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Formulation, Optimization and characterization of Simvastatin Nanosuspension prepared by nanoprecipitation technique

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ABSTRACT

Poor water solubility and slow dissolution rate are issues for the majority of upcoming and existing biologically active compounds. Simvastatin is poorly water-soluble drug and its bioavailability is very low from its crystalline form. The purpose of the present investigation was to increase the solubility and dissolution rate of simvastatin by the preparation of nanosuspension by nanoprecipitation technique at laboratory scale. Prepared nanosuspension was evaluated for its particle size and in vitro dissolution study and characterized by differential scanning calorimetry (DSC) and scanning electron microscopy (SEM). A 2^3 factorial design was employed to study the effect of independent variables, amount of PVPK-30 (X_1), amount of SLS (X_2) and organic to aqueous solvent ratio (X3) on dependent variables, particle size (nm) and time required to release 80% of drug (t_{80}). The relationship between the dependent and independent variables was further elucidated using multiple liner regression analysis (MLRA). The obtained results showed that particle size (nm) and rate of dissolution has been improved when nanosuspension prepared with the higher concentration of PVPK-30 with the higher concentration of SLS and lower concentration of organic to aqueous ratio, The rate of dissolution of the optimized nanosuspension was enhanced (80% in 20min), relative to micronized suspension of simvastatin (7.03% in 20 min), mainly due to the formation of nanosized particles. These results indicate the suitability of 2^3 factorial design for preparation of simvastatin loaded nanosuspension significantly improved in vitro dissolution rate, and thus possibly enhance fast onset of therapeutic drug effect.

Key words: Simvastatin, Nanosuspension, Nanoprecipitation, Factorial design.

INTRODUCTION

It is estimated that more than 1/3 of the compounds being developed by the pharmaceutical industry are poorly water soluble. An important property of a drug substance is solubility,

especially aqueous system solubility [1]. The solubility/dissolution behavior of a drug is key factor to its oral bioavailability. The bioavailability of these drugs is limited by their low dissolution rates. An improvement of oral bioavailability of poor water-soluble drugs remains one of the most challenging tasks of drug development. To overcome poor solubility, many approaches have been studied. They are generally salt formation, use of surfactant, use of prodrugs and micronization. In micronization, the particle size of a drug powder is reduced to a micron scale size (typically 2-10 micron), which increases the specific surface area and dissolution rates. However, many new drugs are so poorly soluble that micronization is not sufficient, which motivated the development of nanoscale systems. By decreasing the particle size from a micron to a nanometer scale, there is a significant increase in the surface area and related dissolution rate [2, 3]. Nanosuspensions are sub-micron colloidal dispersions of pure drug particles in an outer liquid phase. Nanoparticle engineering enables poorly soluble drugs to be formulated as nanosuspensions alone, or with a combination of pharmaceutical excipients. Nanosuspension engineering processes currently used are precipitation [4], high pressure homogenization [5] and pearl milling [6], either in water or in mixtures of water and water-miscible liquids or non-aqueous media [7].

Nanoprecipitation method presents numerous advantages, in that it is a straightforward technique, rapid and easy to perform. In this method, the drug is dissolved in an organic solvent such as acetone, acetonitrile, methanol or ethyl acetate. The organic solvent is evaporated either by reducing the pressure or by continuous stirring. Particle size was found to be influenced by the type of stabilizer, concentrations of stabilizer, and homogenizer speed. In order to produce small particle size, often a high-speed homogenization or ultrasonication may be employed. The super saturation is further accentuated by evaporation of drug solvent. This yields to the precipitation of the drug. High shear force prevents nucleus growth and Oswald's ripening [8].

Simvastatin (SS) is a lipid lowering agent derived synthetically from a fermentation product of *Aspergillus terreus*. After oral ingestion, SS, an inactive lactone, is hydrolyzed to the corresponding β-hydroxyacid form. This is a principal metabolite and an inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme-A (HMG Co-A) reductase, the enzyme that catalyses an early and rate-limiting step in the biosynthesis of cholesterol [9]. SS is a white, crystalline, non-hygroscopic powder, insoluble in water and 0.1N HCl (30µg/ml and 60µg/ml, respectively). It is generally considered that compounds with very low aqueous solubility will show dissolution rate-limited absorption. Improvement of aqueous solubility in such case is a valuable goal to improve therapeutic efficacy. The dissolution rate is a function of the solubility and the surface area of the drug, thus, dissolution rate will increase if the solubility of the drug is increased, and it will also increase with an increase in the surface area of the drug [10, 11].

In this present study, nanoprecipitation precipitation technique is used where a drug solution in a water miscible organic solvent is mixed with an aqueous solution containing a surfactant(s). Upon mixing, the supersaturated solution leads to nucleation and growth of drug particles, which may be stabilized by surfactants.

The aim of this work is to optimize and characterize the formulation prepared by nanoprecipitation method for the preparation of nanosuspensions in order to identify formulation

parameters. A 2^3 factorial design was applied to investigate the combined effect of 3 formulation variables i.e. amount of amount of PVPK-30*(X_1)*, amount SLS (X_2) and organic to aqueous solvent ratio *(X_3)*. The particle size (nm, Y_1), and time required to release 80 percentage of drug (t_{80}, $Y_{2)}$ were taken as responses. Multiple linear regression analysis (MLRA) was employed to construct polynomial equations relating each response to the factors affecting it. Characterization of optimized nanoparticles was carried out by differential scanning calorimetry (DSC), and fourier transform infrared spectroscopy (FT-IR). Dissolution study of nanosuspension formulations was performed in distilled water and was compared to that of micronized suspension of the simvastatin.

MATERIALS AND METHODS

Materials

Simvastatin and polyvinyl pyrrolidone (PVPK-30) was obtained as a gift sample from Torrent Pharmaceutical Ltd., Ahmedabad, India. Ethyl acetate, methanol, acetone, acetonitrile, sodium lauryl sulphate (SLS) were obtained as a gift sample from S.D.Fine Chemicals Ltd., Mumbai, India. Bidistilled water was prepared in laboratory for study. All materials used for study conformed to USP-24 standards.

Preparation of simvastatin nanosuspensions by nanoprecipitation

Nanosuspensions were prepared by the solvent evaporation technique. Simvastatin was dissolved in an acetone at room temperature. This was poured into different amount of water containing different amount of PVPK-30 and SLS 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.

Determination of solubility for simvastatin and its nanosuspension

The aqueous solubility of simvastatin in powder form was determined by a shake-flask method. Briefly, an excess amount of simvastatin was suspended in 10ml of water, and the suspensions were shaken at 37°C. Aliquots were withdrawn and filtered through a 0.22µm Whatmen filter. The filtered solution was suitably diluted and the simvastatin concentration in the filtrate was analyzed by UV analysis method at 238nm (Systronic 2203, Japan).

The aqueous solubility of the optimized nanosuspension of simvastatin was measured by centrifugal method. Briefly, 10ml of nanosuspension was loaded into centrifugal tubes. Samples were centrifuged at 10000 rpm for 10 min at 20°C (C-24BL, Remi, India). Simvastatin concentration in a sample of the clear supernatant was measured UV analysis method at 238nm (Systronic 2203, Japan).

Particle size and its morphology

Particle size was determined by photon correlation spectroscopy (PCS) using a Zetasizer 3000 (Malvern Instruments, UK). This analysis yields the mean diameter (z-average, measuring range: 20–1000 nm). All the data presented are the mean values of three independent samples produced

under identical production conditions. Particle morphology was examined by SEM, The particle morphology was determined by the IBAS I/II Image Analyzer System (Germany).

Optimization of formulation using 2^3 factorial design

2^3 factorial design is one of the tools to study the effect of different variables on the quality determinant parameters of any formulation. Based on the principle of design of experiments, this design was employed to investigate the effect of three independent factors. A 2^3 factorial design for three factors at two levels each was selected to optimize the varied response variables. The three factors, amount of PVPK-30 *(X_1)*, amount of SLS *(X_2)* and organic to aqueous solvent ratio *(X_3)* were varied and the factor levels were suitably coded (Table 1). Particle size (nm) and time required for 80 percentage of drug release (t_{80}) were taken as the response variables. In this design, 3 factors are evaluated, each at 2 levels. Experimental trials were performed at all 8 possible combinations (Table 2). All other formulation variables and processing variables were kept invariant throughout the study. Statistical analysis of the 2^3 factorial design batches was performed by multiple regression analysis using Microsoft Excel 2007[12].

Table 1. Variable level of 2^3 factorial design for simvastatin nanosuspension.

Variable level	-1 (low)	+1 (high)
PVPK-30(mg) *(X_1)*	20	40
SLS(%w/v) *(X_2)*	0.01	0.02
Organic to aqueous ratio *(X_3)*	0.025	0.1

Table 2.Formulation of Simvastatin nanosuspension using 2^3 factorial design

Ingredients	**A1**	**A2**	**A3**	**A4**	**A5**	**A6**	**A7**	**A8**
Simvastatin(mg)	10	10	10	10	10	10	10	10
PVPK-30 (mg)	20	40	20	40	20	40	20	40
SLS (%w/v)	0.01	0.01	0.02	0.02	0.01	0.01	0.02	0.02
Acetone(ml)	1	1	1	1	1	1	1	1
Water(ml)	40	40	40	40	10	10	10	10
Organic to Aqueous ratio	0.025	0.025	0.025	0.025	0.1	0.1	0.1	0.1

Lyophilization

The optimized nanosuspension (A_4) was lyophilized using mannitol (1:1 ratio) as a cryoprotectant. Nanosuspension containing ampoules were freeze in deep freezer at –20°C for 8h (EIE, India) for primary freezing. The ampoules were then transferred to flask and the flask was attached to the vacuum adapter of lyophilizer (hetodry winner). The solvent was sublimed under a pressure of 80 mmHg for 24h [13].

Dissolution study

Invitro drug release studies were performed in USP apparatus-Type II using paddle method at rotation speed of 50 rpm. Dissolution was carried out in distilled water as a dissolution medium. The volume and temperature of the dissolution medium were 900ml and 37.0 $\pm$ 0.5°C. 5 ml of sample was withdrawn periodically (after 10 minutes) and replaced with an equal volume of fresh distilled water 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 SS was calculated based on the standard UV calibration curve at 233nm (Systronic 2203, Japan).

Differential Scanning calorimetry (DSC) Analysis

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.

FTIR Spectroscopic Analysis

Fourier–transform infrared (FT–IR) spectra of moisture free powdered samples of SS, its lyophilized nanoparticles and PVPK-30 were obtained using a spectrophotometer (FTIR-8300, Shimadzu Co., Kyoto, Japan) by potassium bromide (KBr) pellet method. The scanning range was 750–4000 cm-1 and the resolution was 1 cm-1.

RESULT AND DISCUSSION

Simvastatin is a BCS class-II drug having low solubility and high permeability. Thus, it is challenging to enhance the solubility of simvastatin particles in an aqueous solution. Solvent evaporation with homogenization has been employed to produce nanosuspension of simvastatin. The different formulative variables (1) amount of PVPK-30 (2) amount of SLS and (3) organic to aqueous solvent ratio were contribute much towards the change in particle size in nanosuspension preparation.Nanosuspension of simvastatin was prepared as formulation design shown in table 2. Formation of a colloidal nanodispersion can be visualized by the bluish opalescence.

Figure 1 Photograph of the nanosuspension A4 showing bluish opalescence.

In the prescreening study, Pluronic-F68, Pluronic-F127, PVP-K30, PVA, SLS and Tween-80 were selected as stabilizers. Before the stastical experiment design was employed, three factors (amount of stabilizer, amount of SLS and organic to aqueous solvent ratio) were prescreened by varying one factor at a time. From this study, it was found that higher conc. of PVPK-30 with

higher conc. of SLS and lower conc. of organic to aqueous solvent ratio gives desired particle size (300 nm) with lower polydispersivity index (0.218) compare to other formulations. 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 induces agglomeration or aggregation and too much stabilizer promotes Oswald's ripening. It was observed that particle size (nm) and rate of dissolution has been improved when nanosuspension prepared with the higher concentration of PVPK-30 with the higher concentration of SLS and lower concentration of organic to aqueous ratio, The rate of dissolution of the optimized nanosuspension was enhanced (80% in 20 min), relative to micronized suspension of simvastatin (7.03% in 20 min), mainly due to the formation of nanosized particles.

Solubility study

It was observed that the solubility of prepared nanosuspension has been increase up to 36.14 fold due to the formation of stabilized nanoparticles.

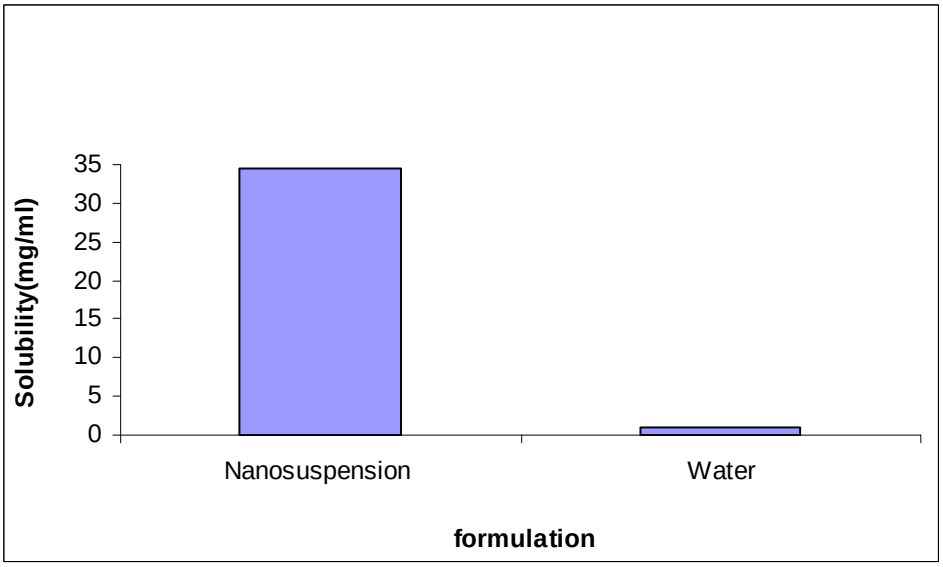

Figure 2. Comparison of solubility of simvastatin suspension with its nanosuspension

Particle size

The optimized batch (A_4) had a Z-average particle size of 300.3nm with 0.218 poly- dispersivity index which indicate the particles are in uniform distribution. The particle size distribution pattern of the optimized nanosuspension formulation is given in figure 3.

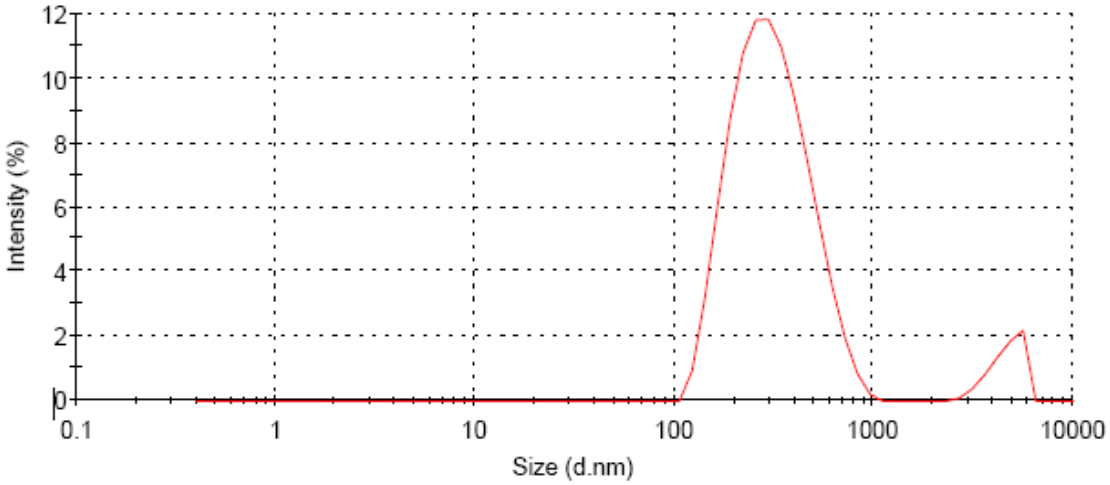

Figure3. Particle size graph of optimized formulation A_4.

Screening Electron Microscopy

Pure drug and Optimized nanoparticles surface appearance and shape were analyzed by SEM as shown in figure 4(a) and 4(b) respectively. Micronized SS powder showed irregular shapes with particle size generally larger (5-50µm) than the SS prepared nanoparticles and had the different morphology. The SS prepared nanoparticles were more uniform as compared to pure SS.

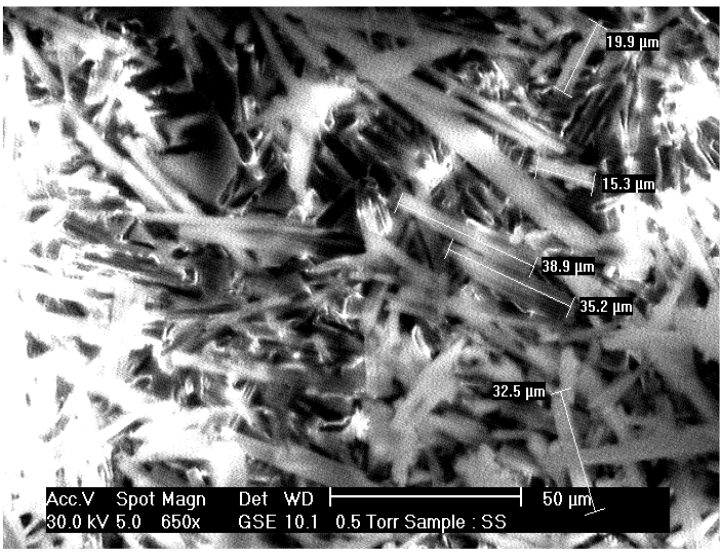

Figure 4(a). Screening electron microscopy of pure drug

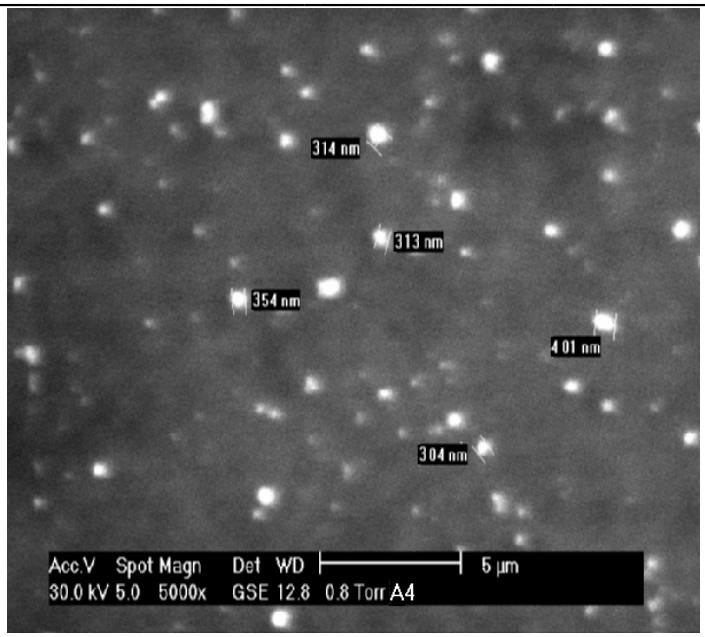

Figure 4(b). Screening electron microscopy of optimized formulation A_4

Experimental data analysis

A three factor, two level full factorial design was adopted for optimization employing the amount of PVPK-30, amount of SLS and Organic to Aqueous solvent ratio as independent variables. Particle size (nm) and time required to 80 percentage drug release as dependent variables. Experimental trials were performed at all 8 possible combinations.

Table 3. Formulation and dissolution characteristics of 2^3 factorial design

Run	Coded values			Actual values			Dependent Variables	
	X1	X2	X3	X1	X2	X3	Y1	Y2
A_1	-1	-1	-1	20	0.01	0.025	409.7	28
A_2	1	-1	-1	40	0.01	0.025	323	24
A_3	-1	1	-1	20	0.02	0.025	398	25
A_4	1	1	-1	40	0.02	0.025	300.3	20
A_5	-1	-1	1	20	0.01	0.1	521.6	33
A_6	1	-1	1	40	0.01	0.1	435	30
A_7	-1	1	1	20	0.02	0.1	505	32
A_8	1	1	1	40	0.02	0.1	408.5	27

Y_1 is indicating particle size (nm), whereas Y_2 is time taken for 80% drug dissolve.
X_1= amount of PVPK-30, X_2= amount of SLS and X_3= organic solvent to aqueous solvent ratio. Each batch contains 10 mg of simvastatin. Standard deviation of the responses did not exceed 3% of the measured value.

In order to investigate the factors systematically, a 2^3 factorial design was employed. As shown in equation (1), a statistical model incorporating interactive and polynomial terms was used to evaluate the responses.

$$Y=bo + b_1X_1 + b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{23}X_2X_3 + b_{13}X_1X_3 \qquad (1)$$

Seven coefficients (b_1 to b_7) were calculated representing b_0 as the intercept, and b_1 to b_7, various quadratic and interaction terms. Mathematical relationships generated using MLRA for the studied response variables are expressed as equations. The polynomial equations comprise the coefficients for intercept, first-order main effects, interaction terms, and higher order effects. The sign and magnitude of the main effects signify the relative influence of each factor on the response.

Concerning particle size, the results of multiple linear regression analysis showed that both the coefficients b_1 and b_2 bear a negative sign and coefficients b_3 bear positive sign (R^2=0.99). It can be concluded from the equation (2) that when the concentration of X1 and X_2 was increase with decrease in the concentration of X3 then desired particle size could be obtained and its controlling the stabilization to the nanoparticles for coalescence.

$$Y= 412.63 -45.93 X1 -9.68X2 + 54.88X3 -2.61X1X2 -1.0875X2X3 + 0.1625 X1X3. \quad (2)$$

The coefficients β_1, β_2, and β_3 were found to be significant at $P < 0.05$. Concerning $t_{80\%}$, the results of MLRA showed that both the coefficients b_1 and b_2 bear a negative sign and coefficient b_3 bear positive sign (R^2=0.99). It can be concluded from the equation (3) that complete adsorption of the stabilizers can takes place on the formed nano particle which leads to formation of stabilized nanosuspension and desired $t_{80\%}$ was observe after 20 min in the formulation A4. The coefficients β_1, and β_3 were found to be significant at $P < 0.05$.

$$Y= 27.375 -2.125X1 -1.375X2 + 3.125X3 -0.37X1X2 + 0.375X2X3 + 0.125X1X3 \quad (3)$$

The improvement of dissolution rate in formed nanosuspension was observed because of increased surface area, which enhances strong hydrophilic character of drug toward PVPK-30 due to the formation of intermolecular hydrogen bonds and improve wettability of hydrophobic SS.

Differential Scanning calorimetry (DSC) Analysis:

The physical state of raw simvastatin and lyophilized drug nanoparticles was examined by DSC and their thermo grams are shown in Fig. 5. Raw simvastatin exhibited a melting point at 141.47°C with fusion enthalpy of 76.379 J/g. whereas DSC scan of PVP, a broad endotherm ranging from 80 to 110°C was observed, due to the presence of residual moisture in PVP. This complete absence of SS peak indicates that SS is present as amorphous after being precipitated as nanoparticles; its melting point was decreased to 93.4 °C indicating reduced crystallinity.

In vitro drug release study

Fig. 6 shows the dissolution behavior of simvastatin with its nanosuspensions. The release rate profiles were drawn as the percentage simvastatin dissolved from the nanosuspension and pure drug versus time. Dissolution studies of pure simvastatin and all other prepared nanosuspension (A1- A8) were carried out in distilled water. T80% (time to dissolve 80% drug) values calculated from release profile are reported in Table 3. From this data, it was evident that onset of dissolution of pure simvastatin was very low. Dissolution of simvastatin nanoparticles was affected by different surfactant concentrations and organic to aqueous solvent ratio. It can be

observed that, 80% of the simvastatin nanosuspension was dissolved in 20min; while in the same period, 7.06% of the raw simvastatin was dissolved. The dissolution rate of simvastatin nanoparticles is 11.33 times that of raw drugs. According to Noyes–Whitney equation, the dissolution rate is directly proportional to its surface area exposed to the dissolution medium. The increase dissolution for drug nanoparticles could thus be mainly ascribed to their greater surface area in comparison with raw drug.

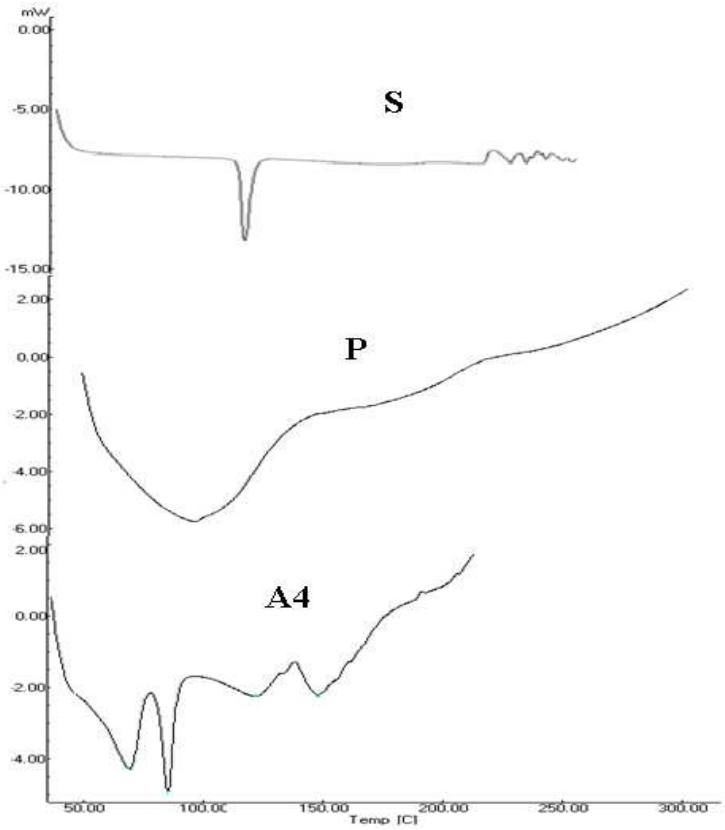

Fig. 5 DSC thermograms of raw simvastatin(S), PVPK-30(P) and drug nanoparticles (A4).

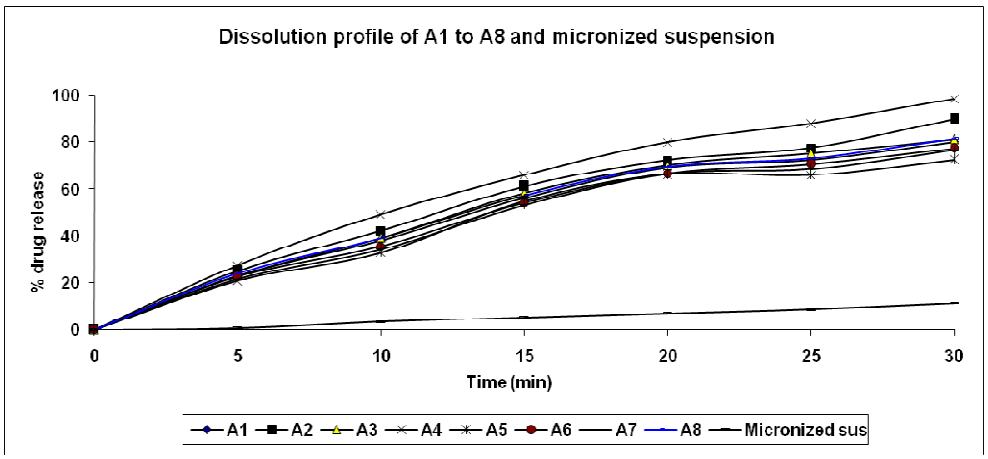

Figure 6. Dissolution profile of A1 to A8 and micronized suspension.

Fourier transforms infrared spectroscopy (FT-IR)

Fourier transform infrared spectroscopy (FT-IR) has been used to assess the interaction between carrier and guest molecules in the solid state. Upon preparing nanoparticles, the peak band of the guest shifts in the absorption spectrum. The FT-IR spectra's of all samples are shown in Figure 7. Raw simvastatin and precipitated nanoparticles exhibited same FTIR spectrum as shown in Fig. 7, which demonstrates that the chemical structure of the drug is not changed before and after the precipitation process.

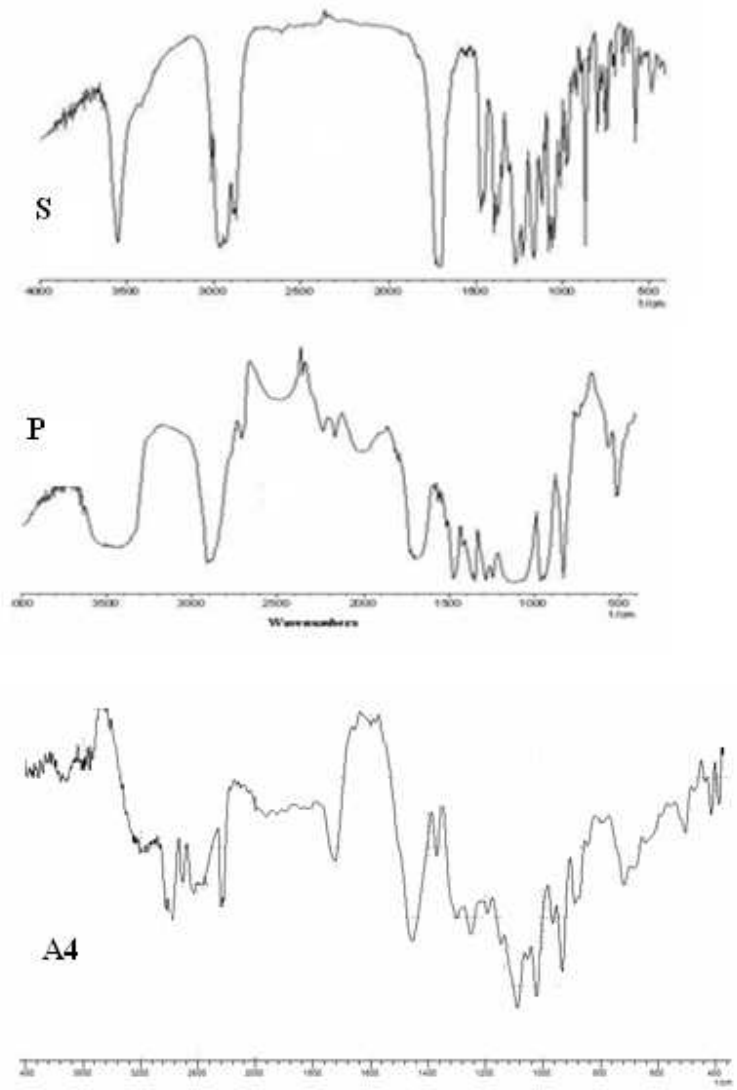

Figure 7. FTIR spectra of SS (S), PVPK-30 (P) and optimized formulation (A4)

CONCLUSION

Nanoprecipitation technique was employed to producing nanoparticles of simvastatin, a poorly water-soluble drug, for the improvement of solubility and dissolution velocity. In this process, the particle size of simvastatin can be obtained in the micron and nano-size ranges, by adjusting the operation parameters, such as the stabilizer concentration and the organic to aqueous solvent

ratio. The best nanosuspension of simvastatin can be obtained by 40mg PVPK-30, 0.02%w/v SLS and 0.025 organic to aqueous ratio using solvent evaporation technique at laboratory scale. The dissolution of nanosized simvastatin is significantly enhanced compare with the pure simvastatin suspension. Nanoprecipitation can thus be a simple and effective approach to produce submicron particles of poorly water-soluble drugs.

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