The objective of the present study was to formulate and characterize the poorly soluble drug simvastatin in order to enhance the solubility and dissolution characteristics. Simvastatin is a Biopharmaceutical classification system (BCS) Class II drug having very less solubility and therefore low oral bioavailability (5%). In the present study, simvastatin nanosuspensions were prepared by homogenization technique by using poloxamer 188 and poloxamer 407 with different ratios. The Lyophilized nanosuspensions were characterized by differential scanning calorimetry (DSC), powder X-ray diffractometry (pXRD), scanning electron microscopy (SEM) and infra red spectroscopy (FTIR). The nanosuspension was evaluated for drug content, saturation solubility and in-vitro dissolution studies. The pXRD profile of nanosuspension suggests that transformation of crystalline drug into amorphous form. DSC studies revealed that there was no interaction between drug and carrier. The effect of particle size was found to be significant on the saturation solubility of the drug and in-vitro drug release studies showed significant increase in the dissolution rate of nanosuspensions as compared with pure drug. This study has shown that initial crystalline state is changed to amorphous form due to particle size reduction. Saturation solubility with poloxamer 188 and poloxamer 407 was found to be 317.7 µg/ml and 218 µg/ml respectively when compared with pure drug. The dissolution profile with poloxamer 188 was better than with poloxamer 407, with former releasing 99.14 % drug in 60 minutes. In conclusion the results indicated nanosuspension technique by using poloxamer successfully used for enhancing the solubility of simvastatin. nanosuspension with poloxamer 188 showed suitability, as a stabilizer in the formulation of nanosuspension.
INTRODUCTION

Fairly soluble drugs in gastrointestinal media exhibit complete oral absorption, and thus good bioavailability. About 40% of drugs are not soluble in water in practice and therefore are slowly absorbed, which results in insufficient and uneven bioavailability and GI toxicity [1]. Thus, most exigent phase of drug development practice particularly for oral dosage forms is the enhancement of drug solubility thereby increases its oral bioavailability. Bioavailability refers to the limit of therapeutically active drug that approaches the systematic circulation and thus, is available at the site of action [2]. There are two reasons proposed for poor aqueous solubility of drugs [3]: (i) high lipophilicity and (ii) strong intermolecular forces which cause the insolubilization of drugs [2]. Various approaches have been proposed to enhance solubilization of poorly water soluble drugs for the improvement of their bioavailability [4]. The methodologies commonly used for drug solubilization includes micronization, chemical modification, pH adjustment, solid dispersion, complexation, co-solvency, micellar solubilization and hydrotropy [5]. This is preferred to narrate different traditional and novel methodologies for the increase in solubility of hydrophobic drugs for converting to oral dosage forms.

In this study, HPH was evaluated for nanoparticle preparation. Scientists use nanotechnology for classical and novel drug delivery applications. Nanoparticles can be defined as structure that have at least one length dimension less than or equal to 500nm, and exhibit novel and unique chemical, physical, or biological behavior because of their small size [6]. Nanosuspension, a carrier-free colloidal drug delivery system, consists essentially of pure drug nanoparticles (100-1000 nm) and a minimum amount of surface active agent required for stabilization. By definition, drug nanocrystals are nanoparticles composed of 100% drug without any matrix material, with a mean diameter below 1000 nm. The dispersion medium can be water, aqueous solutions or non-aqueous media. Surfactants and/or polymeric stabilizers are used for the stabilization of these systems. Nanonization of drug powders increase the surface of the particles, leading to an increase of the dissolution velocity. Another important aspect is the increase in saturation solubility. In addition, the distance of diffusion on the surface of drug nanoparticles is decreased, thus leading to an increased concentration gradient. The increased concentration gradient leads too much higher increase in the dissolution velocity as well [7].

Simvastatin (SV) is a cholesterol lowering agent that is derived synthetically from a fermentation product of Aspergillus terreus and widely used to treat hypercholesterolemia. SV is a white, crystalline and non-hygroscopic powder having log P= 4.4 and glass transition temperature of 25°C. Its molecular weight is 418.56. When given orally, SV (a lactone) is readily hydrolyzed into the corresponding β, δ-dihydroxy acid form, a potent competitive inhibitor of 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) - the enzyme that catalyzes the conversion of HMG-CoA to mevalonate, which is an early and rate-limiting step in the biosynthesis of cholesterol [8]. However, it has a short half life and is practically insoluble in water. It is also generally considered that compounds with poor water solubility will show dissolution rate–limited absorptions in vivo due to its poor absorption, distribution [9,10]. Therefore, it is very important to introduce effective methods to enhance the solubility and dissolution rate of drug, substantially leading to its improved oral bioavailability.

Poloxamer have been recently widely used as wetting and solubilizing agents as well as surface adsorption excipients. They have been employed to enhance the solubility, dissolution and bioavailability of many hydrophobic drugs using various techniques, for some drugs, the improvement of solubility using Poloxamer was higher compared to the order meltable polymers. In the present study, Poloxamer was thus empirically selected as a hydrophilic carrier for its excellent surfactant properties and oral safety [11].

The main possibilities for improving dissolution according to analysis are to increase the surface area available for dissolution rate by decreasing the particle size of the solid compound and or by optimizing the wetting characteristics of the compound surface, to decrease the boundary layer thickness, to ensure sink condition for dissolution and last but definitely not least, to improve the apparent solubility of drug under physiologically relevant condition [12]. In the present study simvastatin nanosuspension was prepared by high pressure homogenization method using two different carriers in different ratios.

MATERIALS AND METHODS

Simvastatin was collected as a gift samples from micro labs limited, Hosur, India. Poloxamer 188 and 407 was gift samples from Micro labs Bangalore. All other chemicals and solvents used are of analytical graded.

Preparation of Nanosuspension

Different formulations of simvastatin Nanosuspension was prepared by high pressure homogenization technique by using different concentration of the stabilizer like poloxamer 188 the formulations F4, F5, and F6 with different ratios 0.5 %, 1 % and 1.5 % respectively and poloxamer 407 F7, F8, F9 contains 0.5 %, 1 % and 1.5 % respectively, but in all the formulation drug concentration remain constant (5 g). Simvastatin powder (5 % w/v) was dispersed in aqueous surfactant solution using magnetic stirrer. After drug dispersion in the surfactant solution first size reduction step was done using an Ultra- Turrax T 25 basic homogenizer at 9500 rpm for 10 minutes [13-15]. The obtained mixtures were homogenized using Micron-LAB 40 high pressure homogenizer, the homogenization step includes first two cycles at 100 bar and next two cycles at 500 bars pressure as initial step. Finally the suspension was homogenized for 15 cycles at 1500 bar pressure.

Production of dry nanoparticles

The nanosuspension was lyophilized using a Virtis freeze dryer, USA. The freeze dried nanosuspension is used to increase the shelf life of nanosuspension and to study the dissolution behavior. 1% mannitol is added to each formulation as a cryoprotectant at the time of lyophilization. The sample is kept in deep freezer at -70°C overnight and then the sample is kept in Virtis freeze dryer for 2 days at -50°C at 2 millitorr.
Particle size analysis
The particle size analysis was performed by microtace blue wave particle size analyzer (Germany). First, the lyophilized powder was diluted with de-ionized water to obtain a suitable concentration for measurement [16]. The results observed from particle size distribution were used to confirm the formation of Nano size particles.

In vitro drug release studies
The in vitro release of pure simvastatin and the nanosuspension was determined in USP dissolution test apparatus using paddle method at a rotation speed of 50 rpm. The dissolution profile was carried out in freshly prepared acidic buffer (pH 1.2) and also in phosphate buffer (pH 7.0) containing 0.5 % sodium lauryl sulphate. 10 mg of pure drug and nanosuspension containing 10 mg of simvastatin equivalent was taken and placed in dissolution medium. The volume and temperature of dissolution medium were 900 ml and 37.0 ± 0.2°C, respectively. Samples were withdrawn at fixed time intervals and were filtered. The filtered samples were analyzed at 238 nm using Shimadzu UV-Visible spectrophotometer [17]. The results observed from different batches of formulation were compared with the dissolution profile of unprocessed drug.

Saturation solubility studies
Saturation solubility measurements were analyzed by ultraviolet absorbance determination at 238 nm using a Shimadzu UV-Visible spectrophotometer. The saturation solubility studies were carried out for both unprocessed pure drug and different batches of lyophilized Nanosuspension. 10 mg of unprocessed pure drug and Nanosuspension equivalent to 10 mg of Simvastatin were weighed and introduced separately into 25 ml stoppered conical flask containing 10 ml distilled water. The flasks were sealed and placed in a rotary shaker for 24 hrs at 37°C and equilibrated for 2 days. The samples obtained were collected after the specified time interval, and it is filtered and analyzed. The diluted samples were analyzed using UV spectrophotometer at 238 nm [18]. The results were analyzed in triplicate and standard deviations are reported.

CHARACTERIZATION OF NANOPARTICLES
Differential scanning calorimetry (DSC)
Thermal properties of powder samples were assessed by Differential scanning calorimeter (DSCQ20 V24.4) Germany). The 5 mg of sample was placed in the aluminum vial and kept in the instrument and then heated from 20°C to 200°C at heating rate of 10°C/min under a stream of nitrogen at a flow rate of 50 mm/min [16]. Enthalpy changes (H) were calculated from peak areas of samples and study the polymeric changes of formulation.

Scanning electron microscopy
Scanning electron microscopy is a type of electron microscopy that images the surface of solid specimen by using focused beam of high-energy electrons. The scanning process and image formation in SEM depends on signal produced by elastic and inelastic interactions between high energy electron beam and specimen surface. The particle size analysis of lyophilized Nanosuspension was carried out to confirm the Nano-size of formulation. The samples were lightly sprinkled on a double sided adhesive tape stuck to an aluminum stub and the stubs were coated with platinum. The stub containing sample was placed in scanning electron microscope chamber and analyzes the surface morphology.

Fourier Transform Infra-Red Spectroscopy
FT-IR spectra nanosuspension were recorded on the sample prepared in KBr disks (2 mg sample in 200 mg KBr disks) using Shimadzu Fourier Transform Infra-Red spectrometer [19]. The samples were scanned over a frequency range 4000-400 cm⁻¹.

Re-Dispensibility and Percentage Drug Content Determination
The prepared Nanosuspensions were analyzed for drug content by UV spectroscopic method. Different batches of nanosuspension equivalent to 10 mg of simvastatin weighed accurately and dissolved in 10 ml ethanol. The stock solutions were diluted with distilled water and analyzed by UV spectrophotometry at 238 nm [17, 20].

RESULTS AND DISCUSSION
Drug content estimation
The drug content of all formulation was found to be in the range of 98.67% to 99.83 % and these values are within the limit.

Particle size analysis
The particle size analysis of nanosuspension was carried out by using Malvern particle size analyzer. The mean particle size of formulation F4 to F9 was found to be 116.6, 281.6, 92.02, 138.3, 190.1 and 131.1 respectively. The particle size of nanosuspension poloxamer 188 (F6) was significantly decreased than Poloxamer 407. Polydispersity index of formulation F4 to F9 was found to be 0.167, 0.523, 0.293, 0.126, 0.026 and 0.301.

Zeta potential
Zeta potential can greatly influence the stability of nanoparticles. Extremely positive or negative zeta potential values cause larger repulsive forces, whereas repulsion between particles with similar electric charge prevents aggregation of the particles and thus ensures easy redispersion. In the case of combined electrostatic and steric stabilization, a minimum zeta potential of ± 20 mV is
Formulations F6 and F9 were showed nearly similar ζ potential of F6 –38.5 mV and F9 –37.1 mV respectively which indicates a good physical stability of nanoparticles. The ζ potential graphs are presented in Figures 1.

Saturation solubility

Saturation solubility data of the nanosuspension and plain simvastatin were used in different dissolution media. Pure simvastatin showed a solubility of 14.16 µg /ml in distilled water, 20.49 µm /ml in pH (1.2) buffer and 46.7 µg /ml in pH 7.0 buffer. As saturation solubility of nanosuspension increases the carrier proportion in nanosuspension also increases. The solubility of simvastatin nanosuspension showed poloxamer 188, 1.5 % concentration F6 317.7 µg/ml, and poloxamer 407, F9 218 µg/ml respectively. 1.5 % concentration with poloxamer 188 Nanosuspension showed highest solubility (317.7µg/ml). This may be due to increase in solubilization effect of carriers present in lyophilized nanoparticles

FT-IR

The interaction study was carried out between simvastatin and poloxamers. The following IR peaks were observed from simvastatin: 3549.14 cm⁻¹ (free OH stretching), 2968.55 cm⁻¹, 2955.04 cm⁻¹ (CH stretching), 1697.41 cm⁻¹ (CO stretching). The similar peaks were observed in formulation and polymers. From the above study revealed that there was no interaction between drug and polymers.
Differential scanning calorimetry

The DSC thermogram of simvastatin lyophilized formulation showed indicates only endothermic peak. The DSC thermograms of SIM and SIM loaded Poloxamer 188 and Poloxamer 407 nanoparticles are shown in Fig 3. Simvastatin was characterized by a single, sharp melting endothermic peak at 131.8 J/g and melting point range at 135.66°C. Suggesting that there was no physical or chemical interaction in SIM and HPH Nanosuspension.
Figure 3: DSC Thermograms of F4-F6 formulations and Simvastatin

**Powder X Ray Diffractometry (PXRD)**

The PXRD patterns of pure drug and nanosuspensions are depicted in Fig 4. The diffraction pattern of the pure drug simvastatin shows a highly crystalline nature, indicated by numerous distinctive peaks at a diffraction angle of 2theta (5.31°, 10.66°, 16.04°, 21.47°) throughout the scanning range; on the other hand, PXRD of nanosuspensions showed a significant decrease in the degree of crystallinity as the disappearance of sharp distinctive peaks. It can be predicted that the larger proportion of simvastatin has been converted to the amorphous form. The relative reduction in the diffraction intensities in the surface nanosuspension can be attributed to the change in orientation during the crystal growth phase.
Scanning electron microscopy

SEM was carried out to study the surface morphology of particles. It was found that simvastatin nanoparticles revealed a smooth texture. The SEM picture of pure drug particles consisted of a mixture of large crystals, indicating its crystalline nature. However, the prepared simvastatin nanoparticles had a nearly spherical shape with relatively uniform size and no drug crystals were present. (Figure 5)

In-vitro dissolution studies

The in-vitro release profile of nanosuspensions is shown in Figure 5. The release rate of simvastatin in physical mixture as well as nanosuspensions was higher as compared to the plain simvastatin for both carriers. Pure simvastatin showed poor dissolution profile i.e. it was observed that only 29.06 % of drug was released at the end of 60 min, whereas PMs showed slight improvement due to effect of carrier present in the respective mixtures. The increase in dissolution rate was seen to be proportionate with increase in carrier proportion. In case of Poloxamer 407, F7, F8, F9 the drug released was 87.67 %, 89.91 % and 91.54 % respectively and Poloxamer188, F4, F5, F6 the drug was released 91.32 %, 93.71 % and 99.14 % respectively at the end of 60 min with increasing carrier proportion. The nanosuspension with poloxamer 188 showed maximum 99.14 % drug release within 60 min, whereas nanosuspension with Poloxamer 407 released 91.64 % after 60 min. indicating that nanosuspension with Poloxamer 188 showed better dissolution profile than Poloxamer 407. This might be due to miceller solubilization effect of poloxamer 188. The increased dissolution rate for poloxamer 188 was due to reduced particle size of nanoparticles. This is due to high pressure homogenization process decreased the size of solid particles and increased the surface area of particles significantly which led to the increase in dissolution.
In vitro dissolution study of nanosuspension: (A) Poloxamer 188  
(B) Poloxamer 407

CONCLUSION
In conclusion, nanosuspension of simvastatin with two different carriers prepared by High pressure Homogenization method, to enhance the dissolution rate of drug thus improving its solubility and bioavailability. All formulation was prepared using Poloxamer 188 and Poloxamer 407 surfactant, without any physical and chemical interaction. Studies showed that the nanosuspensions of Poloxamer 188 with simvastatin showed an enhancement in dissolution profile as compared to physical mixtures as well as nanosuspension with Poloxamer 407 and in-vitro studies proved that simvastatin with Poloxamer 188 (F6) showed best results among the formulations. From the above results, the formulation of simvastatin as a nanosuspension is highly successful in enhancing dissolution rate and oral bioavailability of the drug.

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