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FORMULATION, CHARACTERIZATION AND IN-VITRO EVALUATION FOR SOLUBILITY ENHANCEMENT OF A POORLY WATER SOLUBLE DRUG USING NANOEDGE TECHNIQUE

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ABSTRACT

The purpose of the present investigation was to increase the solubility and dissolution rate of Clopidogrel bisulphate by the preparation of nanosuspension by precipitation with High-pressure homogenization (HPH) i.e. Nanoedge technique. Clopidogrel bisulphate (CPS) is a crystalline poorly water soluble drug having fair bioavailability of 50%. In this present work different batches were fabricated using different polymers such as PVP K-30 and PVA with different surfactants such as SLS and Tween 80. Preformulation study was carried out different techniques such as FTIR and differential scanning calorimetry (DSC). Prepared nanosuspension was evaluated for its particle size using scanning electron microscopy (SEM), Zeta- potential, in-vitro dissolution study and short term stability study was done for the drug profile. The results showed that nanosuspension prepared with polymer PVP K-30 and surfactant sodium lauryl sulfate (SLS) proved to be better optimized batch compared to the available marketed product. This can be explained due to greater surface area of the nanosuspension compared to the conventional techniques.

KEYWORDS: Clopidogrel bisulphate, Nanosuspension, HPH, Zeta-potential, SEM, DSC.

1.INTRODUCTION

An important fraction (~40%) of the new drug candidates emerging from drug discovery programs has poor water solubility and this trend is not expected to change in the future [1]. Nowadays, large portion of new molecules come from combinatorial chemistry which focuses on target-receptor geometry, target identification and lead candidate generation. However, candidates emerging from these screens invariably have high molecular mass and high Log P, which contribute to insolubility [2]. Also, high affinity and high specificity binding to molecular targets generally entails some degree of hydrophobic interactions which leads to solubility constraints [3]. The basic challenges associated with poorly soluble drugs are low bioavailability and/or erratic absorption. Many approaches are used to solve the problems of poor solubility and poor bioavailability of drugs. The conventional approaches include micronization, use of fatty solutions, use of penetration enhancer or co solvents, surfactant dispersion method, salt formation, precipitation, liposome, dispersion of solids, emulsion and micro emulsion methods, inclusion complexes with cyclodextrins [4]. These techniques shows beneficial effect as drug delivery system but major problems of these techniques are lack of universal applicability to all drugs. Among the most promising solutions to this challenge are nanosuspensions. Nanosuspension technology can be used to improve the bioavailability of poorly soluble drugs and also provide stability to the drug [5]. Nanosuspensions (NS) are defined as biphasic systems consisting of submicron-sized crystalline drug particles dispersed in an aqueous vehicle in which the particles are stabilized by different polymers and coatings of surfactants (surface-active agent which reduces surface tension) to produce stable pharmaceutical formulations [6].

Clopidogrel bisulphate (CPS) being very slightly soluble was investigated as the model drug for this study. Clopidogrel bisulphate is an oral, thioenopyridine, anti-platelet agent used to inhibit blood clots in coronary artery disease, peripheral vascular disease. The drugs works by irreversibly inhibiting the receptor called P_2Y_{12}, an adenosine diphosphate (ADP) chemoreceptor on platelet cell membranes [7].

Because of having poor water solubility, its absorption is dissolution rate limited, which often results in irregular and delayed absorption. Reports in the literature reveal that CPS has got low oral bioavailability 50 %; plasma protein binding (94-98%) with the half-life is 8 hrs [8]. Presently, CPS is available on the market in conventional tablet forms which can't increase the oral bioavailability and have multiple of therapeutic effects. Therefore, an alternative

route of drug delivery and dosage form that can selectively target the drug directly into various regions of the body was selected [9].

In the present study, an attempt was made to improve the dissolution of CPS using precipitation with high- pressure homogenization (NANOEDGE) technique method, where a drug solution in a water miscible organic solvent is mixed with an aqueous solution containing different amounts of surfactants and polymers. The mixing of two solutions done by using magnetic stirrer followed by high-pressure homogenization leads to formation of nanoparticles. The major drawback of the precipitation technique, such as crystal growth and long-term stability, can be resolved using the Nanoedge technology. The nano suspension can further used as a granulating fluid in the formation of a stable solid dosage forms such as tablets and capsules [10].

2. MATERIALS AND METHODS

2.1. Materials

Clopidogrel bisulphate was obtained as a gift sample from Cadila pharmaceuticals, Gujarat. PVP K-30 from BASF, Mumbai; PVA and Tween-80 from signet, Mumbai; SLS from Stephan, Germany and ethanol from Merck Millipore. All reagents used were of analytical grade.

2.2. Methods

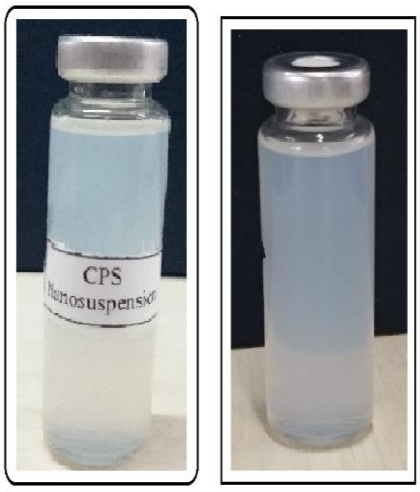

Figure 1. Images of prepared CPS nanosuspension

The nanosuspension was obtained by the precipitation with high-pressure homogenization technology. The drug clopidogrel bisulphate (CPS) was initially dissolved in 2 ml of organic

solvent such as ethanol (organic phase). The carriers PVA, PVP K-30 and surfactants Tween 80, SLS were added to 20 ml of purified water (aqueous phase). Then the organic phase was slowly added drop wise with syringe into the aqueous phase which is kept at room temperature and stirred with a speed of 600-800 rpm for 1hr to evaporate organic solvents using Magnetic stirrers. Different batches of nanosuspension with polymers and surfactants ratios were shown in Table 2.

2.3. Solubility studies

The solubility of clopidogrel bisulphate was determined by mixing an excess quantity of the drug with approximately 2 ml of the solvent taken in a glass vials with rubber stopper. Then the vials were shaking with mechanical shaker (Remi, Mumbai, India) for 24 hrs at room temperature. After 24 hrs the vials were centrifuge with (Remi, Mumbai, India) at rpm 1500-3000 for 10 mins. Then the supernatant liquid was pipette out from each vials followed by dilution with suitable solvent and the solubility was determine in UV-Visible spectrophotometer(Shimadzu-1800,Japan) at 200-800 nm respectively [11].

2.3.1. Size reduction of particles

2.3.1.1. High pressure homogenization

Nanosuspension of CPS was further passed through high-pressure homogenizer (GEA Niro soavi, Model type NH 3006-15) in order to get smaller particle size of the prepared nanosuspension. The homogenization steps includes first two steps with 200 bar pressure and next two cycles with 500 bar pressure as initial step. Finally the suspension is homogenized for 10 cycles with 1500 bar pressure to obtain final nanosuspensions [12]. Then the sample was cool down and placed in the refrigerator at 4 to 6 °C for further analysis such as surface characteristics, particle size, SEM etc.

Scanning Electron Microscopy

The morphology study of the raw CPS powder and the nano-sized CPS were determined using a scanning electron microscope (Model JSM 84 0A, JEOI, Japan) for roundness, smoothness and the formation of aggregates characteristics. Freshly prepared CPS nanosuspensions in small amount were placed on a glass slides following the evaporation of solvent and photomicrographs were taken with scanning electron microscope [13].

Particle size, Polydispersity index, and Zeta potential

The particle charge has importance in the study of the stability of the nano suspensions. Generally the zeta potential of more than ±40mV will be considered to be required for the stabilization of the dispersions. For electrostatically stabilized nanosuspension a minimum zeta potential of ±30mV is required and in case of combined steric and electrostatic stabilization it should be a minimum of ± 20mV of zeta potential is required. The average diameter of CPS nanosuspensions was determined by photon correlation spectroscopy (PCS) (Zeta- sizer Nano ZS, Malvern Instruments, UK) at room temperature. Nanosuspension was added to the sample dispersion unit (deionized water) and stirred at 1500-2000 rpm with magnet in order to reduce the inter-particulate aggregation and laser obscuration range was maintained between 10-20 %. The samples were adequately diluted with deionized water and placed in an electrophoretic cell [14]. Nanoparticles were characterized by a mean z-average diameter and Polydispersity index, zeta potential results were shown in Table 3.

FTIR Spectroscopy

Compatibility of the clopidogrel bisulphate with different excipients used to formulate the nanosuspension was established by Fourier Transform Infrared spectral analysis. Clopidogrel powder alone and along with different excipients was compressed into a pellet along with IR grade KBr (KBr pellet technique) using Shimadzu hydraulic press. The FTIR Spectrum were recorded in the wave number 400-4000 cm^{-1} on a Shimadzu 8400-S FTIR spectrophotometer.

Drug entrapment efficiency

To determine drug entrapment efficiency after preparing the fresh nanosuspension, 10ml of each formulation was centrifuged and the free drug present in the supernatant liquid was analyzed by UV-Visible spectrophotometer at 200-800 nm. The amount free drug can be calculated by using calibration curve. For separation of nanoparticle from un-entrapped active ingredient, a portion of the nanosuspension was transferred to 3 ml thick wall polycarbonate centrifuge tubes. The samples were then centrifuged. Centrifugation was done by using BECKMAN COULTER centrifuge. The centrifugation was done at speed of 15000 rpm for 30 minutes [15]. The entrapment efficiency was calculated using the following equation

$$\text{Entrapment efficiency} = \frac{\text{Total Drug content} - \text{Free dissolved Drug} \times 100}{\text{Drug amount used}}$$

Differential scanning calorimetry

The differential scanning calorimeter was done to evaluate the compatibility study of drug with excipients. DSC scans of the powdered samples of CPS drug; PVP K-30, PVA, SLS and formulation mixtures were studied using DSC- Shimadzu 60 with TDA trend line software. All samples were weighed (10-12 mg) and heated at a scanning rate of 20°C/min under dry air flow (100 ml/min) between 50° C and 300° C [16].

In-vitro study

The *in-vitro* drug release studies of clopidogrel bisulphate nanosuspension was performed by dialysis method in an open end tube sealed with dialysis membrane (Himedia laboratories Pvt. Ltd., Mumbai, India. pore diameter 2.5 nm) was fitted in an USP dissolution apparatus containing 900 ml of buffer solution as dissolution medium at pH 1.2 with stirring at 50 rpm at 37±0.5^{0}C. CPS nanosuspension (5ml) was added into the dialysis tube and samples (1ml) were withdrawn at predetermined time intervals from the external release medium for a period of 1 hour and replaced by same volume of fresh medium to maintain sink condition [17].Absorbances of withdrawn samples were measured using a double beam UV-visible spectrophotometer at 222 nm. The amount of drug present in each aliquot was determined from standard calibration curve. The *in-vitro* drug release study was performed for all optimized formulation and compared the observed data to obtain the better bioavailability of drug.

Short term stability study of nanosuspension

Stability study was performed for physical appearance of the nanosuspension. Samples were stored at room temperature and 4^{0}C for 2 weeks, 1 and 2 months. The observation was done to check the physical appearance. Sedimentation of layer can be observed when nanosuspension was stored at room temperature for 1 and 2 months .If sediment were disappeared with slight hand shaking then the good stability of the formulation was achieved. If sediment did not disappeared with slight hand shaking then the poor stability of the formulation was achieved and that formulation batch was rejected. The optimization of the suitable batch on the basis of stability parameter can be done also by evaluating the average particle diameters when samples were stored at stability parameters. The particle size for the batches was evaluated before performing stability study and after performing stability study (Samples were stored at room temperature, 4^{0}C for 2 weeks, 1 and 2 months). Then the observed data was compared to show the stability of formulation.

RESULTS AND DISCUSSION

Clopidogrel bisulphate (CPS) is a BCS class-II drug having low solubility and high permeability. Thus, it was challenging to enhance the solubility of CPS particles in an aqueous solution. Precipitation with High-pressure homogenization (NANOEDGE) method has been employed to produce nanosuspension of CPS. The different formulate variables such as Surfactant: Polymer ratios were contributing much towards the change in particle size 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 nanosuspensions were evaluated for particle mean diameter, Polydispersity index (PI), Zeta-potential, Drug entrapment efficiency (DEE), In-vitro dissolution study and short term stability study. The solubility rate of clopidogrel bisulphate in different solvent is shown in Table-1. FTIR spectra of pure drug CPS, SLS, PVP K-30, drug with excipients were obtained which shows no chemical interaction between drug and excipients. The results of FTIR study shown in Figure no 6 to 11. Pure clopidogrel bisulphate exhibited a melting point at 180^0C which was confirmed by DSC spectra (Figure no-12) and also the DSC of optimized formulation was shown in (Figure no-13). The CPS nanosuspension prepared by HPH was found to be Bluish opalescence in color (Figure no-1). In the nanoscale, which is normally seen as going from 100 nm to 1000 nanometers range, is suitable for the nanoparticle suspension formulation. In this nano formulation the particle mean diameter was found to be in range of 245.1 nm to 995.6 nm. 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), >0.7 (very polydisperse). In this nano formulation the PDI was found to be in range of 0.138 to 0.842. In order to obtain a nanosuspension exhibiting good stability, for an electrostatically stabilized nanosuspension a minimum zeta potential of ± 30 mv is required whereas in the case of a combined electrostatic and steric stabilization, a minimum zeta potential of ± 20 mV is required. In this nano formulation the zeta potential was found to be in range of -5.9 to -26.7. Batch no F7 and F8 were taken as optimized formulation because these batches were found under the suitable range for nanosuspension formulation. Particle size of F7 was 430.0 nm, PDI was 0.235 and zeta potential was -19.6 mv whereas particle size of F8 was 245.1 nm and PDI was 0.138 and zeta potential was -26.7 mv. DEE of the CPS loaded nanosuspension was found to be 32.52 %, 42.43 %, 67.42 %,67.42 % and 82.61 %, for the selected batches on the basis of particle size, polydispersity index, zeta potential. The low DEE values indicate

relatively low affinity of the drug with the polymer matrix; also poor entrapment is probably solubility and ionization of the drug.

Because Clopidogrel is less soluble in water and SLS (surfactant) is present in the aqueous phase. Therefore, when the organic phase is added drop wise into the aqueous surfactant solution, part of the drug is ionized and escapes from the nanoparticles during diffusion of the ethanol into the aqueous phase. Batch no F8 was found to be DEE in range of 82.61 % in which the polymer and surfactant conc. was used as 5 mg and 10 mg respectively and this batch was found to be the robust formulation for clopidogrel nanosuspension.

The SEM picture of formulation F5, F6, F7 nanoparticles were found to be spherical with a smooth surface and less aggregate while F8 have spherical smooth surface and no aggregation (Figure no-5). According to all kind of information which is mentioned above regarding to particle size, zeta potential and PDI, and SEM image, the F8 preparation was proved as a best formulation among all batches. In vitro drug release from the nanosuspension was performed by the dialysis experiment using the static Franz diffusion cell. The release rate profiles were drawn as the percentage CPS dissolved from the nanosuspension and pure drug vs. time. Dissolution studies of pure CPS and all other prepared nanosuspension (F5-F8) were carried out in SGF pH 1.2. t50% (time to dissolve 50% drug) values calculated from release profile are reported in Table no-6. From this data, it was evident that onset of dissolution of pure CPS was very low as compare to its nanosuspension. Physical appearance of the F8 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. Before performing stability study the particle size of the formulation F8 was 245.1 and it was found to be 299.3, 245.2, 244.5, 245 nm (in average) when samples stored at room temperature and 4^0C for 2 weeks, 1 and 2 months. It can be inferred from the observed data that the prepared nanosuspension F8 was passed the stability test and was stable at different conditions.

Table -1: Solubility data of Clopidogrel bisulphate in different medium

Sl. No	Medium	Solubility
1	Normal pH water	Practically insoluble
2	0.1 N HCl (pH1.2)	More soluble
3	pH 4.5 Acetate buffer	Less soluble
4	pH 6.8 Phosphate buffer	Less soluble
5	Ethanol	Highly soluble

Table-2: Formulation batches of clopidogrel bisulphate nanosuspension

Ingredients	Functions	F1	F2	F3	F4	F5	F6	F7	F8
CPS (mg)	API	10	10	10	10	10	10	10	10
Tween 80 (ml)	S-1	0.5	1						
SLS (mg)	S-2			1	2	3	4	5	5
PVA (mg)	P-1					5	10		
PVP K-30 (mg)	P-2							5	10
Ethanol (ml)	Solvent	2	2	2	2	2	2	2	2
Purified water (ml)	Solvent	20	20	20	20	20	20	20	20

Table-3: Particle diameter, Polydispersity index, Zeta potential of CPS nanosuspension

SL no.	Batch No.	Particle diameter (nm)	Polydispersity index(PI)	Zeta potential (mv)
1	F1	995.6	0.819	-5.9
2	F2	866.8	0.842	-8.22
3	F3	868.5	0.816	-9.7
4	F4	777.3	0.789	-8.9
5	F5	676.6	0.561	-11.63
6	F6	599.4	0.443	-12.8
7	F7	430.0	0.235	-19.6
8	F8	245.1	0.138	-26.7

Table-4: Selected batches on the basis of Particle size, polydispersity index and Zeta potential of CPS nanosuspension

Batch No.	Amt. of Drug (mg)	Amt. of surfactant (mg)	Amt. of polymer (mg)	Stirring speed (mg)
F5	10	3	5	1500
F6	10	4	10	1500
F7	10	5	5	1500
F8	10	5	10	1500

Table-5: Physical properties of CPS nanosuspension of optimized formulation

Sl. No	Batch No.	Shape of particles
1	F5	Spherical and less aggregation
2	F6	Spherical and less aggregation
3	F7	Spherical and less aggregation
4	F8	Spherical and no aggregation

Table-6: Time to dissolve 50% drug from pure CPS and its Nanosuspension

Batch No.	Pure drug	F5	F6	F7	F8
t 50 %	>> 8 hr	28	25	18	15

Table-7: Drug Entrapment efficiency (%) of the nanosuspension of CPS

Batch No.	Total amount of Drug taken (mg)	Amount of Drug in Supernatant (mg)	% Drug Entrapment efficiency
F5	10	7.25	32.52
F6	10	6.24	42.43
F7	10	4.74	67.42
F8	10	2.26	82.61

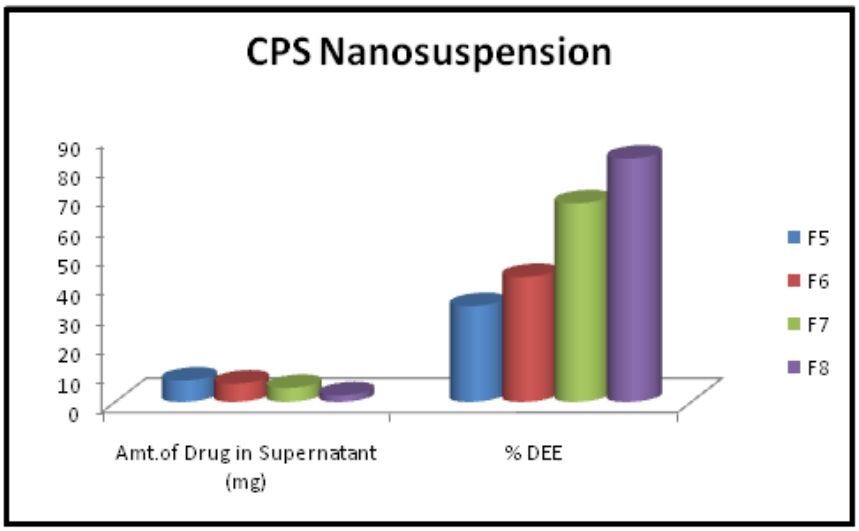

Figure-2: Drug Entrapment efficiency of CPS Nanosuspension

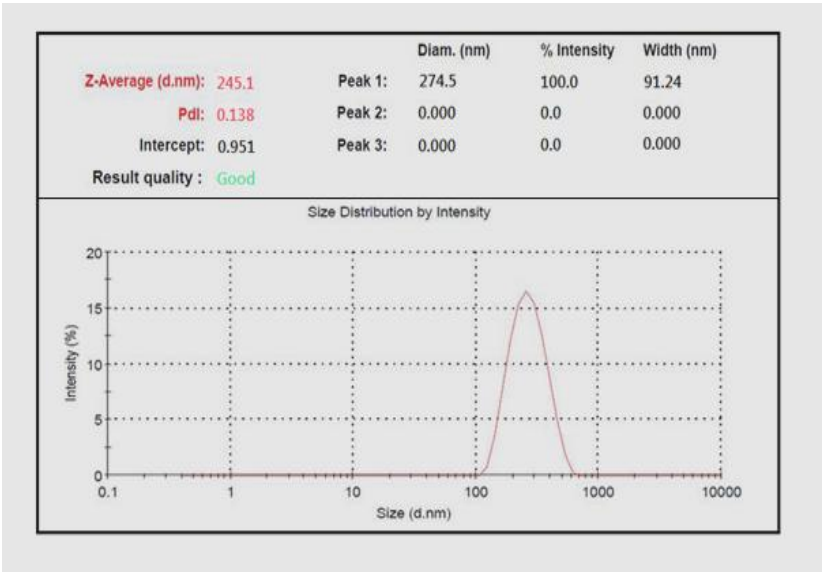

Figure-3: Particle size of Formulation F8

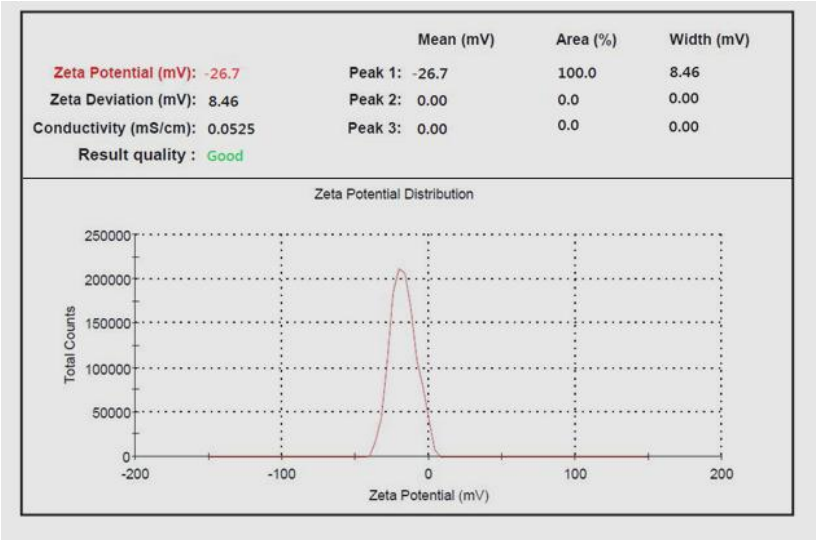

Figure-4: Zeta potential of Formulation F8

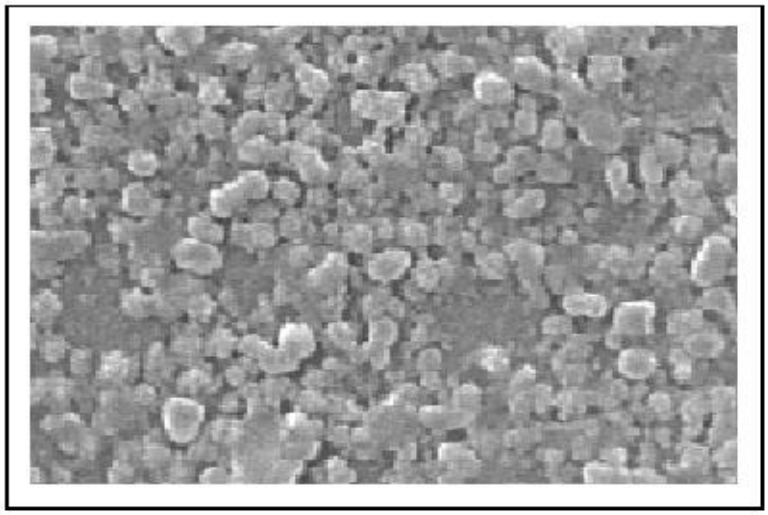

Figure-5: SEM picture of F8 formulation

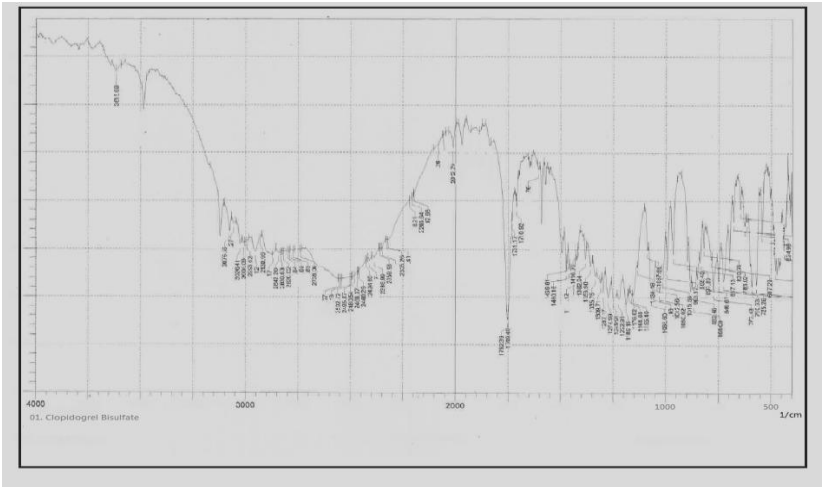

Figure-6: FTIR Spectra of pure Clopidogrel bisulphate

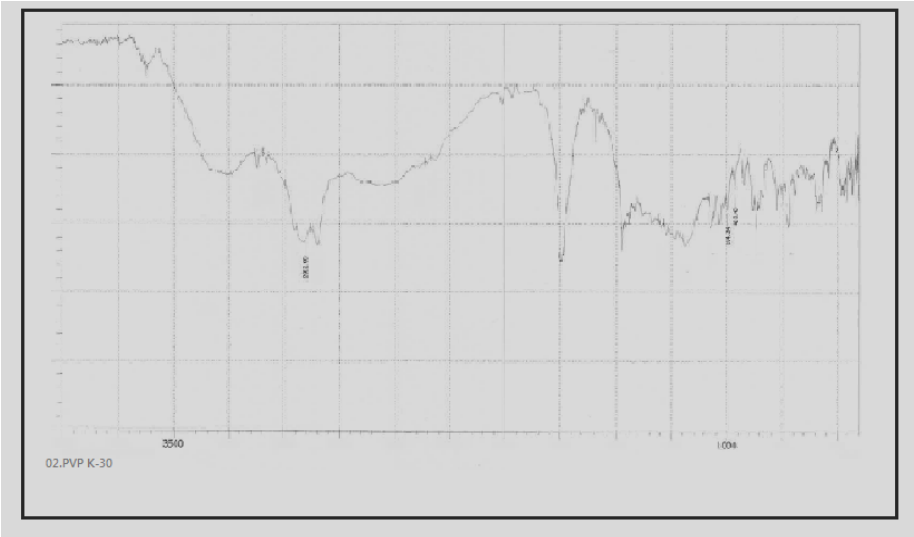

Figure-7: FTIR Spectra of PVP K-30

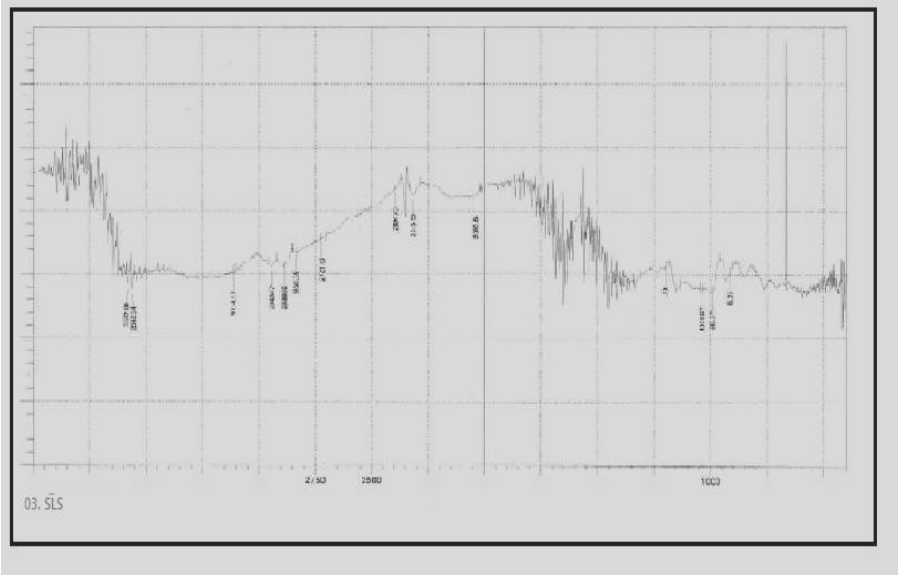

Figure-8: FTIR Spectra of SLS

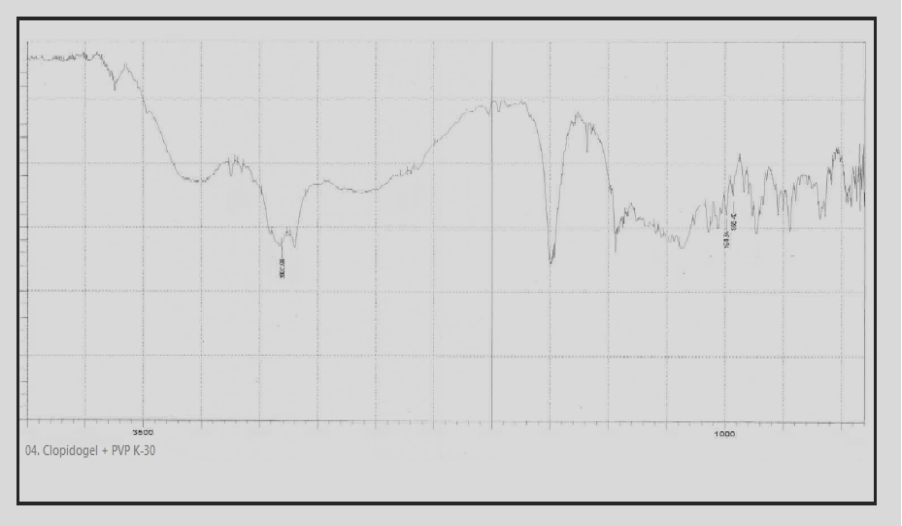

Figure-9: FTIR Spectra of Clopidogrel bisulphate + PVP K-30

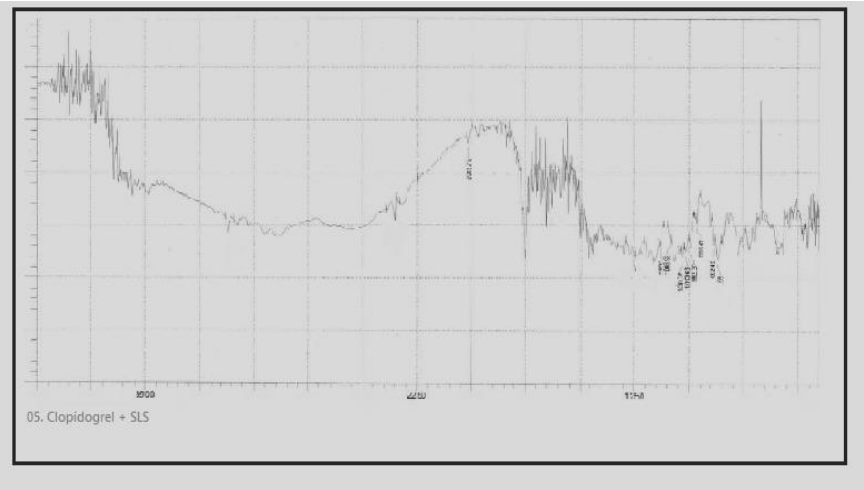

Figure-10: FTIR Spectra of Clopidogrel bisulphate + SLS

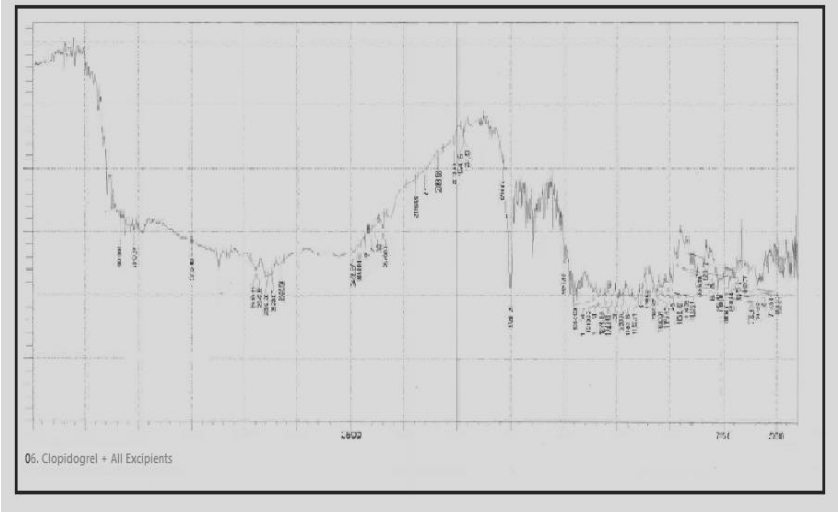

Figure-11: FTIR Spectra of Clopidogrel bisulphate + All Excipients

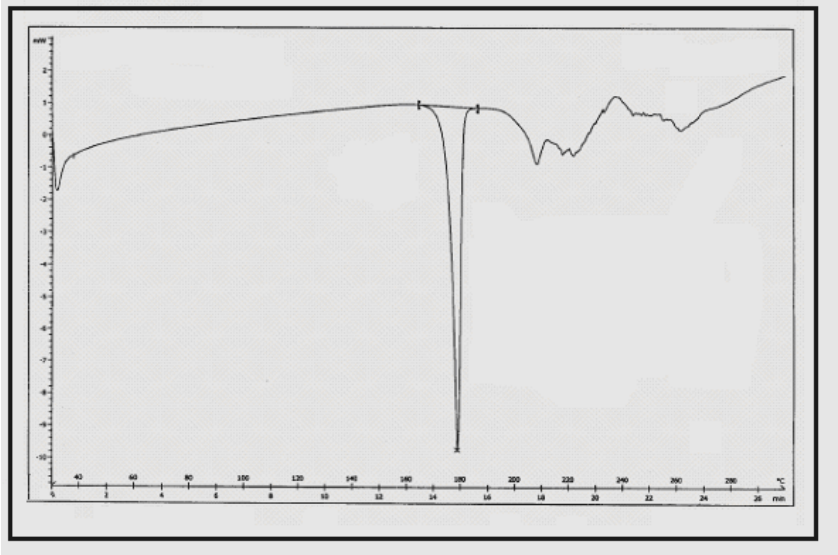

Figure-12: DSC of pure Clopidogrel bisulphate

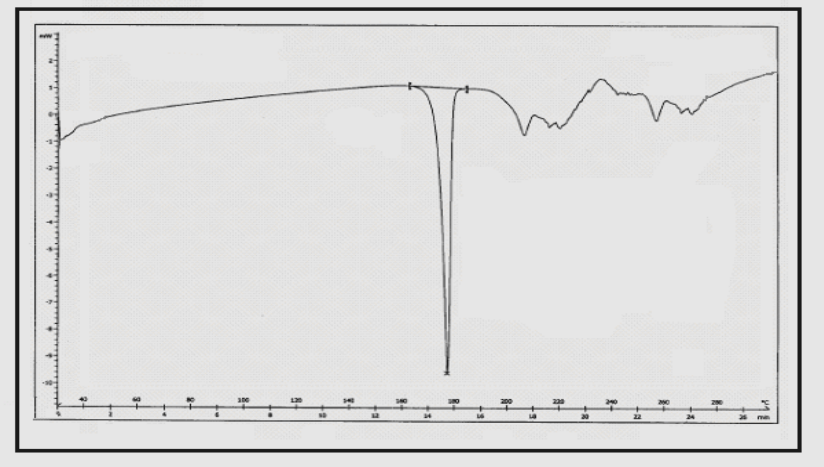

Figure-13: DSC of pure CPS Nanosuspension

CONCLUSION

A nanoprecipitation with HPH method was developed to prepare Clopidogrel bisulphate nanoparticles using different surfactants and polymers. In this process, the particle size of CPS can be obtained in the nano-size ranges by selecting proper stabilizer. The best nanosuspension of CPS can be obtained by SLS as a surfactant and PVP K-30 as polymer using Nanoedge technique. The dissolution of nanosized CPS is significantly enhanced compare with the marketed drug. In conclusion, the Nanoedge method offers a direct process to obtain drug nanoparticles of desirable size, amenable for continuous and consistent production. Clopidogrel cardiovascular drug (anti-platelet) in nano-suspension formulation can overcome the limitation of low solubility, dissolution and bioavailability.

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