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FORMULATION, CHARACTERIZATION AND IN-VITRO EVALUATION OF NANOSUSPENSION FORMULATION OF CLOPIDOGREL USING SOLVENT-ANTISOLVENT TECHNIQUE

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Abstract:

Aim: *The main aim of our study was to improve solubility of Clopidogrel bisulphate by preparing nanosuspensions using solvent-antisolvent (bottom up) technology.*

Methods: *Clopidogrel nanosuspensions were formed by solvent antisolvent method. 15 formulations were prepared with different concentrations at different ratios. These formulations were evaluated for mean particle size, solubility, drug content and maximum yield. The selected formulation was then compared with pure drug for various parameters such as X-ray diffraction, Scanning Electron Microscopy, in-vitro Dissolution studies, Fourier Transform – Infrared Radiation (FT-IR) etc. Release kinetics and stability studies were performed for the optimized formulation.*

Results: *Out of 15 formulations, F15 comply well with all the parameters. On comparison with pure drug, F15 showed better characteristics such as Fourier Transform- Infrared Radiation (FT-IR), Solubility, particle size, Scanning electron microscopy, in-vitro dissolution, X-ray diffraction etc. Optimized formulation showed first order kinetics and stability was shown for over 3 months.*

Conclusion: *Clopidogrel (anti-platelet) in nanosuspension formulation can overcome the limitation of low solubility, dissolution, bioavailability and explore further.*

Keywords: *Clopidogrel, Nanosuspension, Solvent-antisolvent, Bioavailability, Release Kinetics*

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INTRODUCTION:

Heart attack (myocardial infarction) is found to be the most common cause of death in the developed countries. Formation of a blood clot within the heart artery forms the etiology of MI. Today, arrays of antiplatelet drugs are available that can be used to prevent heart attack by preventing clump and clot formation. However, the poor solubility of these drugs remains one of the major challenges addressed by pharmaceutical scientists [1]. At present, about 10% of the drugs in clinical use have bioavailability problems due to poor solubility. The poor solubility of drug causes hinderances in pharmacological screening of compounds for potential drug effects. Hence, improving the saturation solubility and dissolution rate of poorly water-soluble drugs is very important and significantly challenging to pharmaceutical researchers [2].

Clopidogrel, a potent anti-platelet drug, selectively inhibits the binding of adenosinediphosphate (ADP) to its platelet receptor [3]. It is indicated for the prevention of vascular thrombotic events in patients at risk [4]. According to the biopharmaceutics classification system (BCS), Clopidogrel is categorized as a class II agent (poorly water soluble and highly permeable) [5]. Oral bioavailability of Clopidogrel is very low (less than 50%), due to this poor water solubility. It is practically insoluble in water at neutral pH [6].

To overcome this problem, one possible way could be the formulation of this drug using nanotechnology. Nanosuspension consists of the poorly water soluble drug without any matrix material suspended in dispersion [7]. These can be used to enhance the solubility of drugs that are poorly soluble in water as well as lipid media. This approach can be useful for Clopidogrel like drugs to improve their antithrombotic activity which possess a significant challenge for the formulators.

The most important feature of nanosuspension is the increase in saturation solubility as well as dissolution velocity of the compound. The aim of this work is to formulate the Clopidogrel nanosuspension 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 [8][9].

The main aim of our study is to improve solubility of potent BCS Class-II drug (Clopidogrel) by preparing nanosuspensions using solvent-antisolvent (bottom up) technology. The above objectives were framed to enhance drug release in shorter duration of action as well as improve solubility and oral bioavailability.

MATERIALS AND METHODS:

Clopidogrel was obtained as a gift sample from Tirupati Medicare, Baddi (Himachal Pradesh). All the other raw materials were of analytical grade and of high purity, purchased from local source. Instruments were validated and laboratory conditions were well maintained throughout the entire procedure.

Before starting with the formulation development, the pre-formulation studies were conducted to characterize the drug and to select the excipients based on the pre-formulation studies. Preformulation studies involves hygroscopicity, solubility, partition coefficient as well as compatibility studies [10][11].

The hygroscopicity of Clopidogrel was determined as per “European Pharmacopoeia” and slight modification of Callan procedure. Solubility studies utilise shake flask method to determine solubility of the drug. To determine the partition coefficient of the API the shake flask method was used; it is the classical and the most useful method of determination of partition coefficient. The partition coefficient of Clopidogrel between n-octanol & water was determined by slight modification of “Shake Flask Method”, at room temperature The samples prepared for physical compatibility study were also evaluated for chemical incompatibility after 28 days of physical evaluation using FT-IR analysis.

Formulation development of nanosuspension was started with the selection of main ingredients (excipients) involved in its preparation. The main excipients of nanosupesion include stabilizers, solvents and antisolvents. Ethanol, acetone and methanol were selected on the basis of solubility of drug and rapid miscibility with solvent-miscible antisolvent. For the effective size reduction of the drug particles, water soluble polymers and stabiizers have been used to inhibit the particles agglomeration and improve the physicochemical properties of the drug. Common pharmaceutical excipients that are suitable for use as polymeric stabilizers include the cellulosics, such as Hydroxypropyl methyl cellulose (HPMC), and pluronics (F68 and F127) and surfactant like tween 80.The Stabilizers were added in ratio of 0.1 % (w/v) in the antisolvent (water in most of the cases).

Clopidogrel nanosuspensions were prepared in accordance with the liquid antisolvent precipitation technique. Clopidogrel and surfactants were dissolved in organic solvents such as methanol, ethanol or acetone at different concentrations to form the organic phase. The antisolvent phase was prepared by dispersing the stabilizers in water. The organic solution was rapidly introduced into antisolvent solution under vigorous agitation. The vigorous agitation results in the formation of clopidogrel nanosuspensions [12].

Formulations design for Nanosuspension

In this section, eight formulations were taken in which different solvents and antisolvents with different concentrations were added. The drug concentrations in these formulations (F1 to F8) were added in an unchanged amount i.e 20 mg as shown in Table 1. In the formulations F1 to F4, acetone is added as a solvent and water is added as an antisolvent in different concentrations. In the formulations F5 to F8, ethanol is added as a solvent and water as an antisolvent in different concentrations. The ratio of antisovent to solvent taken is 1:10, 1:20, 1:50 and 1:100.

In this section, the ratio of antisolvent to solvent was kept constant i.e 1:20 and ethanol (as solvent) and water (as antisolvent) were added to formulations F9 to F12. In this parameter, different concentrations (10 mg, 20 mg, 50 mg and 100 mg) of drug Clopidogrel were added in the formulations F9 to F12 as shown in Table 1. After this, the best drug concentration was selected for further parameters. In this section, different stabilizing agents were added in the premeasured quantity. In formulation F13, HPMC E5 is added in 0.1% (w/v), Tween 80 and Poloxomer were added to the formulations F14 and F15 in the same quantity. The ratio of antisolvent to solvent is kept constant i.e 1:20 for all three formulations. Ethanol and Water were added as solvent and antisolvent(F13 to F15) as shown in Table 1.

Table 1: Formulation of clopidogrel nanosuspension using different stabilizer at different ratios.

Formulation code	Solvent	Anti-solvent	Clopidogrel (mg)	Solvent-antisolvent ratio	Stabilising agent % w/v	Stabilising agent % w/v	Stabilising agent % w/v
					HPMC E5	Tween-80	Poloxamer F-68
F1	Acetone	Water	20	01:10	-	-	-
F2	Acetone	Water	20	01:20	-	-	-
F3	Acetone	Water	20	01:50	-	-	-
F4	Acetone	Water	20	1:100	-	-	-
F5	Ethanol	Water	20	01:10	-	-	-
F6	Ethanol	Water	20	01:20	-	-	-
F7	Ethanol	Water	20	01:50	-	-	-
F8	Ethanol	Water	20	1:100	-	-	-
F9	Ethanol	Water	10	01:20	-	-	-
F10	Ethanol	Water	20	01:20	-	-	-
F11	Ethanol	Water	30	01:20	-	-	-
F12	Ethanol	Water	50	01:20	-	-	-
F13	Ethanol	Water	20	01:20	0.1	-	-
F14	Ethanol	Water	20	01:20	-	0.1	-
F15	Ethanol	Water	20	01:20	-	-	0.1

F: Formulations; HPMC: Hydroxypropylmethyl cellulose; Clopidogrel drug was used in milligrams

Characterization

Percentage Drug Content: To determine the encapsulation efficiency dissolved 10 mg of nanosuspension in 30 ml methanol and then sonicate under vigorous shaking for 1 hour. The resultant solution was centrifuged. The drug content in supernatant solution was analyzed spectrophotometically by using ultraviolet-visible (UV-VIS) spectrophotometer at 271 nm with further dilutions against appropriate blank.

Percentage Solubility: The percentage solubility of clopidogrel bisulphate was determined by mixing 10mg of the nanosuspension formulation with approximately 2 ml of the distilled water taken in a glass vials with rubber stopper. Then the vials were kept on shaking on a water bath shaker for 24 hrs at room temperature. After 24 hrs the vials were centrifuge with centrifugal machine at rpm 1500-3000 for 10 mins. Then the supernatant liquid was pipette out from each vials and the solubility was determine in UV-Visible spectrophotometer (Shimadzu 1800, Japan) at 200-800 nm respectively. For each formulation the experiment was repeated in triplicate.

Particle Size: The mean particle size was determined using optical microscope. In this method, the size of 250 particles was determined and the average particle size was calculated. Thus, a particle size analyser is being further used for the accurate size determination [13].

Percentage Yield: To determine the yield, the weight of nanosuspension obtained at the end of preparation was determined. The total weight of raw materials used to obtain this nanosuspension was determined to obtain the theoretical yield.

On the basis of above parameters, an optimum formulation was selected which complies with in all the parameters and will be evaluated against marketed formulataion.

Evaluation of optimised formulation

Based on the parameters discussed above, an optimised formulation was selected and evaluated with pure drug. The evaluation was done using various parameters such as X-ray diffraction studies, Differential Scanning colorimeter, Scanning electron microscopy, *in-vitro* Dissolution studies etc.

Particle Size

The mean particle size was determined using optical microscope. In this method, the size of 250 particles was determined and the average particle size was calculated. Thus, a particle size analyser is being further used for the accurate size determination [13].

Zeta Potential

In this study, the zeta potential was assessed by determining the electrophoretic mobility of the particles. The zeta potential was measured using a Zetasizer Nano 1000 HS (Malvern Instruments, Malvern, UK). Samples were diluted with the respective original dispersion medium, which provides information regarding the thickness of the diffuse layer. Diluted nanosuspension was added to the sample cell (quartz cuvette) and was put into the sample holder unit and zeta potential was measured. The Zetasizer range provides exceptionally high performance that can measure a particle size from less than a nanometer in size to several microns. This system measures particle size using dynamic light scattering [14].

FT-IR Studies

An FTIR spectrometer simultaneously collects high spectral resolution data over a wide spectral range. This confers a significant advantage over a dispersive spectrometer which measures intensity over a narrow range of wavelengths at a time. The term Fourier transform infrared spectroscopy originates from the fact that a Fourier transform (a mathematical process) is required to convert the raw data into the actual spectrum. The prepared samples were scanned in the range from 650 cm-1-4000 cm-1 [15][16].

X-ray Diffraction Analysis

X-ray diffraction (XRD) is the basic characterization technique for structural and phase analysis. Crystal nature of a sample is determined by X-ray diffraction analysis. This diffractometer uses copper-potassium radiation having a wavelength of 1.5418 Å. The sample is kept horizontally and can be rotated using a spinner sample stage. The X-ray tube was operated at 45 kV and 40 mA current [17].

SEM (Scanning electron microscopy)

In order to examine the particle surface morphology and shape, scanning electron microscopy (SEM) was used. A concentrated aqueous suspension was spread over a slab and dried under vacuum. It gives information about the particle size distributed in the nanosuspension. Schematic diagram of SEM is shown in Fig.5.7 (left). SEM micrographs of the samples were obtained using ZEISS EVO-50 scanning electron microscopes presented in Fig.5.8 (right). SEM measurements are based on the principle of irradiating the specimen with a fine focused electron beam. The secondary electrons, backscattered electrons, auger electrons, characteristic X-rays and several other radiations are released from the specimen. Generally, the secondary electrons are collected to form the image in the SEM mode [18].

Differential Scanning Calorimetry (DSC)
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 DSC 4000 from Perkin Elmer, USA. 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 25 ºC to 300 ºC [19].

***In-vitro* Dissolution Studies**
The dissolution studies of powder nanosuspension were carried out in dissolution apparatus (USP apparatus II) in 100 ml of phosphate buffer pH 6.8 as a dissolution medium, maintained at 37 ± 0.5°C. The medium stirred at 100 rpm. Aliquots 1 ml of the dissolution medium was withdrawn at 15, 30, 45, 60, 90, 120 and 180 mins time interval and the same amount was added with the fresh medium in order to maintain the sink conditions. Samples were assayed spectrophotometerically on UV-Visible spectrophotometer [13][20].

Release kinetic model
The in vitro release data obtained were fitted in to various kinetic equations. The value of R^2 was more in first order drug release. The value on n is 0.45<n<0.89 that is non ficckian diffusion [21].

Stability Studies of Optimized Formulation as per ICH guidelines
The stability studies were carried out as per ICH Q1A/(R2) guidelines for the optimized formulation. The formulation was stored at 40° ± 2°C/75% ± 5% RH for the duration of 3 months. The samples was analysed for physical changes and drug content after interval of 0, 30, 60, and 90 days. Physical stability was analysed by appearance, colour and the chemical stability was analysed by percent drug entrapment [13].

RESULTS:

Preformulation study of drug is an important step for formulation development. The preformulation studies of Clopidogrel include determination of hygroscopicity, solubility profile, partitioning etc. Hygroscopicity of Clopidogrel to moisture was determined according to European Pharmacopeia and procedure defined by Callan et al. The total moisture absorbed was assessed in terms of total weight gain by Clopidogrel when exposed to 25°C/80±2% RH for 24 hrs & 25±1°C/90±2% RH for 7 days in humidity chamber as shown in Table 2. The total weight gain after 7 days was only 0.067±0.012 % at 25±1°C/90±2% RH whereas negligible weight gain was observed at 25±1°C/80±2% RH in 24 hrs. Therefore, it could be concluded that the procured Clopidogrel sample is non hygroscopic in nature as per specifications of European Pharmacopoeia and Callan classification.

Table 2: Hygroscopicity of Clopidogrel

Original Weight Taken (mg)	Weight (in mg) ± S.D. after exposure at		Total weight gain (in mg) ± S.D after		% weight gain (in mg) ± S.D after		Inference
	25±1°C/80±2 %RH for 24 hrs	25±1°C/90°C ±2%RH for 7 days	24 hrs	7 days	24 hr	7 days	
300	300.01 ± 0.028	300.02 ± 0.039	0.01 ± 0.028	0.02 ± 0.039	0.003 ± 0.007	0.0067 ± 0.012	Non-hygroscopic

S.D: Standard Deviation

The solubility studies of drug were conducted in different solvents and the results of which are listed below in Table 3. It was observed that the drug Clopidogrel has higher solubility in ethanol as compare to other solvents.

Table 3: Solubility of drug in various solvents

S.No.	Name of solvent	Solubility (mg/ml)
1	Water	0.04±0.0023
2	Ethanol	1.50±0.0078
3	Acetone	0.81±0.012
4	Methanol	0.84±0.0023

The Partition coefficient of Clopidogrel was determined between n-Octanol and water using 'shake flash method'. Thus experimentally observed value and theoretical value of partition coefficient is tabulated in Table 4.

Table 4: Partition coefficient of drug

Organic Phase	Aqueous Phase	Theoretical value	Practical value
n-Octanol	Water	3.89	4.15±0.72

The drug-excipients sample was taken in 1:1 ratio and tested for its physical characterization at three different temperatures and humidity levels such as 25 °C /60%RH, 30°C /65%RH, 40°C /75%RH for 4 weeks. After completion of physical compatibility, the samples were analyzed for physicochemical interaction between drug and polymer by FT-IR analysis.

The FT-IR spectra obtained for pure drug, excipients and the physical mixture of drug and excipients is given in the Figure 1. Excipients, e.g. Poloxomer shown in Figure 1(b) have shown characteristic peaks at 1100 cm-1 corresponding to its functional group -C-O, indicating its purity and authenticity. Similarly, Tween 80 and HPMC also show their respective characteristic peaks well matched with literature, shown in Figure 1(c)-(d). No appreciable change was observed in absorption peaks of drug when loaded with the physical mixture of excipients (Figure 1(e)-(g). Therefore, it can be concluded that there was no chemical interaction existing between drug and excipients.

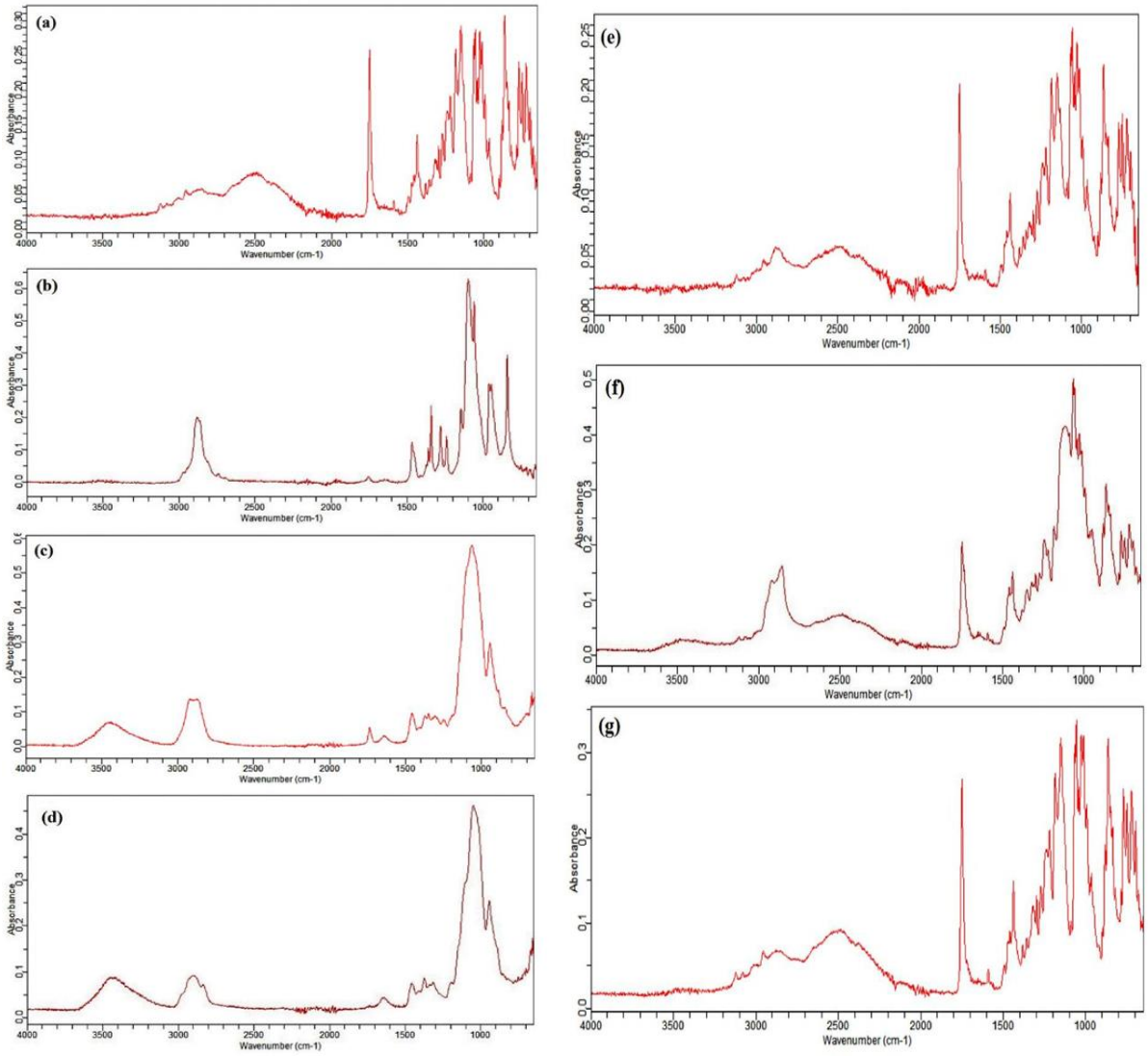


Fig 1: FT-IR spectrum of (a) pure Clopidogrel drug (b) Poloxomer (c) Tween 80, (d) HPMC; physical mixture of (e) Clopidogrel drug and Poloxomer (f) Clopidogrel drug and Tween 80 (g) Clopidogrel drug and HPMC

Formulation Development of Nanosuspension

In order to develop Nanosuspenion of Clopidogrel, various excipients were selected based on preformulation studies. The main excipients (ingredients other than API) of nanosupension include stabilizer, solvent and antisolvent. Effect of anti-solvent to solvent ratio on particle and percentage solubility is given in a Table 5. In this section, eight formulations were taken in which different solvents and antisolvents with different concentrations were added. The drug concentration added in these formulations (F1 to F8) was added in an unchanged amount i.e 20 mg. In the formulations F1 to F4, acetone is added as a solvent and water is added as an antisolvent in different concentrations. In the formulations F5 to F8, ethanol is added as a solvent and water as an antisolvent in different concentrations. It was also found that Formulation code i.e. F1 to F8 has percentage drug content 67.45±3.89 to 88.23±3.02, percentage solubility 60.87±3.65 to 82.89±2.54, percentage yield 70.25±0.075 to 82.74±0.016 and particle size 0.98±0.21to 1.39±0.25µm. It was found that formulation F6 has highest percentage solubility, highest percentage yield and smaller particle size. Now it shows that anti +solvent to solvent ratio in ethanol is showing better results i.e. 1:20 as comparison to anti solvent to solvent ratio in acetone on increasing anti-solvent to solvent ratio particle size was reduced. If the ratio of antisolvent to solvent is increased, the degree of supersaturation ratio is increased. This increases the nucleation rate and increase the particle size; possibly due to equilibration of nucleation and growth kinetics.

Effect of drug concentration on particle size, percentage % Drug content, and percentage yield and percentage solubility is given in Table 5. For this purpose, ethanol (as solvent) and water (as antisolvent) were added (chosen from results of the Table 3) in the constant ratio of (solvent to antisolvent) 1:20 in formulations F9 to F12. Then, different concentrations (10 mg, 20 mg, 50mg and 100mg) of drug Clopidogrel were added in these formulations F9 to F12. From the table it was found that Formulation code i.e. F9 to F12 has percentage drug content 83.65±4.32 to 88.23±3.02, percentage solubility 81.48±4.25 to 87.98±2.01, percentage yield 74.28±1.56 to 82.74±2.01 and particle size 0.98±0.21 to 1.54±0.21µm. It was found that formulation F10 has highest percentage solubility, highest percentage yield and smaller particle size. Hence the formulation F10 contains the best drug concentration for Clopidogrel nanosuspension and it was selected for further optimization.

Effect of different stabilizer on particle size, percentage % Drug content, percentage yield and percentage solubility was given in a Table 5. For this, different stabilizing agents were added in the premeasured quantity.In formulation F13, HPMC E5 is added in 0.1% (w/v), Tween 80 and Poloxamer were added to the formulations F14 and F15 in the same quantity. The ratio of antisolvent to solvent is kept constant i.e 1:20 for all three formulations. Ethanol and Water were added as solvent and antisolvent (F13 to F15).Three stabilizers Poloxamer, Tween80 and HPMC E5 were added in a premeasured quantity of the drug. As it is clear from the table that among these three formulations, F15 formulation (that contain poloxamer as a stabilizer) was found to exhibit smallest particle size, highest drug content, maximum percentage yield and greatest solubility. Therefore, F15 formulation is considered as the best formulation among all formulations used to formulate Clopidogrel nanosuspension and will undergo for the evaluation study

Table 5: Particle size, Entrapment Efficiency, Yield and solubility of different nanosuspension formulation

S.No.	Formulation code	% Drug content	Solubility (in % age)	Yield (in % age)	Particle size (µm)
1	F1	78.34±3.32	64.32±3.74	72.35±3.17	1.11±0.25
2	F2	75.23±2.96	63.78±3.12	76.85±3.45	1.15±0.12
3	F3	68.23±3.25	65.23±3.78	74.85±4.25	1.56±0.18
4	F4	67.45±3.89	60.87±3.65	70.25±3.75	1.01±0.21
5	F5	79.68±4.01	76.98±3.89	71.58±3.14	1.06±0.41
6	F6	88.23±3.02	87.98±2.01	82.74±2.16	0.98±0.21
7	F7	81.45±3.58	82.89±2.54	79.65±3.78	1.32±0.38
8	F8	80.78±4.11	79.68±3.85	74.89±3.21	1.39±0.25
9	F9	83.65±4.32	84.21±2.65	74.28±1.56	1.58±0.25
10	F10	88.23±3.02	87.98±2.01	82.74±2.01	0.98±0.21
11	F11	87.55±2.58	82.46±3.25	80.36±2.65	1.33±0.18
12	F12	84.52±4.25	81.48±4.25	79.89±2.87	1.54±0.21
13	F13	84.15±4.32	86.24±2.89	83.38±2.58	1.25±0.35
14	F14	86.56±3.02	89.98±1.98	86.74±3.33	1.052±0.19
15	F15	91.84±3.54	92.58±3.12	90.36±1.98	1.04±0.16

Evaluation of Nanosuspension

Particle Size Analysis

Formulation code F15 was selected for size determination using particle size analyzer. On evaluation particle size formulation was found to be around 358 nm and Polydispersity Index (PDI) of the formulation was 0.200 as shown in Figure 2.

Zeta potential

In general, zeta potential of particles should be at least ±30 mV for electrostatically stabilized systems or ±20 mV for sterically stabilized systems to obtain a physically stable nanosuspension. The zeta potential of optimized nanosuspension was found to be -13.5 mV as shown in Figure 3, indicating that the prepared nanosuspension do not suffer from instability problems. Zeta potential of nanosuspension exhibited no essential changes before and after the storage and stability studies.

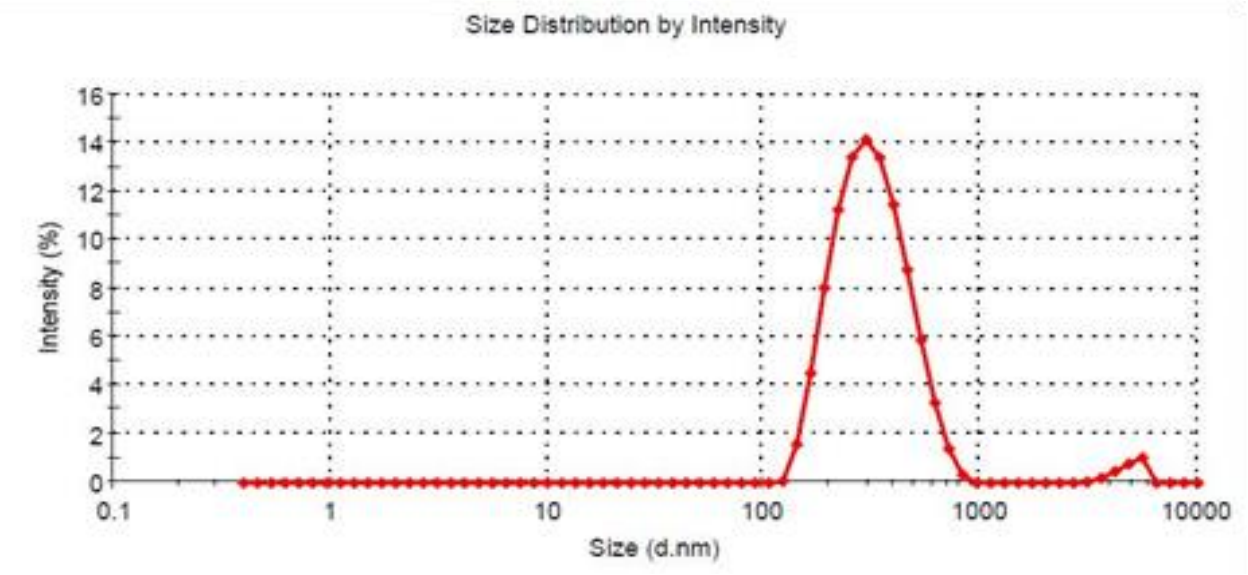


Fig 2: Particle size distribution of formulation F15

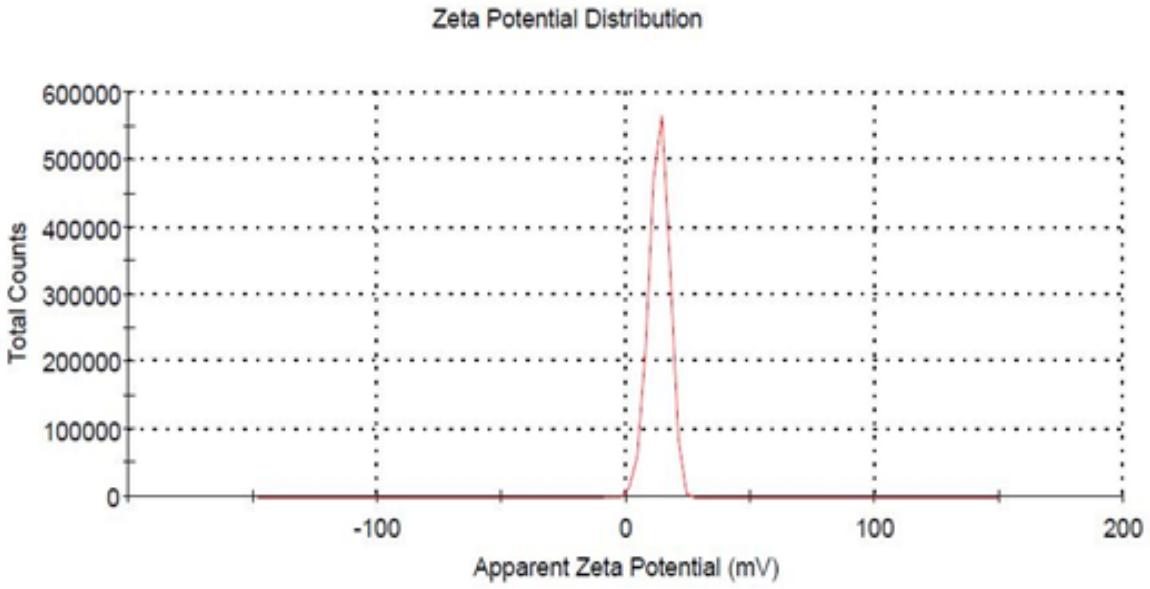
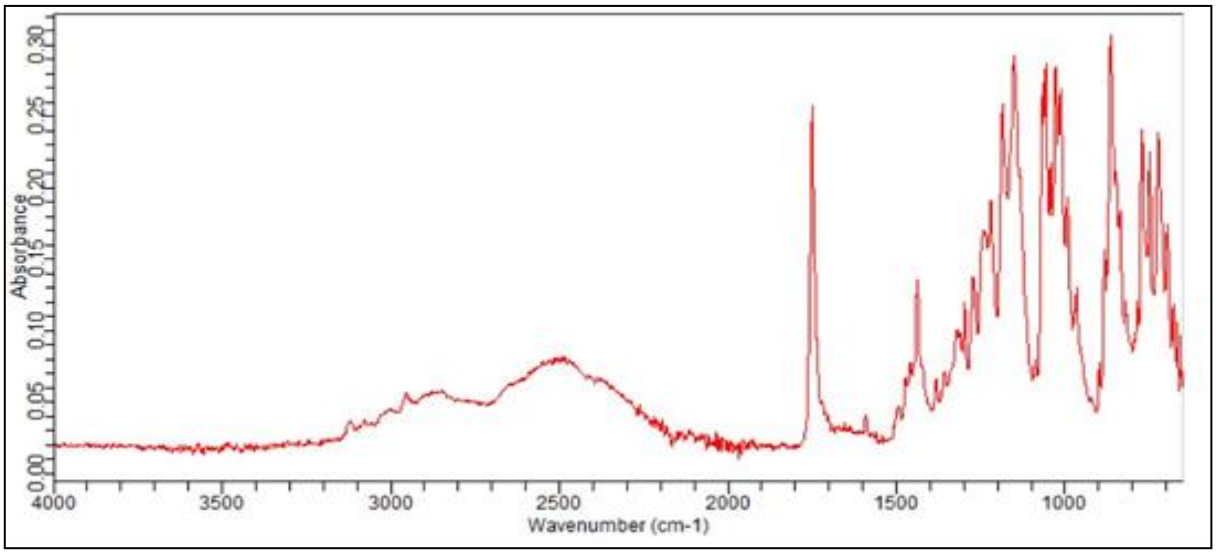


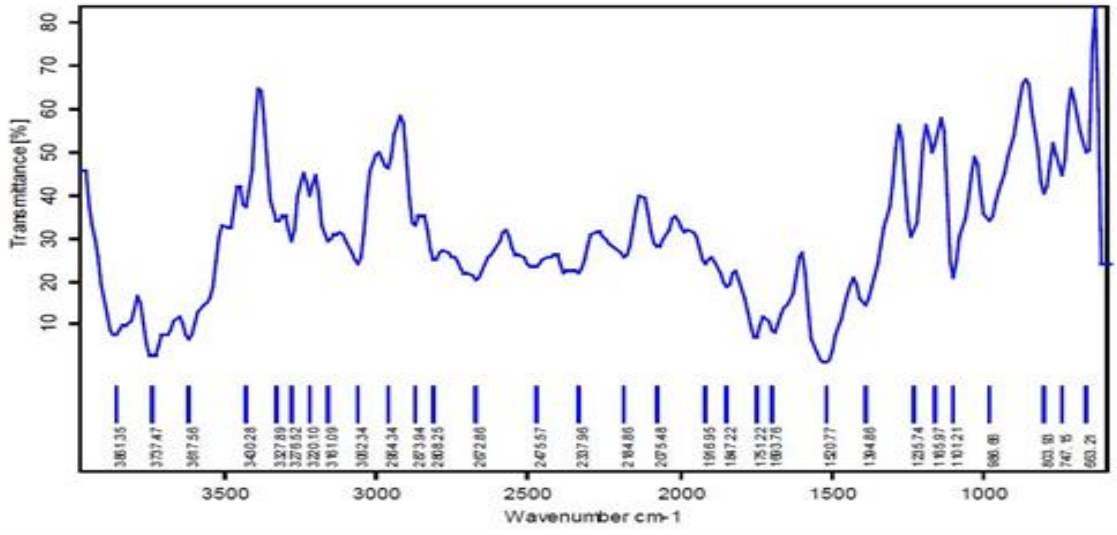
Fig 3: Zeta Potential of formulation F15.

FT-IR Studies

There was no incompatibility between drug and other solvents and ingredients as shown in Figure 4(A) and 4 (B). The results showed that the characteristic peak of Clopidogrel was 1747 cm-1 which is due to C=O 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. IR spectrum of Clopidogrel is characterized by principal absorption peaks at 3113.16cm-1 (C-H aromatic), 1748.41.36cm-1 (C=O stretching), 1176.36cm-1 (C-O stretching) as shown in Table 6.



A



B

Fig 4: A) FTIR of Drug Clopidogrel and B) Formulation (F15)

Table 6: FT-IR spectral peaks or pure drug and formulation (f15)

S.No.	Observed peak in pure drug (cm^{-1})	Observed peak in Formulation (F15) (cm^{-1})	Inferences
1	1176.36	1165.97	C-O stretching
2	1747	1751.11	C=O stretching of the ester
3	1748.41	1752	C=O stretching
4	3113.16	3161	C-H aromatic

X-ray Diffraction (XRD)

XRD pattern (in 2θ range 10° ≤ 2θ ≤ 70°) of pure drug and and the nanosuspension F15 was shown in Figure 5. These patterns were taken at very slow scan rate (step size 0.01°, scan time per step 50 s). The X-ray patterns of the Clopidogrel powder displayed the presence of numerous narrow and symmetrical diffraction peaks, indicated the crystalline structure of the drug, while XRD for nanosuspension powder F15, as expected, no sharp peak drug was observed. This indicates that the crystalline structure of Clopidogrel was lost because of the precipitation and lyophilization of the drug.

Scanning electron microscopy

Well defined grains represent polycrystalline nature of the pure drug sample as shown in Figure 6(A). However, the SEM of formulation F15 as shown in Figure 6(B) did not show any particular granular structure, thus represents amorphous nature of formulation.

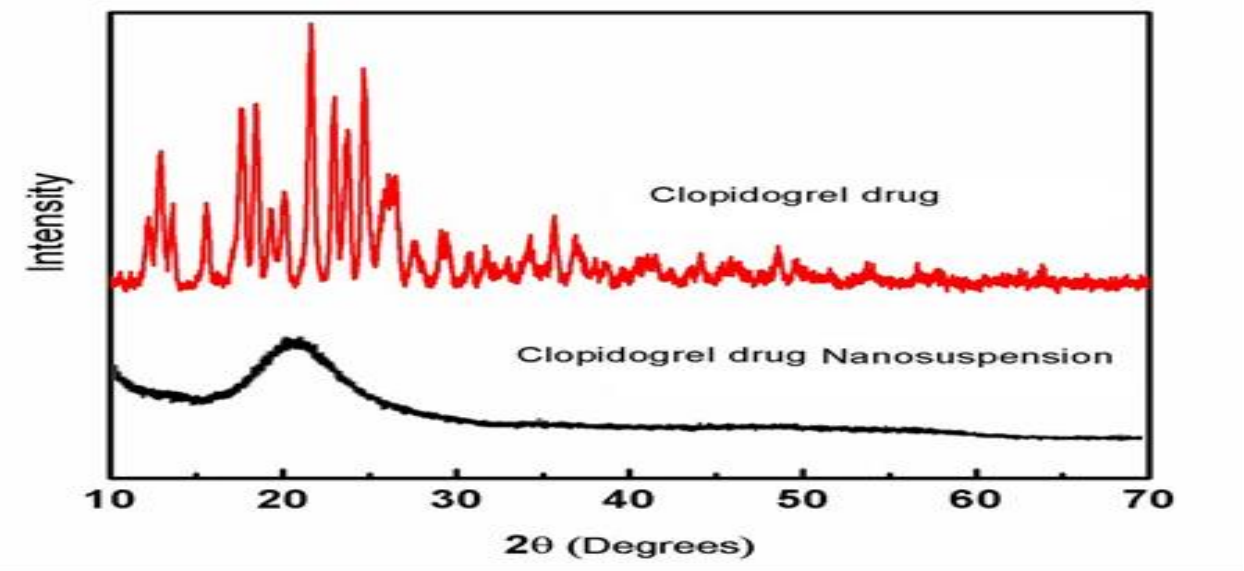
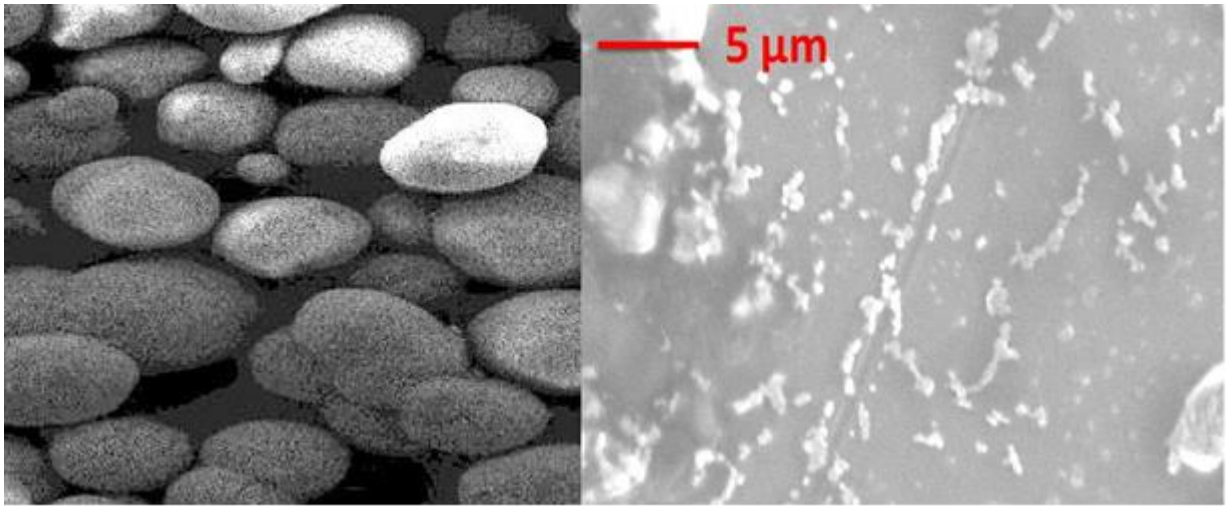


Figure 5: X-Ray diffraction patterns of pure drug and nanosuspension F15 formulation



A B

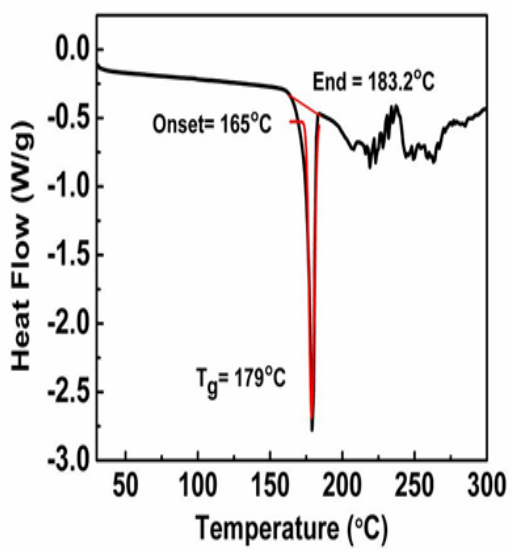
Figure 6: A) SEM of drug and (B) F15 formulation

Differential Scanning Calorimetry (DSC)

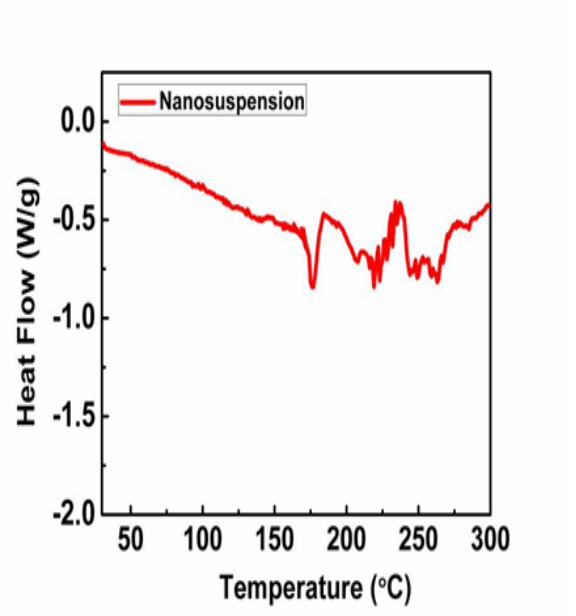
The solvent -antisolvent process of nanosuspension formation may change the physical state of Clopidogrel drug. Figure 7(A) shows the DSC curves of raw clopidogrel showed a single sharp endothermic peak at 179 °C which corresponded to its melting point. This result is also supported by our melting point measurement discussed earlier. Figure 7(B) represent the DSC curve for nanosuspension formulation F15. In this lyophilized powder, the melting point peak of Clopidogrel disappeared which reveals that the drug lose the crystalline state and converted to an amorphous form, as also indicated in the XRD data.

In-vitro drug release study

Percentage Drug release of F15 formulation and pure drug was given in below Table 7. Form the Figure 8, it was found that in vitro drug release nanosuspension was higher and faster as compare to pure drug.



A



B

Figure 7: (A) DSC of clopidogrel drug (B)DSC of Clopidogrel Nanosuspension formulation F15.

Table 7: Percentage Drug release of F15 formulation

S. No.	Time (Min.)	% Drug release (Pure Drug)	% Drug release (F15 formulation)
1	15	0±0	25.36±1.26
2	30	3.64±0.22	38.74±2.14
3	45	7.2±0.81	52.65±3.12
4	60	14.79±0.62	66.74±3.41
5	90	18.2±1.48	72.36±3.11
6	120	23.62±1.02	74.85±2.96
7	180	29.04±1.32	76.52±2.85

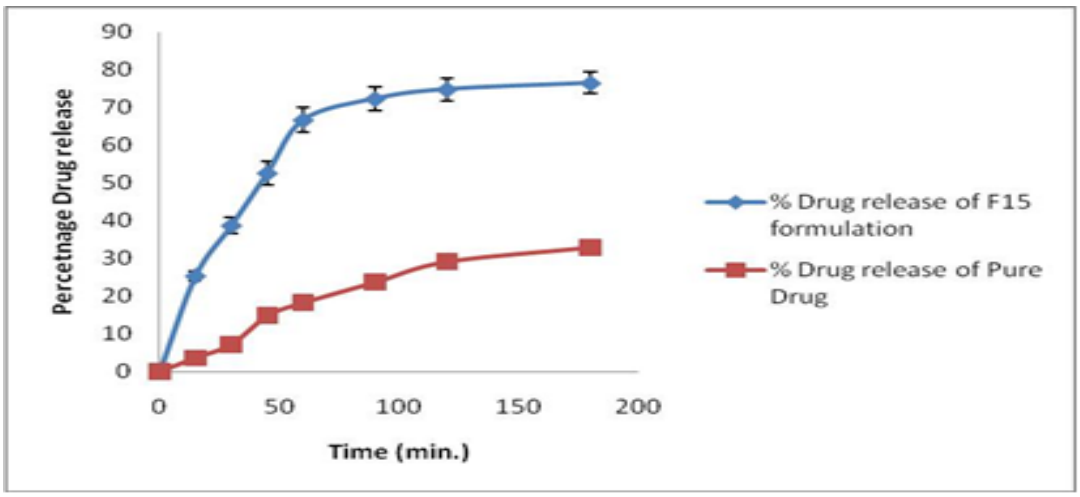


Figure 8: In-vitro drug release of F15 formulation and pure drug

Drug release and kinetics

Model dependent methods are based on different mathematical functions, which describe the release profile. Once a suitable function has been selected, the release profiles are evaluated depending on the derived model parameters. In vitro kinetic analysis showed that drug release was best explained by First order kinetics equation, with highest value of linearity (R2 > 0.9) for F15 formulation.

Stability Studies

The stability studies were carried out as per ICH guidelines for the optimized formulations. The nanosuspension (F 15) was stored at 40°C ± 2°C/75%± 5%RH for the duration of 3 months.The stability studies showed that there was no change in the appearance and colour of nanosuspension indicating that the optimized formulation F15 was physically stable at the conditions to which they were exposed as shown in Table 8. It was observed that there was very slight reduction in the drug content of the nanosuspension which were stored at 40°C ± 2°C/75%± 5%RH after storage for 90 days and the optimized formulation F15 did not show any significant change in drug content during stability study and is found to be stable.

Table 8: Evaluation of physical stability and chemical stability (% drug content) of optimized formulation

Time (days)	Physical change		Chemical Change	
	Appearance	Colour	% drug entrapment	Absorption maxima (nm)
0	NC	White	91.84 ± 3.54	271
30	NC	White	91.33 ± 3.40	271
60	NC	White	90.89 ± 1.98	271
90	NC	White	90.37 ± 2.65	271

NC: No Change

DISCUSSION:

Poor solubility of drug is one of the major problems currently being addressed by pharmaceutical scientists at industry and academia. At present, about 10% of the drugs in clinical use have bioavailability problems due to poor solubility. The decreased solubility of drugs makes it very difficult to perform the pharmacological screening of compounds for potential drug effects. Hence, improving the saturation solubility and dissolution rate of poorly water-soluble drugs is very important and significantly challenging to pharmaceutical researchers seeking to achieve optimum absorption of new drug candidates.

The purpose of this work was to enhance solubility of Clopidogrel drug and provide rapid onset of action. The effort has been made to find out the best formulation for Clopidogrel nanosuspension. Clopidogrel is a BCS class-II drug having low solubility and high permeability. Thus, it was challenging to enhance the solubility of Clopidogrel in an aqueous solution. Precipitation antisolvent method has been employed to produce nanosuspension of Clopidogrel. The different formulate variables such as sovent, antisolvent and Surfactant ratios were contributing much towards the change in particle size and solubility in nanosuspension preparation.

Initially solubility study was carried out by taking drug in different solvents and drug-excipient compatibility study was determined by FTIR method. The physical state of pure drug was examined by DSC technique and the prepared nanosuspension was evaluated for particle size, Polydispersity index (PDI), Zeta potential, percentage yield , percentage drug content, In-vitro dissolution study and short term stability study. The solubility rate of Clopidogrel in different solvent is shown in Figure 1. FTIR spectra of pure drug Clopidogrel and physical mixture of drug with excipients like poloxomer, Tween 80, HPMC E5 shows no chemical interaction between drug and excipients. On preformulation evaluation melting point and partition coefficient was found to be 176-180°C±0.95oC and 4.15±0.72, represent lipophilic nature of drug. Ethanol has higher drug solubility as compare to other solvents. Standard calibration curve was prepared in methanol using concentration range 1µ/ml-10µg/ml. R^2 value was found to be 0.998.

Fifteen different batches (F1 –F15) with different solvent antisolvent ratio, different drug concentration and different type of stabilizer. Among all formulation F15 was optimized; % drug content, % Solubility, % Yield, Particle size (µm) was found to be 91.84±3.54, 92.58±3.12, 90.36±1.98, 1.34±0.16 respectively. Particle size of F15 formulation was found to be 358.2 nm and PDI of the formulation was 0.200.

Poloxamer F68 was the most effective stabilizer for poorly soluble drugs. Poloxamers have a linear ABA triblock polymer chain (A stands for hydrophilic polyethylene oxide (PEO) segment and B stands for hydrophobic polypropylene oxide (PPO) segment). The hydrophobic PPO chains can drive the polymer to adsorb on the surface of drug particles, while the hydrophilic PEO chains surround the drug particles providing steric hindrance against aggregation. Pluronic® F68 has a lower molecular weight compared to other pluronics which may exert less kinetic restriction in the adsorption process and faster diffusion. Percentage drug release of nanosuspension was higher and faster as compare to pure drug that was 76.52±2.85 in 180 minutes. Drug release studies of pure Clopidogrel and prepared nanosuspension (F 15) were calculated from release profile are reported in Figure 8. From this data, it was evident that onset of drug release of pure Clopidogrel was very low as compare to its nanosuspension. Physical appearance of the F15 nanosuspension did not change when samples were stored at specified conditions. A loose, thin layer of sediment was observed when nanosuspension was stored at room temperature for 1 and 2 months. However, the sediment layer disappeared with slight hand shaking. On performing stability study, it can be inferred from the observed data that the prepared nanosuspension (F 15) was passed the stability test and was stable at different conditions.

CONCLUSION:

The Precipitation method offers a direct process to obtain drug nanoparticles of desirable size, amenable for continuous and consistent production. Clopidogrel (anti-platelet) in nano-suspension formulation can overcome the limitation of low solubility, dissolution and bioavailability. Hence Antisolvent -precepitation method can be used as an effective tool for preparation of nanosized formulations. Clopidogrel nanosuspension prepared by this method showed significant improvement in aqueous solubility as well as dissolution characteristics which may significantly improve its solubility.

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ABBREVIATIONS

FT-IR: Fourier Transform Infrared Radiation, SEM: Scanning Electron Microscope, DSC: Differential Scanning Calorimetry, HPMC: Hydroxy propyl methyl cellulose, PDI: Polydispersibility Index.

CONFLICT OF INTEREST

There is no conflict of interest/competing interest. This work is original and nothing has been plagiarised to the best of my knowledge.

FINANCIAL ASSISSTANCE: None

REFERENCES:

1.Sahoo N, Kakran M, Shaal L, Li L, Müller R, Pal M. Preparation and characterization of quercetin nanocrystals. J Pharm Sci, 2011; 100 (6): 2379-90.

2.Gao L, Liu G, Wang X, Liu F, Xu Y, Jing M. Preparation of a chemically stable quercetin formulation using nanosuspension technology. Int J Pharm, 2011; 404: 231–37.

3.Patel V, Kukadiya H, Mashru R, Surti N, Mandal S. Development of microemulsion for solubility enhancement of clopidogrel. Iranian J Pharm Res, 2010; 9(4): 327-34.

4.Trifkovic M, Cardoso S, Sheikhzadeh M, Rohani S, Barghi S. Model Assisted Design and Simulation of a Pharmaceutical Batch Process; Manufacturing of Clopidogrel Bisulfate. Proceedings of the Canadian Engineering Education Association. 2011.

5.Takagi TRC, Bermejo M, Yamashita S, Yu LX, Amidon GL. A provisional biopharmaceutical classification of the top 200 oral drug products in the United States, Great Britain, Spain, and Japan. Molecular Pharmaceutics, 2006; 3: 631–43.

6.Patel V, Kukadiya H, Mashru R, Surti N, Mandal S. Development of microemulsion for solubility enhancement of Clopidogrel. Iranian J Pharm Res, 2010; 9 (4): 327-34.

7.Toshi C. A Review on Nanosuspensions promising Drug Delivery Strategy. Current Pharm Res, 2012; 3(1): 764-776.

8.Ezeddin K. Nanodispersions Platform for Solubility Improvement. Int J Res Pharm Biomed Sci, 2013; 4(2): 636-643.

9.Kumar GP. Nanosuspensions: The Solution to Deliver Hydrophobic Drugs. Int J Drug Delivery, 2011; 3: 546-557.

10.Nagare SK, Ghurghure SM, Khade AB, Jadhav SG, Salunkhe SB. A Review on Nanosuspensions - An Innovative Acceptable Approach in Novel Delivery System. Ind J Novel Drug Delivery, 2012; 4(3): 189-201.

11.Mallik S, Kshirsagar MS, Saini V. Studies on physical /chemical compatibility between synthetic and herbal drugs with various pharmaceutical excipients. Scholars Research Library Der Pharmacia Letters 2011; 3(5): 173-178.

12.Hao J, Gao Y, Zhao J, Zhang J, Li Q, Zhao Z, Lin J. Preparation and optimization of resveratrol nanosuspensions by antisolvent precipitation using box-behnken design. AAPS Pharm Sci Tech, 2015; 16(1): 118-128.

13.Verma P, Prashar N, Kumar V, Chaudhary H. Nasal (In-situ) Gel (Phenylepherine HCl) for Allergic Rhinitis Congestion treatment: Development and Characterization. American Journal of PharmTech Research, 2016; 6(3): 299-314. http://www.ajptr.com/archive/volume-6/june-2016-issue-3/63022.html

14.Tao T. Preparation and evaluation of Itraconazole dihydrochloride for the solubility and dissolution rate enhancement. Int J Pharm., 2009, 367: 109–114.

15.Maheswari UR, Mullaicharam AR. Development and In-Vitro Evaluation of Nanosuspension Formulation Containing Acyclovir for the Treatment of Ocular Infections. Res J Pharm Biol Chem Sci, 2013; 4(1): 463-80.

16.Ahmed S, Nazmi M, Hasan I, Sultana S, Haldar S, Reza MS. Fexofenadine HCl Immediate Release Tablets: In vitro Characterization and Evaluation of Excipients. Bang Pharm J, 2013; 16(1): 1-9.

17.Ige PP, Baria RK, Gattani SG. Fabrication of fenofibrate nanocrystals by probe sonication method for enhancement of dissolution rate and oral bioavailability. Colloids and Surfaces B: Biointerfaces. 2013; 108: 366– 73.

18.Park YJ, Hyun CK. Revaprazan-containing solid dispersion and process for the preparation thereof. WO Patent 078922. 2008.

19.Muller RH, Jacobs C, Kayer O. Nanosuspensions for the formulation of poorly soluble drugs. In: F Nielloud, G Marti- Mestres (ed). Pharmaceutical emulsion and suspension. Marcel Dekker, New York. 2000, 383-407.

20.Costa P, Sousa Lobo JM. Modeling and comparison of dissolution profiles. Eur J Pharm Sci. 2001; 13(2): 123–33.

21.Dash S, Murthy PN, Nath L, Chowdhury P. Kinetic modeling on drug release from controlled drug delivery systems. Acta Pol Pharm, 2010; 67(3): 217–23.