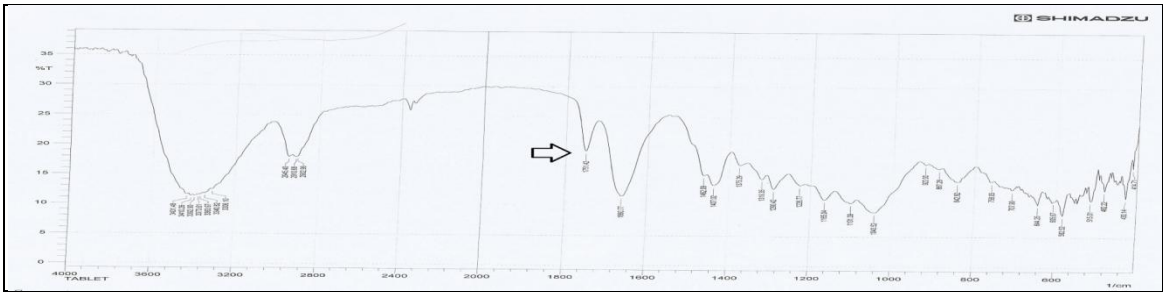

**A: FTIR spectrum of pure clopidogrel bisulfate**




**B: FTIR spectrum of physical mixture (1:1:1) of clopidogrel, PVPK-30 and PVA**




**C: FTIR spectrum of lyophilized powder**



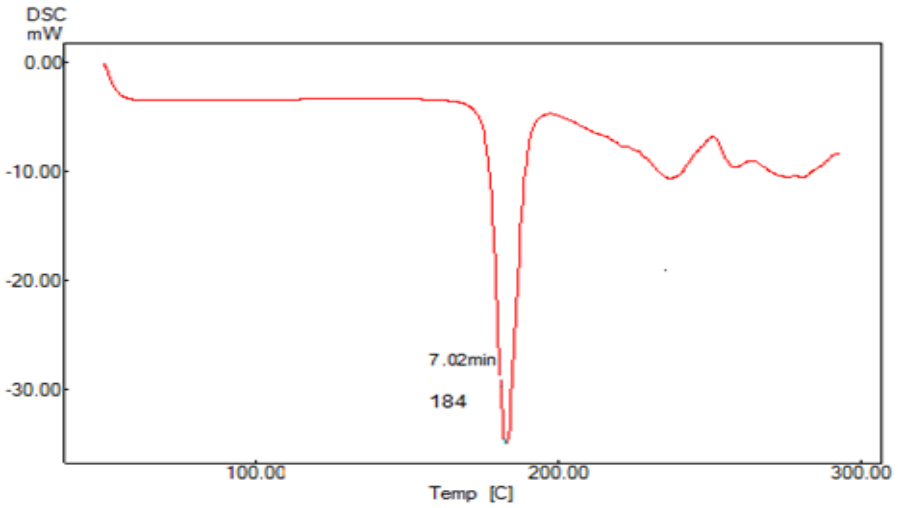

**D: FTIR spectrum of F13a tablet**

**Fig. 7: FTIR spectrum (A-D)**

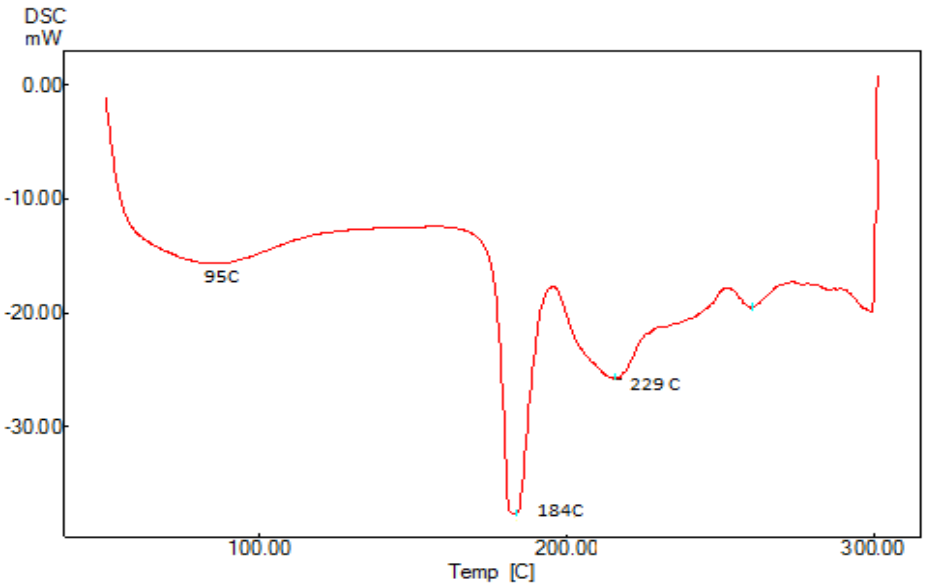
**Differential Scanning Electron Microscopy (DSC)**

Antisolvent precipitation process may change the physical state of clopidogrel. The DSC curves (as figure 8) of raw clopidogrel showed a single sharp endothermic peak at 184 °C which corresponded to its melting point[7].

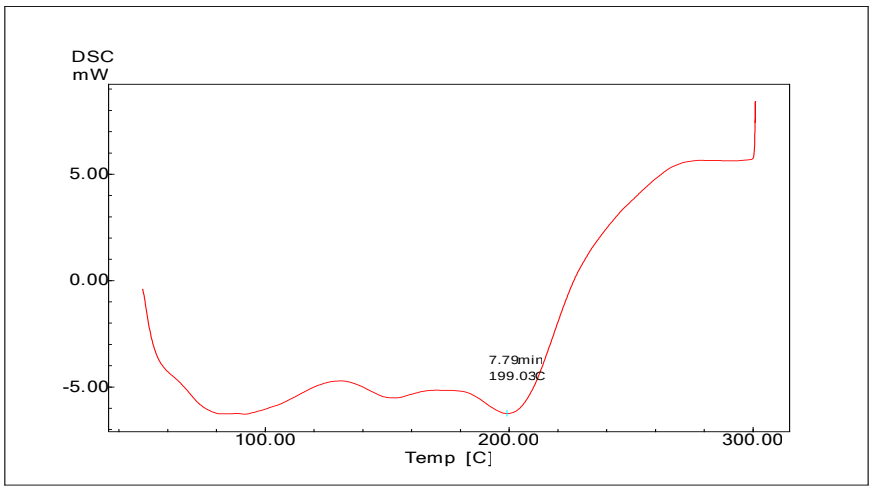
The PM of clopidogrel, PVA and PVP at ratio (1:1:1) exhibited an endothermic peak of raw clopidogrel at 184°C,PVPK-30 at 95 °C and PVA at 229°C which indicated that stabilizers do not change the physical state of clopidogrel in the physical mixture and there is no chemical interaction between drug and the stabilizers. While in the lyophilized powder the melting point of clopidogrel disappeared this mean that the drug lose the crystalline state and converted to an amorphous form, while the peak of PVA showed endothermic peak at 199 °C and PVPK-30 at 95°C. The melting point of clopidogrel also disappeared in F13a tablet this mean that there is a certain loss of the crystallinity, it showed three peak for PVA at 199 ºC, PEG 6000 at 64 ºC and MCC (Avicel)® at 96 ºC [38].



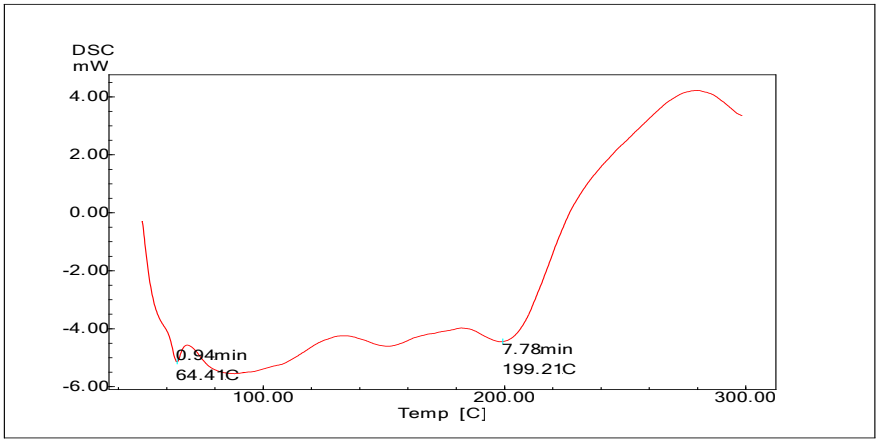

**a. DSC of clopidogrel bisulfate**




**b. DSC of physical mixture (1:1:1) of clopidogrel, PVP and PVA**




**c. DSC of lyophilized powder**




**d. DSC of F13a tablet**

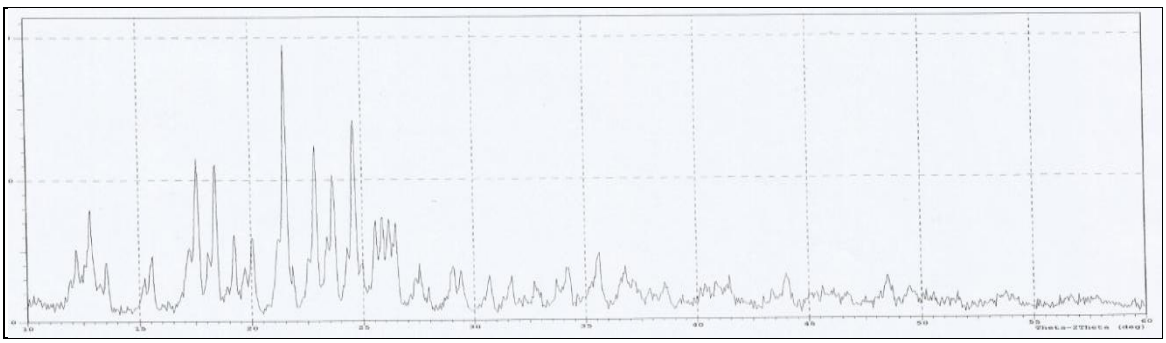
**Fig. 8: DSC of (a-d)**

**Powder X-ray Diffraction**

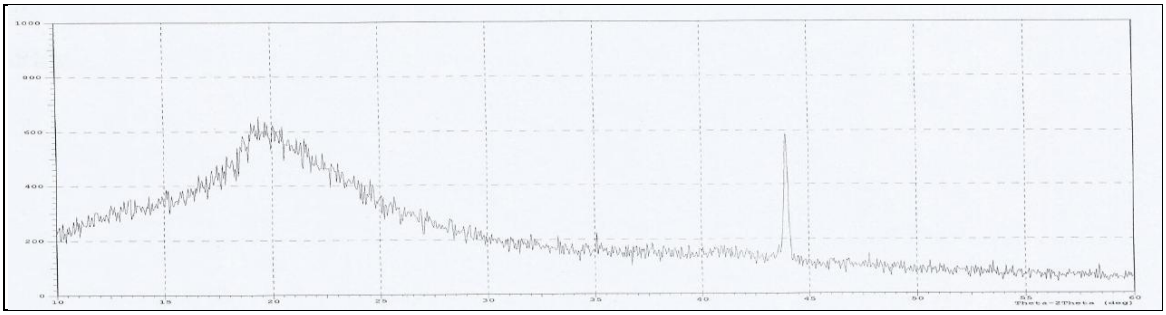
The results obtained from DSC reasonably agreed with the results obtained by PXRD. The change in the crystalline state of the dried clopidogrel nanoparticles was further confirmed by X-ray diffraction. The X-ray patterns of the clopidogrel powder in figure (9 A) displayed the presence of numerous narrow and symmetrical characteristic diffraction peaks,the strongest 3 peaks are 21º, 24 ºand 22 º at 2$\theta$ and with high intensity this indicated the crystalline structure of the drug, while XRD for lyophilized powder and tablet, no sharp peak for pure drug was observed and the strongest peaks were 19, 21 at 2$\theta$ respectively with less intensity of the diffraction peak when compared to that of raw drug indicating that the crystalline structure of clopidogrel was lost because of the precipitation.

**Scanning Electron Microscopy**

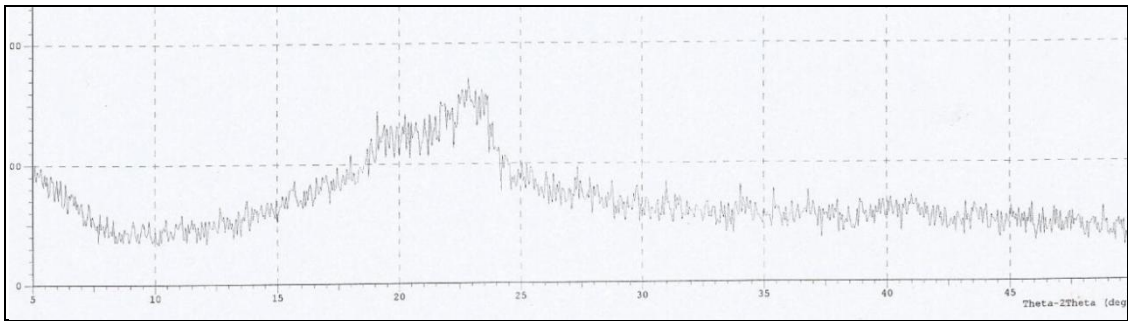
SEM of the pure drug and optimized formula F13 is shown in figure (10). It can be seen that the raw drug particles have a rough surface with large particle size while the SEM of F13 liquid deposit showed small particle. It was clearly seen that stabilizers were adsorbed onto the drug particle surface inhibiting particle growth.



**A: XRD of pure powder**

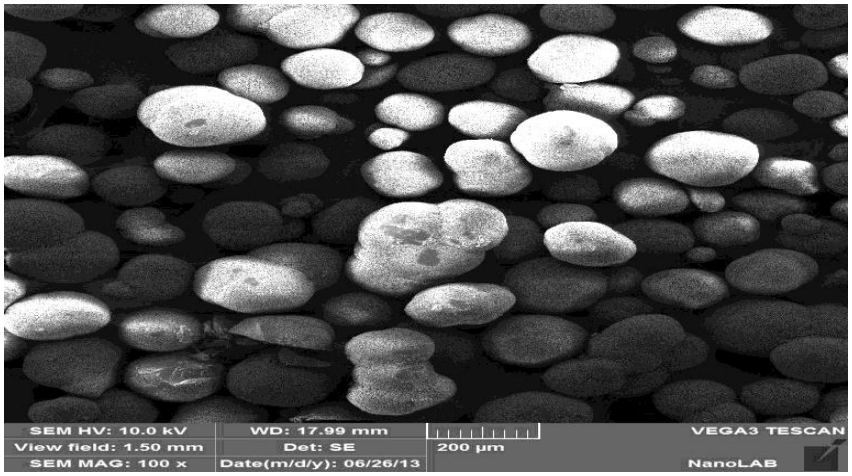


**B: XRD of lyophilized powder**

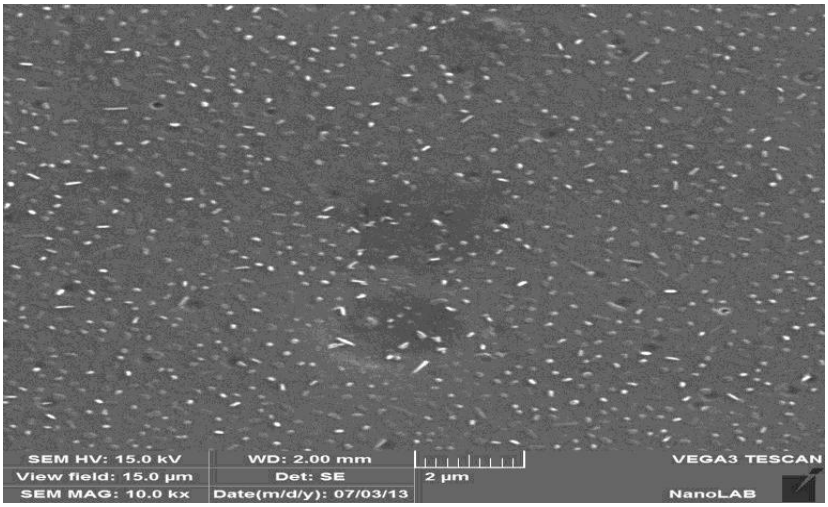


**C: XRD of tablet**

**Fig. 9: XRD of pure drug lyophilized formula and tablet**




a

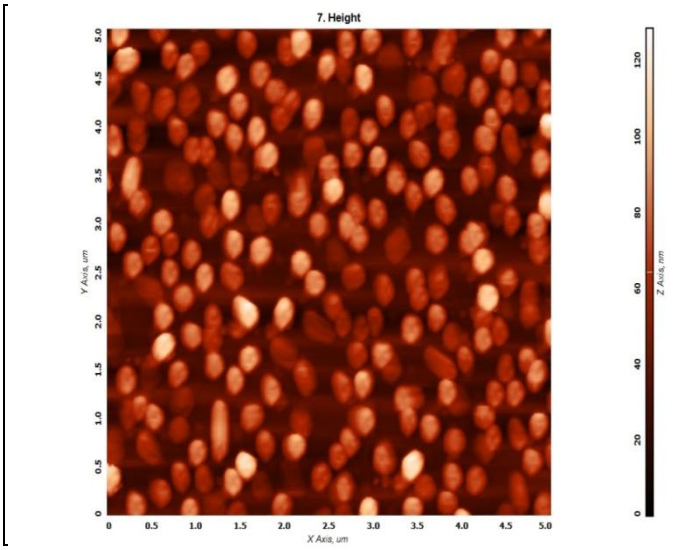



**B**

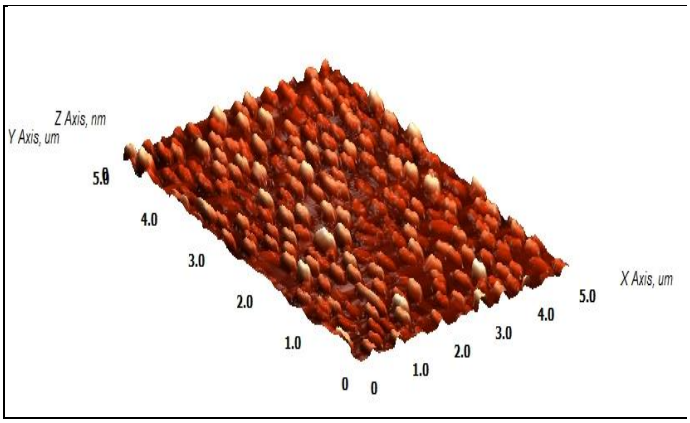
**Fig. 10: SEM of raw drug (a), SEM of F13 (b)**

**Atomic Force Microscope**

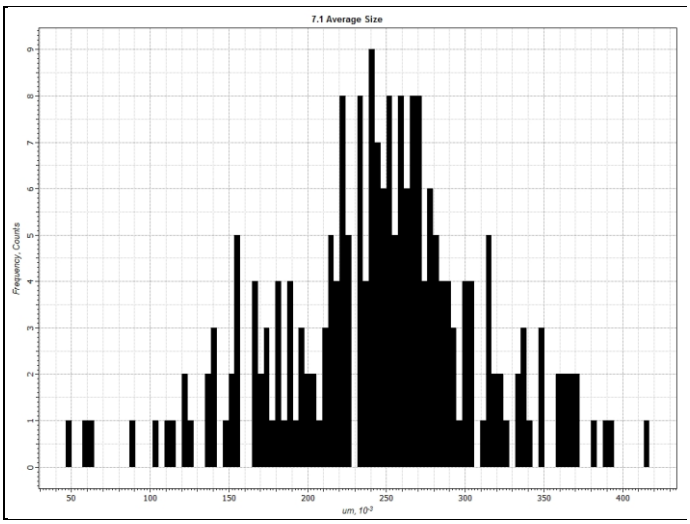
AFM is complementary to SEM imaging. Dispersing nanoparticles on a substrate (mica) is essential to get uniform and smooth surface.3D surface morphology, particle size measurement and distribution of F13 presented in figure (11), 3D morphology of the formulation revealed that the particles was stable and there is no aggregation could be observed. AFM of F13 showed that the particle size of sample was 250 nm and also showed uniform particle size distribution. This particle size is highly larger than the particle size obtained by ABT-9000 nano particle size analyzer. In fact comparatively larger mean particle size was observed when SEM and AFM methods were used. This could be explained due to the broad distribution of the particle sizes present in the system which can be visualized in the SEM and AFM pictures [39] [40].




a




b




C

**Fig. 11: AFM of F13 (a), 3D morphology of F13 (b) and particle size distribution of F13 (c)**

**CONCLUSION**

Antisolvent -precepitation method can be used as an effective tool for preparation of nanosized formulations. Clopidogrel nanoparticles prepared by this method showed significant improvement in aqueous solubility as well as dissolution characteristics which may significantly improve its oral bioavailability.

**ACKNOLEDGEMENT**

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