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Formulation and evaluation of pitavastatin nanosuspension

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Abstract

The main aim of the study was to formulate and characterize nanosuspension of pitavastatin (poorly soluble drug) by precipitation method to improve its dissolution characteristics. The prepared nanosuspension were evaluated by DSC, Zeta potential analysis, SEM, FTIR, Solubility & *in-vitro* drug release studies.DSC curve obtained confirms the transfer of drug crystalline form to amorphous form. Solubility studies and *in-vitro* drug release studies shows that the prepared nanosuspension has increased solubility and dissolution rate compared to pure drug. The technology is easy to scale up and requires less sophistication, the method can be extended to various poorly water soluble drugs.

Key-Words: Pitavastatin, Nanosuspension, Nanoprecipitation

Introduction

Poor solubility of drug substance has always been a challenging problem faced by pharmaceutical scientists and it is increased now because more than 40% of new chemical entities are poorly water soluble. One of the most persistent problems faced by drugs with poor aqueous solubility is that their oral delivery is frequently associated with implication of low bioavailability and lack of dose proportionality. There are number of technologies like solid dispersion[1-2], complexation, co-solvency, use of surfactants, etc., but they lack universal applicability to all drugs. A novel technology that can used to overcome problems associated with this method is nanosuspension, which is based on size reduction mechanism.

In the present research work an attempt was made to improve the solubility and dissolution rate of model drug pitavastatin. Pitavastatin as a synthetic lipid-lowering agent is an inhibitor of 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMGCoA) reductase which catalyzes the conversion of HMG-Co A to mevalonate, an early rate-limiting step in cholesterol biosynthesis[3]. Pitavastatin is a hyperlipidemic drug whose bioavailability is reported has less than 5% [4]. Nanosuspension of pitavastatin is prepared by precipitation method using β-cyclodextrin as stabilizer and Tween 80 as surfactant.

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Material and Methods

Pitavastatin was obtained as a gift sample from spectrum labs, Hyderabad. All other chemicals and solvents used are of analytical grade.

Preparation of pitavastatin nanosuspension by nanoprecipitation

Nanosuspensions were prepared by the precipitation technique. Pitavastatin was dissolved in a methanol at room temperature. This was poured into water containing different amount of β-cyclodextrin and tween 80 maintained at room temperature and subsequently stirred on magnetic stirrer (Remi, India.) to allow the volatile solvent to evaporate. Addition of organic solvents by means of a syringe positioned with the needle directly into stabilizer/surfactant containing water. Organic solvents were left to evaporate off under a slow magnetic stirring of the nanosuspension at room temperature for 1 hour [5].

Particle size

Particle size was determined by using a Zetasizer 3000 (Malvern Instruments, UK). This analysis yields the mean diameter (z-average, measuring range: 20–1000 nm). In order to have a better determination of the particles size, the samples were diluted with deionized water and redispersed by shaking.

Saturation solubility studies

Saturation solubility measurements were assayed through ultraviolet absorbance determination at 241 nm using shimadzu UV-Visible spectrophotometer. The saturation solubility studies were carried out for both the unprocessed pure drug and different batches of

lyophilized nanosuspensions. 10 mg of unprocessed pure drug and nanosuspensions equivalent to 10 mg of pitavastatin were weighed and separately introduced into 25 ml stoppered conical flask containing 10 ml distilled water. The flasks were sealed and placed in rotary shaker for 24 hrs at 37°C and equilibrated for 2 days. The samples were collected after the specified time interval and it is filtered and analyzed. The diluted samples were analyzed using UV spectrophotometer at 241nm [6].

Drug entrapment efficiency (DEE) [7]

The freshly prepared nanosuspension was centrifuged at 20,000 rpm for 20 min at 5°C temperature using cool ultracentrifuge. The amount of unincorporated drug was measured by taking the absorbance of the appropriately diluted 25 ml of supernatant solution at 241 nm using UV spectrophotometer against blank/control nanosuspensions. 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 for each batch and the average was calculated.

The entrapment efficiency (EE %) could be achieved by the following equation:

$$\text{Entrapment efficiency}\,(\%) = \frac{W_{\text{initial drug}} - W_{\text{free drug}}}{W_{\text{initial drug}}} \times 100$$

Fourier transform infra red spectroscopy (FTIR) [8-9]

Compatibility study of pitavastatin with the carrier β-cyclodextrin, and mixture β-cyclodextrin used to formulate nanosuspensions was determined by FTIR Spectroscopy using Perkin Elmer RX1. Spectral analysis of pitavastatin, β-cyclodextrin and combination was carried out to investigate the changes in chemical composition of the drug after combining it with excipients. The compatibility study on FTIR was carried by JASCO FT/IR 4100, MD, and USA in the frequency range 4000-400 cm^{-1}.

Scanning Electron Microscopy (SEM)[10]

Surface morphology of the specimen will be determined by using a scanning electron microscope (SEM), Model JSM 84 0A, JEOI, Japan. The samples are dried thoroughly in vaccum desicator before mounting on brass specimen studies, using double sided adhesive tape. Gold-palladium alloy of 120°A Knees was coated on the sample sputter coating unit (Model E5 100 Polaron U.K) in Argon at ambient of 8-10 with plasma voltage about 20mA. The sputtering was done for nearly 5 minutes to obtain uniform coating on the sample to enable good quality SEM images. The SEM was operated at low accelerating voltage of about 15 KV with load current about 80 mA.The condenser lens position was maintained between 4.4-5.1. The objective lens aperture has a diameter of 240 microns and working distance WD = 39mm.

Zeta Potential measurement[11]

Zeta potential of the suspension is measured by Malvern Zetasizer. The zeta sizer mainly consists of laser which is used to provide a light source to illuminate the particles within the sample. For zeta potential measurements this light splits to provide an incident and reference beam. The incident laser beam passes through the center of the sample cell, and the scattered light at an angle of about 130 is detected. Zetasizer software produces a frequency spectrum from which the electrophoretic mobility hence the zeta potential is calculated.

Thermal analysis by differential scanning calorimetry (DSC)

DSC scans of the prepared lyophilized powdered drug sample and pure drug samples were recorded using DSC- Shimadzu 60 with TDA trend line software. All samples were weighed (8-10 mg) and heated at a scanning rate of 10°C/min under dry nitrogen flow (100 ml/min) between 50 and 300° C. Aluminum pans and lids were used for all samples. Pure water and indium were used to calibrate the DSC temperature scale and enthalpy response.

Dissolution study[12]

In vitro drug release studies were performed in USP apparatus-Type II using paddle method at rotation speed of 50 rpm. Dissolution was carried out in 0.1N HCL as a dissolution medium. The volume and temperature of the dissolution medium were 900 ml and 37.0 ± 0.5° C. 5 ml of sample was withdrawn periodically (after 5minutes) and replaced with an equal volume of fresh 0.1N HCL up to 60min. Samples were suitably diluted and filtered through a filter paper (0.22 µm, Whatman Inc., USA). The filtrate was then subject to the UV analysis against the blank (distilled water). Percent cumulative release of pitavastatin was calculated based on the standard UV calibration curve at 241nm (Systronic 2203, Japan).

Results and Discussion

Pitavastatin is a BCS class-II drug having low solubility and high permeability. Thus, it is challenging to enhance the solubility of pitavastatin particles in an aqueous solution. Solvent evaporation with precipitation has been employed to produce nanosuspension of pitavastatin. The different formulative variables (1) amount of β-cyclodextrin (2) amount of Tween 80 and (3) organic to aqueous

solvent ratio were contribute much towards the change in particle size in nanosuspension preparation. Nanosuspension of pitavastatin was prepared as formulation design shown in table 1. Formation of a colloidal nanosuspension can be visualized by the bluish opalescence.

In the prescreening study β-cyclodextrin and Tween 80 were selected as stabilizers. From this study, it was found that higher concentration of β-cyclodextrin with higher concentration of Tween 80 and lower concentration of organic to aqueous solvent ratio gives desire particle size(446 nm) compared to other formulation. The stabilizer's characteristics and concentration played an important role in creating a stable formulation. It must be capable of wetting the surface of the drug crystals and providing a steric or ionic barrier. Too little stabilizer include agglomeration or aggregation and too much stabilizer promotes Ostwald's ripening. It was observed that particle size (nm) and rate of dissolution has been improved when nanosuspension prepared with the higher concentration of β-cyclodextrin with the higher concentration of Tween 80 and lower concentration of organic to aqueous ratio.

The particle size of pitavastatin was significantly reduced from 50µm to 446nm for the formulation F5.The least particle size was observed with formulation F5 containing β-cyclodextrin and Tween 80.Our finding suggest that beyond the particle size change some other factors related to the carrier used may be predominantly influencing the solubility of pitavastatin. Overall analysis by pitavastatin nanosuspension suggests the particle size or the solubility of nanosuspension appears dependent on the carrier characteristics and the concentration of the carrier.

The drug entrapment of the nanosuspension was found to high (92%) with F5 containing higher concentration of β-cyclodextrin and Tween 80 whereas the least drug entrapment was observed in F1 (54%) containing lesser concentration of β-cyclodextrin and Tween 80. All the formulation showed drug entrapment above 54%.An increase interaction between the drug and the carrier results in increased in drug entrapment.

The saturation solubility studies indicating that nanosuspension showing maximum solubility compare to unprocessed drug which is due to the amorphous nature of drug after precipitation. The physical state of raw pitavastatin and lyophilized drug nanoparticles was examined by DSC. The DSC of pitavastatin shows an endothermic curve at its melting point 184.9°C (ΔH = -15.80 J/g) and the precipitated drug shows an endothermic peak at 300. 7°C (ΔH = -16.43J/g). The complete absence of pitavastatin peak indicates that pitavastatin is present as amorphous after being precipitated as nanoparticles it reduced crystallinity.

The zeta potential analysis value of prepared nanosuspension was found to be -34.4, which indicates that the formulation having good stability. Nanoparticle surface morphology and shape were visualized using SEM (JSM-T330A, JEOL).The drug loaded nanoparticles were found to be irregular with smooth surface.

In-vitro drug data shows the increased dissolution rate of formulations compared to unprocessed drug. The formulations show a maximum cumulative percentage drug release of 98.73% within 10 mins, where as the unprocessed drug having maximum release of 29.16% only. This shows that the precipitated drug has better dissolution rate, solubility and permeability compared to unprocessed drug.

Conclusion

The results of the present study demonstrate that nanoprecipitation technique was employed to produce nanoparticles of pitavastatin, a poorly water-soluble drug, for the improvement of solubility and dissolution velocity. In this process, the particle size of pitavastatin can be obtained in the nano-size ranges, by adjusting the operation parameters. The best nanosuspension of pitavastatin can be obtained by 10mg, β-cyclodextrin 30mg, and 0.2% w/v Tween 80. Thus it can be concluded that the dissolution of pitavastatin is significantly increased when it is nanosized and thus the bioavailability can be increased compared with the pure pitavastatin.

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Table 1: Formulation of pitavastatin nanosuspension

Formulation of pitavastatin nanosuspension					
Ingredients	**F_1**	**F_2**	**F_3**	**F_4**	**F_5**
Pitavastatin	10	10	10	10	10
β-cyclodextrin	10	20	20	30	30
Tween 80	0.1	0.1	0.2	0.1	0.2
Methanol	5	5	5	5	5
Water	10	10	10	10	10

Table 2: Results of solubility and drug entrapment efficiency. Studies of Formulation and Pure Drug

Sample	Solubility (µg/ml)	Drug entrapment
F0	132	----
F1	287	54
F2	378	70
F3	628	78
F4	548	86
F5	651	92

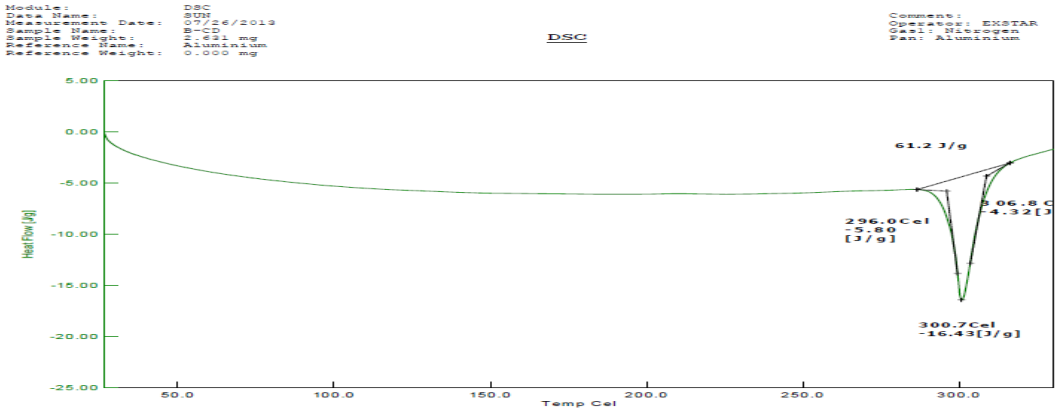


Fig. 1: DSC thermograph of formulation F5

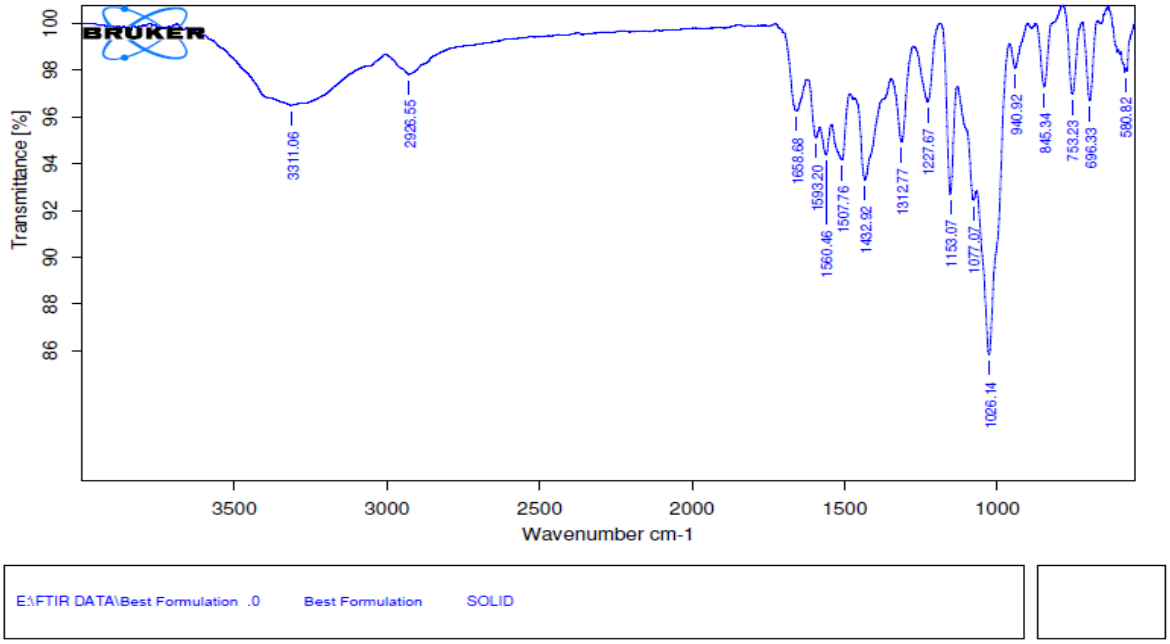


Fig. 2: FTIR spectra of pitavastatin,β-cyclodextrin,Tween 80 formulation F5

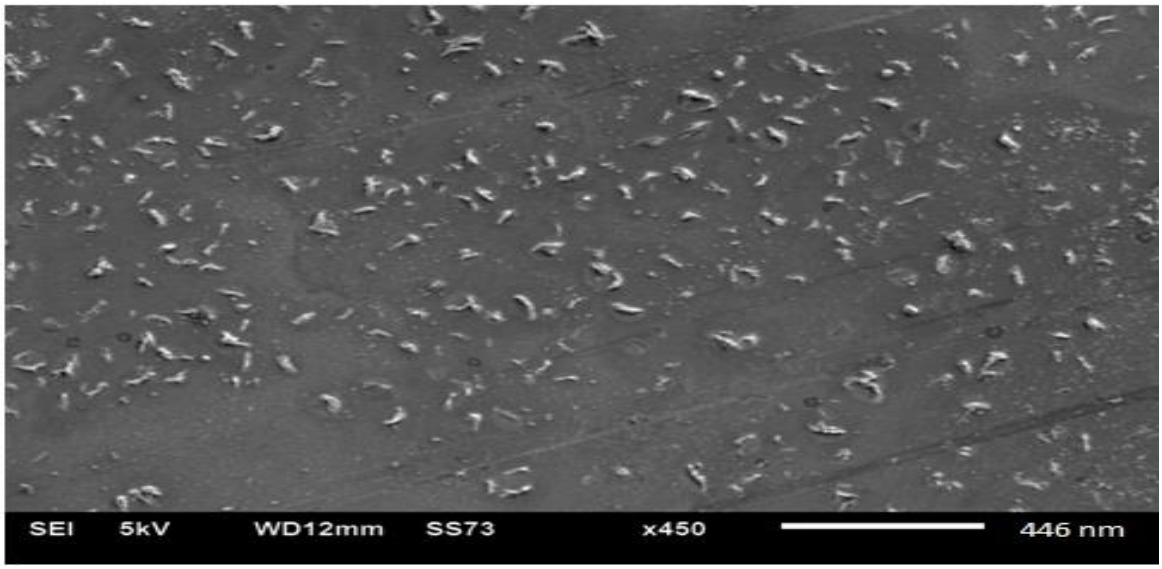


Fig. 3: Screening electron microscopy of formulation F5

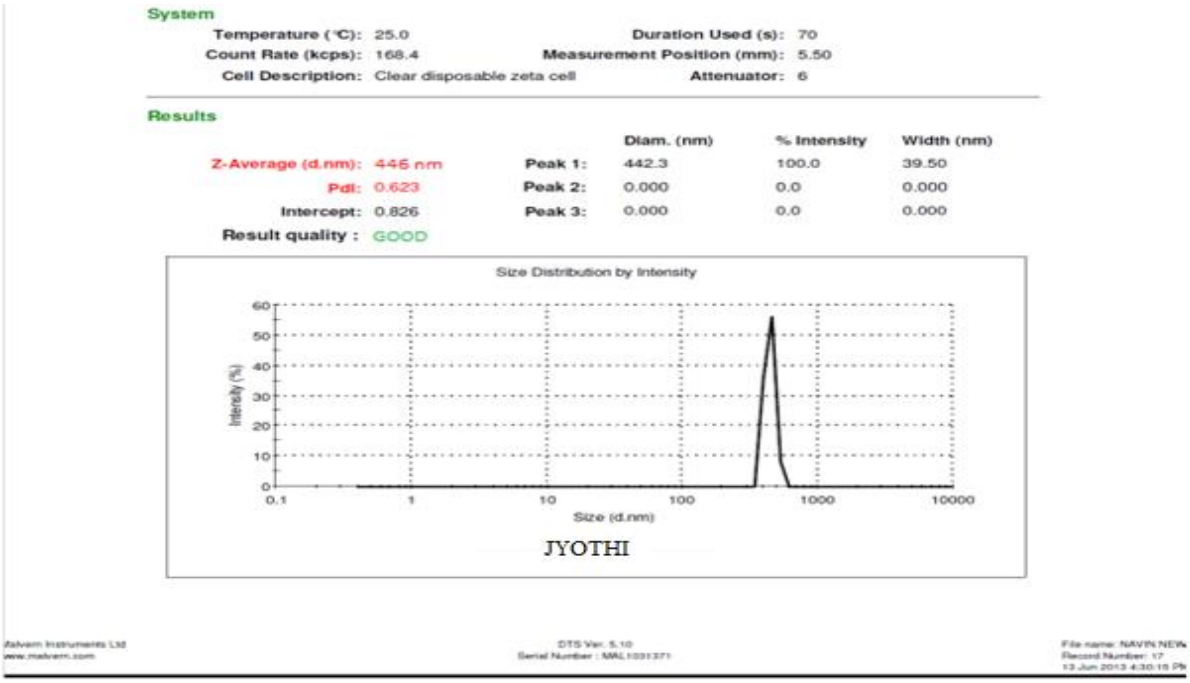


Fig. 4: Zeta potential analysis of formulation F5

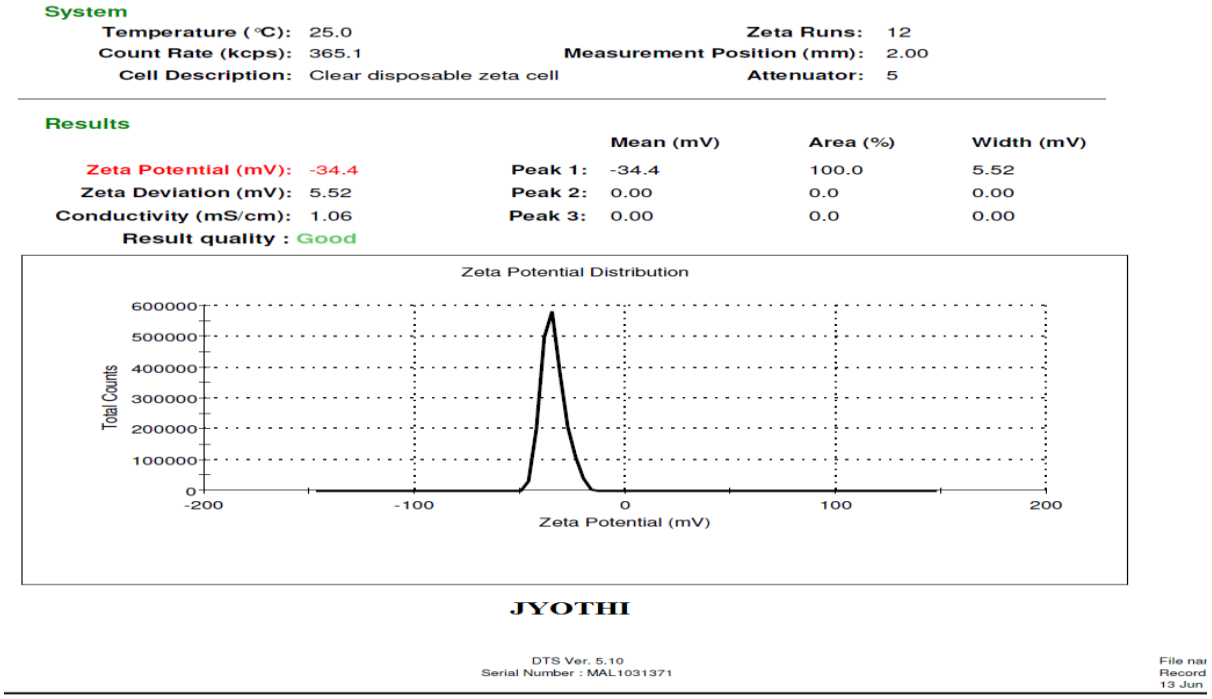
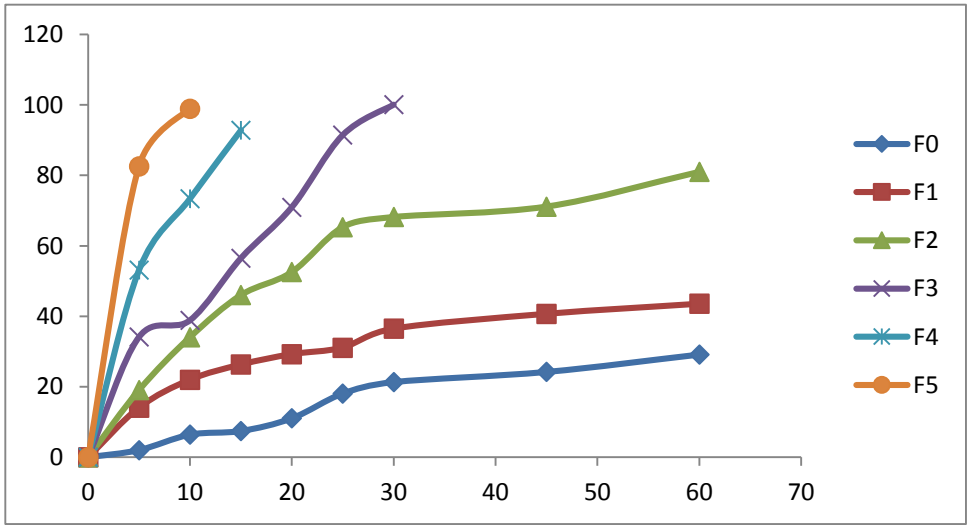


Fig. 5: Zeta potential analysis of formulation F5



Graph 1: Comparative study of nanosuspension formulations

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