REPORT

Improved physicochemical characteristics of artemisinin-nicotinamide solid dispersions by solvent evaporation and freeze dried methods

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Abstract: Artemisinin (ARMN) is a drug of choice against drug-resistant malaria especially due to *Plasmodium falciparum*. Being poorly soluble in water, its solid dispersions with nicotinamide (NA) were prepared at various drug-carrier ratios (1:1, 1:4, 1:6, 1:8, 1:10) by solvent evaporation and freeze drying methods. These solid dispersions were characterized by differential scanning calorimetery (DSC), fourier transform infrared spectroscopy (FTIR), X-ray diffraction patterns (XRD), phase solubility and dissolution studies. Artemisinin and nicotinamide both were found completely crystalline as shown by their XRD patterns. Physical mixtures (PMs) showed decreased intensity in their XRD patterns while solid dispersions by solvent evaporation method (SLVPs) exhibited displaced angles and decreased intensity whereas freeze dried solid dispersions (FDSDs) showed least number of peaks having low intensity and maximum displaced angles. DSC thermograms of drug-carrier ratios at 1:1-1:4 showed lower melting temperature than artemisinin and nicotinamide in all preparations. Endothermic temperature of artemisinin in PMs and SLVPs increased with rise of nicotinamide content upto 1:6 ratio followed by decline. All samples showed crystallization temperature below the artemisinin except drug-carrier ratio 1:6 of PMs while ΔH value was minimum at this ratio. FDSDs produced lowest endothermic temperature than corresponding PMs and SLVPs. SLVPs exhibited band shifting in both functional and fingerprint region compared to respective PMs as exhibited by their FTIR spectra. FDSDs and SLVPs showed different nature of bonding among artemisinin and nicotinamide. FDSDs produced strongest $CONH_2$ bonding followed by SLVPs and PMs respectively. PMs produced significantly higher aqueous solubility and rate of dissolution as compared to artemisinin alone. SLVPs exhibited improved solubility and dissolution profile corresponding to PMs. FDSDs showed highest release rate and aqueous solubility followed by SLVPs and PMs at all ratios. PMs and SLVPs showed their highest dissolution profile at 1:6 drug-carrier ratio followed by gradual decrease while FDSDs progressed in dissolution rate with increase of nicotinamide content successively upto maximum at 1:10 ratio.

Keywords: Artemisinin, nicotinamide, solid dispersions, freeze dried, dissolution, phase solubility.

INTRODUCTION

Artemisinin (ARMN) like quinine has been originated from herb named *Artemisia annua* (Qinghaosu) but is structurally a more distinct compound containing endoperoxide group having antimalarial activity (fig. 1). It is useful for treating drug-resistant malaria caused by *Plasmodium falciparum*. It act faster, has a broad stage-specificity of action and is extremely well tolerated. Evidence of its safety and efficacy comes from large randomised trials in tens of thousands of patients. This artemisinin family is drug of choice for treatment of uncomplicated malaria at the moment (Elizabeth *et al*, 2005). Being poorly soluble in water, it is not completely absorbed by orally dosage forms and typically exhibits dissolution rate limited absorption. The poor dissolution characteristics of relatively insoluble drugs have been a challenge for formulation development scientists. Numerous solid dispersion systems have been demonstrated in the pharmaceutical literature to improve the dissolution properties of poorly soluble drugs (Aggarwal *et al*., 2010; Dhirendra *et al*, 2009; Varma and Pandi, 2005). Solid dispersions of artemisinin with polyvinylpyrrolidone (Nijlen *et al*., 2003), Eudragit (Hoa and Longl, 1999), Hydroxypropylmethylcellulose, Polyethyleneglycol 6000 (Long *et al*, 1999), dihydroartemsinin with polyvinylpyrrolidone (Ansari and Sunderland, 2008), artemether (Ansari *et al*., 2010). To our knowledge there is no report available about preparation and evaluation of artemisinin-nicotinamide solid dispersions.

Nicotinamide (NA), is a non-toxic vitamin, hydrotropic agent that has been used to enhance the aqueous solubility of rofecoxib (Ahuja *et al*., 2007), flurbiprofen (Varma and Pandi, 2005), halofantrine (Lim and Go, 2000), indomethacin (Bogdanova *et al*., 1998), diazepam, griseofulvin, progesterone and testosterone (Rasool *et al*.,

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1991), anticancer nucleosides (Truelove *et al*., 1984) and its structurally related compounds (Chen *et al*., 1994). Nicotinamide has been reported to increase the dissolution rate of piroxicam (Verma *et al*., 2003), indomethacin (Verma *et al*., 2002), nifedipine (Suzuki and Sunada, 1998). From the strong solubilizing effect of nicotinamide, it was assumed that forming a eutectic mixture of drug-nicotinamide might be a less effective means of improving the drug dissolution rate than increasing the proportion of nicotinamide in the solid dispersion (Suzuki and Sunada, 1997).

In our work artemisinin-nicotinamide solid dispersions were prepared by solvent evaporation and freeze dried methods to get comparison and main objectives were to improve the aqueous solubility, dissolution rate of artemisinin and select suitable drug-carrier ratio where maximum benefit can be achieved. The possibility of drug-carrier interactions was studied by phase solubility analysis, phase diagram, fourier transform infrared spectrometry (FTIR), X-ray diffraction (XRD) and differential scanning calorimetry (DSC).

MATERIALS AND METHODS

Materials

Artemisinin (Alchem, New Delhi, India), methanol (Sigma-Aldrich, Germany), nicotinamide (BDH Chemicals Limited, Germany), sodium hydroxide (Merck Ltd, Germany), potassium bromide (FTIR Grade, Fisher Chemicals USA), Acetone (Merck, Germany), Starch (Rafhan Maize, Pakistan), Lactose (DMV international Netherlands), Magnesium stearate (Royal Tiger Products, Taiwan). Demi water was used for the dilution of various samples.

Artemisinin assay

Artemisinin concentrations were measured according to the method described by Zhao & Zeng (1985). After appropriate dilution with demi water, adding 0.2% sodium hydroxide and heating at 50°C for 30 min. The concentration of artemisinin was determined at 290nm with a UV spectrophotometer (JENWAY, 6405 UV/ VIS, UK).

Preparation of physical mixtures (PMs)

Artemisinin and nicotinamide at the ratios 1:1 1:4, 1:6, 1:8, 1:10 respectively were taken in the glass pestle and mortar. These were softly grinded and passed through the sieve (US 180 µm) and transferred to desiccators at 25°C under P_2O_5.

Preparation of solid dispersions by solvent evaporation method (SLVPs)

SLVPs were prepared using drug and NA at 1:1, 1:4, 1:6, 1:8 and 1:10 weight ratios by dissolving the drug and excipient (nicotinamide) in 100 ml of methanol. This solution was shaken on orbit shaker for 4-5 hours at 150 rpm (25°C). The volume of methanol was removed by rotary evaporator. These solid dispersions were pulverized through 180µm mesh sieve and were transferred in colored glass bottles and stored in desiccator for further analysis.

Preparation of freeze dried solid dispersions (FDSDs)

FDSDs were prepared using drug and NA at 1:1, 1:4, 1:6, 1:8 and 1:10 weight ratios by dissolving artemisinin and nicotinamide in 100 ml of methanol. This solution was shaken on orbit shaker for 4-5 hours at 150 rpm (25°C). The methanol was removed and 20 ml of demi water was added and shaken for 5 minutes. Then this solution was frozen at -70 to -80°C in electronic deep freezer. This freezed form was dried in lyophilizer. Freeze dried solid dispersion as pulverized through 180µm mesh sieve. These preparations were transferred in amber glass bottles and stored in desiccator containing P_2O_5 for further analysis.

X-ray diffraction (XRD) studies

X-ray powder diffraction of ARMN, nicotinamide, their PMs, SLVPs and FDSDs were performed using a Siemens D500 apparatus. Measurement conditions included target CuKα, voltage 40 KV and current 30 mA. A system of diverging, receiving and anti-scattering slits of 1°, 1°, 1°, 0.15°, respectively was used. Jade 6.0 were used for data processing (Materials Delta Inc. USA). Patterns were obtained using a step width of 0.04° 2θ between 5 and 50°.

Fourier transform infrared spectrophotometric (FTIR) analysis

Fourier-transform infrared (FTIR) spectra were obtained on a Shimadzu-8400S (Japan) using the KBr disc method (0.5-1% of sample in 200mg KBr disc). The scanning was at 450-4000 cm^{-1} and the resolution set as $1cm^{-1}$. Calibration of the instrument was repeated periodically during operation.

Differential scanning calorimetric (DSC) analysis

DSC of PMs, SLVPs and FDSDs were performed using Setaram 131.The samples were heated at a rate of 10°C/min from 40 to 290ºC under a dry nitrogen gas purge. Indium was used to calibrate the cell constant. All measurements were conducted in sealed non-hermetic aluminum pans. Typical sample weight was 4-8 mg.

Phase solubility studies

For phase solubility studies, excess quantity of each sample was taken in a 25 ml vial containing 10 ml of demi water. It was then placed in shaking incubator at 37±1°C at 100 rpm for five days. Afterwards samples were centrifuged at 6000 rpm for 15 minutes and withdrawn with a syringe equipped with a 0.40µm syringe filter. All samples were diluted to a proper concentration

range and assayed for artemisinin. A control experiment was also performed with pure artemisinin to confirm any degradation in all used solvents. All samples were analyzed triplicate. The apparent stability constants (K_s) of the solid dispersions were calculated from the slope of the phase solubility diagrams according to the following equation (Higuchi and Connors, 1965):

$$K_s = \frac{\text{Slope}}{S_o\,(1\ \text{slope})}$$

Where S_o was the equilibrium solubility of artemisinin at 37°C in the absence of nicotinamide.

Dissolution studies

Drug release was measured using dissolution apparatus (Tablet dissolution tester GDT-7Tv3, Galvano Scientific, Pakistan) at 37ºC and 100 rpm, the paddle apparatus (consisting of six recipients) for high volume by using demi-water as dissolution medium instead of a buffer (Ngo *et al.*, 1996). At pre-determined time intervals (5 min, 15 min, 30 min, 60 min, 90 min, 120 min, and 180 min); 5 ml of sample was taken and replaced with same volume of fresh solvent. Samples were assayed according to analytical procedure of artemisinin described as above. Relative dissolution rate was calculated using following formula:

$$\text{Relative Dissolution rate (RDR)} = \frac{\text{Dissolution of artemisinin with carrier in tablet}}{\text{Dissolution of artemisinin (pure) at the same time interval}}$$

RESULTS

Phase solubility studies

The phase solubility of artemisinin was studied as a function of nicotinamide concentration in demi water and diagrams were drawn by plotting molar concentration of NA versus apparent equilibrium concentration of ARMN as shown in fig. 2. All preparations of physical mixtures, solid dispersions by solvent evaporation and freeze dried method showed increase in aqueous solubility of ARMN with rise of NA percentage (table 1). Aqueous solubility of pure ARMN was found to be 3.68 M × 10^{-6} only. Solubility of physical mixtures was 138.028 M × 10^{-5} at nicotinamide 818.3M × 10^{-2} concentration while solubility in SLVPs was 176.097 M × 10^{-5} and FDSDs 200.225 M × 10^{-5} at the same nicotinamide concentration respectively. The apparent stability constant values were calculated from phase solubility diagram. Stability constant of PMs, SLVPs, FDSDs were 22.128 M^{-1}, 26.87 M^{-1} and 36.06 M^{-1} respectively (fig. 2).

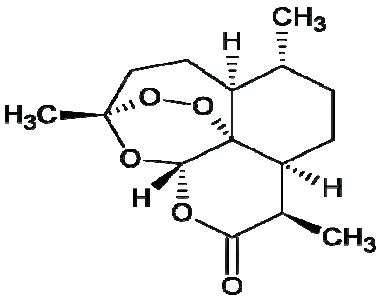

Fig. 1: Structure of artemisinin

Table 1: Graphical values of solubility data of Physical mixtures, solvent evaporation and freeze dried solid dispersions of fig. 2

Nicotinamide M*10^{-2}	Artemisinin (PMs) (M*10^{-5})	Artemisinin (SLVPs) (M*10^{-5})	Artemisinin (FDSDs) (M*10^{-5})
8.18	80.91537622	83.32388819	128.9439383
32.73	105.9745264	113.0052562	157.0491478
49.09	114.5637051	130.718445	167.8874517
65.46	126.0749756	147.9853506	175.644277
81.83	138.0289871	176.0976439	200.2252667

Dissolution profile

Physical mixtures of artemisinin with nicotinamide at different drug-carrier ratios exhibited enhanced dissolution than artemisinin alone in demi water (fig. 3). Physical mixtures showed enhanced dissolution rate with rise of nicotinamide content upto drug-carrier ratio 1:6 followed by decrease in successive ratios i.e. 1:8 and 1:10. Dissolution was substantially increased compared to pure artemisinin i.e., 3.14 times by 1:1 ratio, 3.29 times (1:4), 3.59 times (1:6), 3.41 times (1:8), 3.48 times (1:10). SLVPs exhibited higher rate of dissolution (47.10-65.88%) than respective PMs. They exhibited higher dissolution rate i.e., 4.62 times by 1:1 ratio, 4.96 times by 1:4 ratio, 5.96 times by 1:6 ratio, 5.31 times by 1:8 ratio,

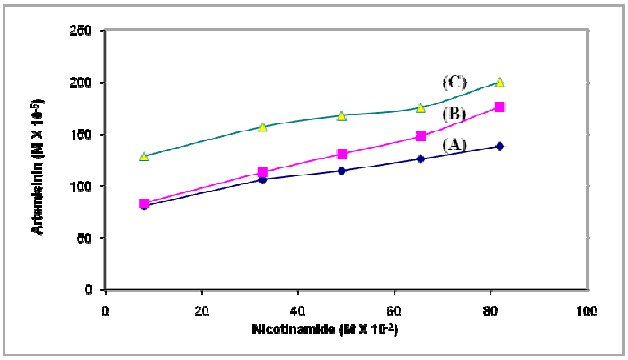

Fig. 2: Phase solubility of physical mixtures of artemisinin-nicotinamde (A), solid dispersions by solvent evaporation (B) and by freeze dried method (C).

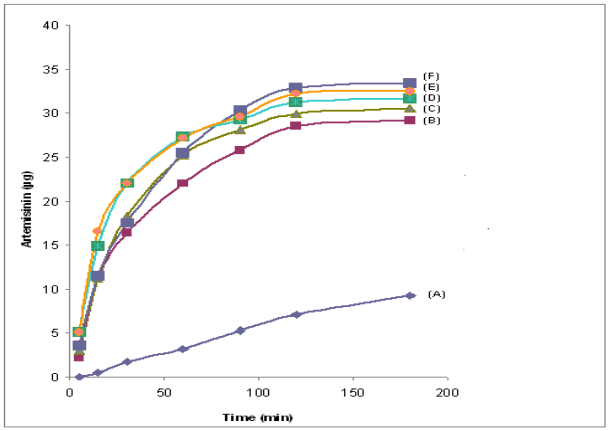

Fig. 3. Dissolution profile of pure artemisinin (A) and various physical mixtures of artemisinin and nicotinamide 1:1 ARMN-Nic (B) 1:4 ARMN-Nic (C), 1:6 ARMN-Nic (F), 1:8 ARMN-Nic (D), 1:10 ARMN-Nic (E)

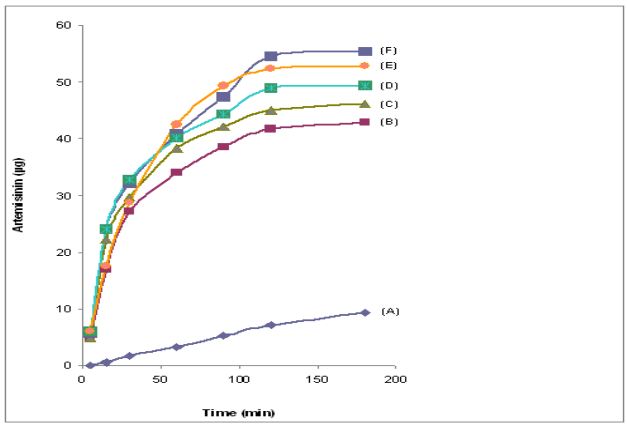

Fig. 4: Dissolution profile of pure artemisinin (A) and artemisinin-nicotinamide solid dispersions by solvent evaporation method in various ratios 1:1 ARMN-Nic (B) 1:4 ARMN-Nic (C), 1:6 ARMN-Nic (F), 1:8 ARMN-Nic (D), 1:10 ARMN-Nic (E)

5.69 times by 1:10 ratio than artemisinin alone respectively. They also showed maximum dissolution rate at 1:6 ratio followed by decrease like corresponding PMs (fig. 4). FDSDs exhibited highest release rate at all ratios compared to corresponding PMs (55.84-78.97%) and SLVPs (5.93-7.88%) but with different statistics. FDSDs showed gradual increase in dissolution rate with rise in NA amount from 1:1-1:10 ratio i.e. 4.89, 5.25, 5.60, 6.07, 6.43 times higher dissolution rate than artemisinin respectively (fig. 5).

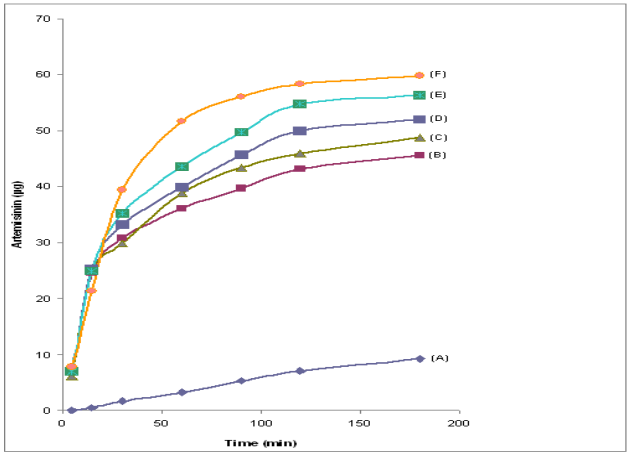

Fig. 5: Dissolution profile of pure artemisinin and Freeze dried solid dispersions of artemisinin-nicotinamide in various ratios 1:1 ARMN-Nic (B) 1:4 ARMN-Nic (C), 1:6 ARMN-Nic (D), 1:8 ARMN-Nic (E), 1:10 ARMN-Nic (F)

Characterization of solid dispersions

Fourier Transform Infrared Spectroscopy

FTIR spectra of pure artemisinin showed characteristic peaks of C=O stretching at 1736 cm^{-1}, C-O stretching at 1011 cm^{-1} and C-O-O-C bending vibrations for endoperoxide bridge at 1123 cm^{-1} whereas pure nicotinamide presented two characteristics stretching bands of N-H at 3159 and 3356 cm^{-1} and carbonyl group showed at 1682 cm^{-1} respectively.

Physical mixtures showed two bands of N-H stretching vibrations at 3159-3161 and at 3354-3360 cm^{-1}, single peak of carbonyl stretching (1735, 1730 cm^{-1} at 1:1 & 1:4 ratio respectively) representative of ARMN and at 1680-1682 cm^{-1} characteristic of NA in 1:6-1:10 ratios respectively. In addition altered bending peaks for C-O-O-C at 1119-1123 cm^{-1} and C-O stretching at 1011-1020 cm^{-1} were also observed during the process of physical mixing.

SLVPs exhibited two bands of N-H stretching at 3159-3163 and at 3358-3360 cm^{-1}, two peaks of carbonyl group were produced by drug-carrier ratio 1:1-1:6 at 1732-1740 and 1680-1684 cm^{-1} respectively while successive ratios exhibited single peak of carbonyl group at 1680 and 1682 cm^{-1} for 1:8-1:10 ratios respectively. Bending vibration of endoperoxide was gradually decreased from 1122 to 1113 cm^{-1} while reverse was true in C-O stretching i.e. 1011 to 1028 cm^{-1} in FTIR spectra with increase of NA ratio.

FDSDs also showed two peaks of N-H stretching at 3159-3163 and at 3356-3362 cm^{-1} respectively; carbonyl group also showed two types of stretching bands i.e. at 1732-1735 cm^{-1} representative of ARMN and at 1678-1684 cm^{-1} characteristic of NA. Mixed band shifting of endoperoxide bending at 1119-1130 cm^{-1} as well as C-O stretching at 1013-1028 cm^{-1} were observed (Figures not shown here).

X-ray diffraction

Artemisinin was found complete crystalline in its XRD patterns, having strong diffraction peaks at 2θ of 10.92°, 11.96° and 12.24°, 14.96°, 22.4°, 24.12° and 38.56° respectively (fig. 5). Nicotinamide exhibited characteristic crystalline diffraction bands at 2θ of 14.96°, 22.4° 27.48° and 37.1° and 38.76° respectively (fig. 6).

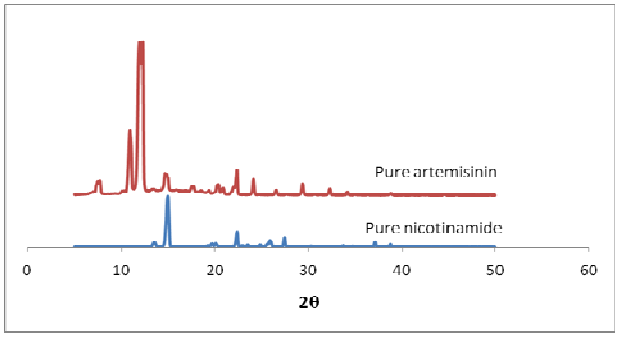

Fig. 6: XRD patterns of artemisinin and nicotinamide only.

Physical mixtures showed characteristic peaks of ARMN but having decreased intensities. All samples of PMs showed a peak characteristic of nicotinamide at 27.1° comparable in size, having low intensity while ARMN-NA at 1:6 ratio attained minimum value (fig. 7). SLVPs of artemisinin-nicotinamide showed displaced angles at 2θ (21.9-22.3°) alongwith reduced intensity as compared to ARMN and respective PMs (fig. 7). Peak heights at this angle was enhanced with rise of nicotinamide content upto 1:6 ratio and decreased in successive ratios (1:8-1:10). Characteristic peak of nicotinamide not only showed displaced angle (26.9-27.2°) but peak height elevated with the increase in NA content. SLVPs showed less peak heights than corresponding PMs at all angles measured. It was noted that a peak at 2θ of 38.5-38.8° was present in all ratios and synergistic effect was shown at this angle by drug-carrier ratio 1:8.

FDSDs produced a shift in diffraction angle at 2θ, 22.12-22.32° having minimum intensity than respective PMs and SLVPs. Characteristic peak of NA was found in all samples with displaced angle (27.32-27.34°) and increased peak heights with rise in NA ratio. It was noted that a peak at 2θ of 38.5° was absent in FDSDs at all ratios whereas it was present in all PMs and SLVPs (fig. 7).

Differential scanning calorimetry

Pure artemisinin exhibited a melting peak at 151.03°C (melting onset temperature at 149.11°C, ΔH= 44.51 J/g) and a strong exothermic peak at 210.04°C having higher intensity while pure nicotinamide has showed sharp endothermic peak at 127.59°C (melting onset temperature at 125.16°C, ΔH=129.2 J/g) attributed to melting respectively.

DSC thermograms of physical mixtures showed one endothermic and one exothermic peak. Drug-carrier ratio 1:1 exhibited peak temperature at 117.52°C (melting onset temperature =114.51°C, ΔH= 62.77 J/g) and strong exothermic peak at 174.2°C respectively; 1:4 ratio showed endothermic band at 122.53°C (ΔH=44.43 J/g) and sharp exothermic curve at 150°C; 1:6 ratio exhibited melting peak temperature (endo-) at 129.83°C (melting onset temperature = 124.85°C, ΔH= 12.51 J/g) and broad exothermic curve at 225°C; 1:8 ratio produced peak temperature (endo-) at 125.15°C (melting onset temperature = 122.68°C and ΔH= 30.35 J/g) and strong exothermic curve at 175°C while at 1:10 ratio peak temperature was at 125.90°C (melting onset temperature = 123.96°C and ΔH= 67.40 J/g) and exothermic band at 150°C respectively (fig. 8).

The DSC thermograms of solubilized form of artemisinin with nicotinamide (SLVPs) produced one endothermic and one exothermic peak also. Thermodynamic parameters at a drug carrier ratio 1:1 were i.e. endothermic peak at 117.04°C (melting onset temperature =114.51°C, ΔH= 66.80 J/g) and strong exotherm at 162°C; at 1:4 ratio peak temperature (endo-) at 123.14°C (melting onset temperature = 117.94°C, ΔH= 34.03J/g) and sharp exothermic curve at 154°C; 1:6 ratio peak temperature (endo-) at 128.44°C (melting onset temperature = 123.84°C and ΔH= 19.09 J/g) and a broad exothermic curve at 208°C; 1:8 ratio peak temperature = 124.21°C (melting onset temperature = 120.15°C and ΔH= 40.07 J/g) and an exotherm at 152°C and 1:10 ratio peak temperature = 126.54°C (melting onset temperature = 123.93°C, ΔH= 67.40 J/g) and a bit weak exothermic curve at 150°C respectively as shown by fig. 8.

Freeze dried solid dispersion (FDSDs) showed one endotherm and one exotherm from 1:1-1:4 while at higher drug-carrier ratios, exotherm was too broad to read. FDSDs at a drug-carrier ratio 1:1 produced thermodynamic parameters i.e. endothermic peak temperature = 116.67°C (melting onset temperature =112.62°C, ΔH= 47.12 J/g) and strong exotherm having high intensity at 152.20°C; 1:4 ratio peak temperature = 122.45°C (melting onset temperature =114.73°C, ΔH= 45.81 J/g) and strong exotherm at 145.5°C; at 1:6 ratio peak temperature = 124.50°C (melting onset temperature = 120.00°C, ΔH= 27.40 J/g) and weak broad exothermic band; at 1:8 ratio peak temperature = 125.14°C (melting onset temperature = 120.83°C, ΔH= 34.32 J/g) and very broad exotherm not readable while at 1:10 ratio peak temperature = 125.82°C (melting onset temperature = 122.22°C, ΔH= 51.76 J/g) and unreadable broad exotherm respectively as shown in fig. 8.

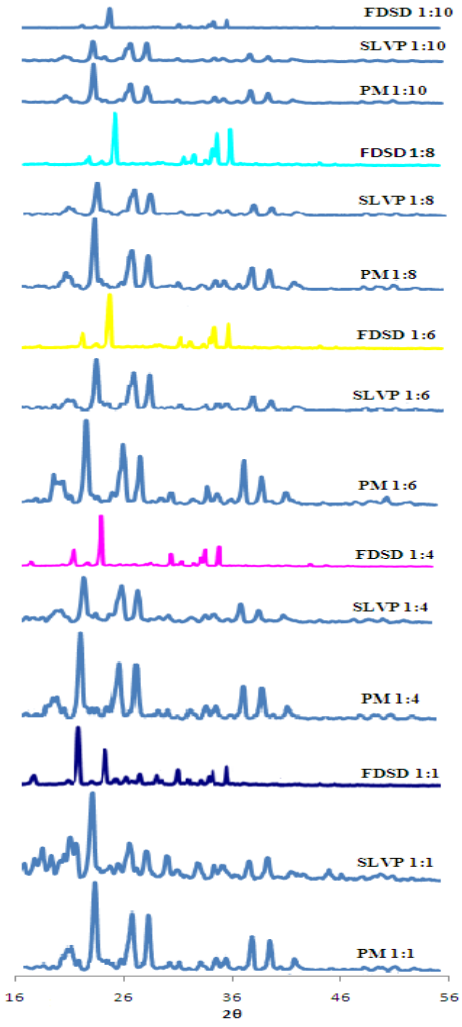

Fig. 7: XRD patterns of PM (Physical mixtures), SLVP (solid dispersions by solvent evaporation method) and FDSDs (Freeze dried solid dispersions) of artemisinin-nicotinamide at various ratios as mentioned in diagram.

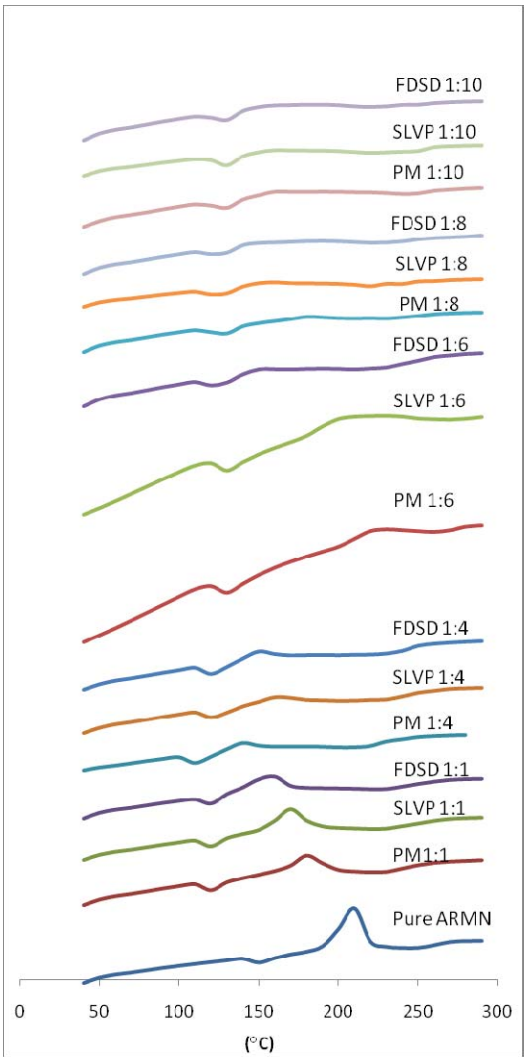

Fig. 8: DSC thermograms of pure artemisinin (ARMN), physical mixtures (PM), solid dispersions of artemisinin-nicotinamide by solvent evaporation (SLVP) and freeze dried method (FDSDs) at various ratios as mentioned in diagram.

DISCUSSION

Dissolution profile studies

Artemisinin showed low as well as slow release profile because of its poor solubility. After 1 h of dissolution study, the pure artemisinin showed dissolution of 8.05% in demi water. Even after 3 h, the pure drug did not show 50% dissolution. This might be attributed to poor wettability and particles agglomeration during the run that caused the powder to float on the surface of dissolution medium. Physical mixtures showed significant increase in rate of dissolution than artemisinin that may be due to solubilizing effect of nicotinamide (Varma and Pandi, 2005; Bogdanova *et al*, 1998), weak interaction among artemisinin and nicotinamide which is reflected by FTIR spectra, lowering of melting temperature in DSC thermograms and mild phase transitions in XRD patterns. This rapid dissolution by physical mixture is similar to nifedipine (Suzuki and Sunada, 1997). In this study, unusual results were found by physical mixture at 1:6 ratio that showed maximum amount of dissolution (33.4mg) as compared to other ratios that may be due to formation of eutectic mixture at this ratio. These findings are against the statistics but our findings were verified by displaced angles, lowest peak intensities in XRD patterns and highest melting temperature among physical mixtures shown by DSC thermograms. FTIR spectra also confirmed this special dissolution profile.

SLVPs exhibited improved dissolution profile as compared to physical mixture but pattern was similar to PMs i.e. SLVP at 1:6 ratio exhibited maximum dissolution rate followed by decrease that was corresponding to increased interaction shown by FTIR

spectra in which maximum peak height and altered wavenumber was noted as compared to other SLVPs. XRD patterns and DSC thermograms supported this profile by showing highest melting peak temperature having minimum peak intensity and minimum ΔH value at this ratio. With the increase in proportion of nicotinamide in SLVPs, there was successive increase in dissolution rate of artemisinin. From the fig. 4, it can be noted that dissolution rate of artemisinin in SLVPs is substantially enhanced compared to pure drug that may be due to encircling of artemisinin by nicotinamide which results decreased aggregation and agglomeration of artemisinin particles allowing a faster dissolution process. Furthermore the hydrotropic solubilization by nicotinamide has been attributed mainly to their ability to destroy water structure, and/or to form complexes with certain drugs on the basis of π-electron donor-acceptor interaction, and/or to undergo hydrogen bonding and/or to undergo hydrogen bonding. According to our results, solid dispersions showed improved dissolution profile than respective physical mixtures similar to piroxicam (Verma *et al*, 2003) and tolbutamide in tolbutamide-nicotinamide eutectic mixture (80% tolbutamide + 20% nicotinamide) revealed that the cumulative drug released from tolbutamide alone at 15 minutes was 30% while the eutectic mixture was 46% (Gebremichael, 2010) but are different from nifedipine where dissolution rate of physical mixtures and solid dispersions were same (Suzuki and Sunada, 1997).

FDSDs produced highest dissolution rate than corresponding PMs and SLVPs at all ratios. They showed increase in dissolution rate with rise in NA amount and produced maximum dissolution value (39.76mg) at 1:10 ratio. Our results are in agreement with freeze dried solid dispersions of meloxicam with PVP (El-Badry and Fathy, 2006) and glyduride lyophilized solid dispersions of PEG4000 and PEG6000 (Betageri and Makarla, 1995) in which freeze dried solid dispersions showed higher dissolution rate than respective solid dispersions by solvent evaporation. FTIR verified the enhanced interaction among the artemisinin and nicotinamide while XRD showed least number of diffraction bands.

Phase solubility studies

Nicotinamide is well known as hydrotropic agent, and its ability to solubilize wide variety of therapeutic compounds has been demonstrated (Suzuki and Sunada, 1997). Keeping this view its ability on artemisinin was undertaken. Physical mixtures showed a linear increase in the aqueous solubility of ARMN with enhanced nicotinamide content. The slopes were lower than one for all ratios indicating the phase solubility profile was typical A_L type and 1:1 molar ratio of artemisinin and nicotinamide combined similar to diazepam (Rasool *et al.*, 1991). SLVPs showed enhanced solubility and stability constant values as compared to corresponding PMs. A different pattern was exhibited by SLVPs in which straight line was found upto concentration of 65.46 M x 10^{-2} (NA) showing A_L type diagram but afterwards non-linear increase in solubility was observed at 81.83 M x 10^{-2} (NA) suggesting the formation of higher order complexes probably via a stepwise interaction between artemisinin and two molecules of nicotinamide (Varma and Pandi, 2005; Rasool *et al.*, 1991). Similarly FDSDs also showed linear and non-linear increase in solubility that confirmed the formation of low and high order of complexation respectively due to dispersion of drug in nicotinamide similar to glipizide (Shukla *et al*, 2010). FDSDs showed highest solubility and stability constant compared to corresponding SLVPs and PMs. This is in accordance with respective dissolution profile discussed in previous section. In many similar studies, freeze drying (Onyeji, *et al.*, 2007; Pose-Vilarnovo *et al.*, 2001; Castillo *et al*, 1999) method was found most effective for enhancing drug solubility and had highest stability constants (K_s) followed by SLVPs and PMs, perhaps due to increase in surface area and the surface free energy. This increase in surface area and energy occurs because in freeze drying, due to primary and secondary drying processes, porous as well as fluffy dry mixture is produced while original starting volume is also maintained (Betageri and Makarla, 1995). This high aqueous solubility of solid dispersions is attributed to high solubilizing effect of nicotinamide similar to parabens (Nicoli *et al.*, 2008).

Characterization studies

Fourier transform infrared spectrophotometric (FTIR) analysis

The method of FTIR spectroscopy is considered to be the most reliable for predicting the possible interactions. Physical mixtures produced single band of carbonyl group representative of ARMN at lower ratios whereas at higher ratios carbonyl group of NA dominated but having lower wave number than the respective carbonyl stretch while other bands occurring in the position as expected. PMs showed clear peaks of NA at higher carrier ratios because of low drug content. This proves that nicotinamide formed hydrogen bonding with carbonyl group of artemisinin similar to indomethacin (Jain, 2008). SLVPs produced two bands of carbonyl group but with shifted values (±4 cm^{-1}) correspond to ARMN & NA upto 1:6 ratios which indicate that N-H group of nicotinamide made hydrogen bond with carbonyl group of ARMN at these ratios. SLVPs showed single band of carbonyl group having displaced value of NA at drug-carrier ratio 1:8-1:10 that signifies that strong $CONH_2$ interaction occurred at these ratios because of absence of carbonyl group characteristic of ARMN. Decrease in frequency of endoperoxide group and increase in C-O group confirmed this interaction. To our knowledge there are very few FTIR reports available about interaction of nicotinamide with drug (Jain, 2008).

FDSDs attained highest band shifting followed by corresponding SLVPs and PMs respectively. These solubilized products produced two carbonyl peaks i.e. carbonyl stretch of pure artemisinin was at lowered wave number similarly carbonyl stretch representative of nicotinamide in majority of prepared drug-carrier ratios were also at lower wave number that indicate presence of hydrogen bonding among $CONH_2$ and probably London forces acting between the aromatic rings (non-polar parts) of both molecules. This way nicotinamide imparted enhanced aqueous solubility in FDSDs through various hydrogen bonding centers on hetero atoms with non-bonded electron pair on it. It was noted that ΰC-O-O-C group was reduced in SLVPs whereas it increased in respective FDSDs with respect to corresponding PMs**.** This indicates that SLVPs and FDSDs have different type of interaction. The intensity of characteristic bands of nicotinamide in all PMs, SLVPs and FDSDs increased with enhanced drug-carrier ratio. FTIR spectra of all PMs, SLVPs, FDSDs showed two bands of N-H stretching vibrations having shifted values at all ratios (characteristic bands of NA) that is indicative of alteration of interaction among artemisinin and nicotinamide. Alteration in the values of stretching and bending vibrations verified this extent of interaction. The band shifting of carbonyl group in our study was similar to artemether (Ansari *et al*, 2010), dihydroartemsinin (Ansari *et al*, 2009), appearance of N-H group due to NA was similar to indomethacin (Bogdanova *et al*, 1998) and peak broadening was found to be analogous with carbamazepine (Sethia and Squillante, 2004). All this behavior verifies the presence of stronger interactions due to $CONH_2$ group.

XRD spectral studies

X-ray diffraction studies were undertaken to see whether nicotinamide could alter diffractogram of artemisinin in solid dispersions. XRD patterns of physical mixtures exhibited gradual decrease of intensity with rise of NA and showed substantial decrement in peak intensity at higher ARMN-NA ratio that is attributed to low drug content similar to flurbiprofen (Varma and Pandi, 2005). SLVPs revealed distinct diffractograms than respective PMs as they exhibited more displaced angles as well as reduced peak intensities as compared to ARMN and PMs that is indicative of stronger interaction. One unusual XRD patterns of SLVPs was observed at drug-carrier ratio 1:6 (21.9-22.3°) while a synergistic effect was found at 2θ of 38.8° at drug-carrier ratio 1:8 also confirms stronger $CONH_2$ interaction between artemisinin and nicotinamide which is verified by their high dissolution rate and DSC thermograms.

FDSDs produced lowest number of peaks, highly displaced angles and least peak intensities as compared to corresponding PMs and SLVPs that indicate strongest interaction among ARMN and NA. In addition a diffraction band at 38.56° which was present in both drug and nicotinamide disappeared in all ratios of FDSDs that confirms the strongest interaction. It can be assumed that FDSDs was partially amorphous and dissolved in carrier similar to indomethacin (Bogdanova *et al.*, 1998), chlorpropamide (Ford and Rubinstein, 1977) and was verified by maximum aqueous solubility at all ratios. Our results show altered XRD patterns of artemisinin in SLVPs and FDSDs. This is different from flurbiprofen where flurbiprofen remained unaltered in solid dispersions (Varma and Pandi, 2005). Rearrangement in diffraction angles, reduced peak intensities, synergistic effect and disappearance of some crystalline peaks verifies the interaction among artemisinin and nicotinamide.

DSC thermograms

Artemisinin was melted at 151.03°C and showed immense crystallization behavior at 210.04°C. These melting and crystallization peaks were found in all samples and at all ratios but having altered melting temperatures than artemisinin and nicotinamide. It indicates that artemisinin was not completely soluble in nicotinamide. DSC thermograms of physical mixtures, solid dispersions by solvent evaporation showed substantial decrease in melting and crystallization temperature except 1:6 ratio of PMs and SLVPs than artemisinin alone. All samples at drug-carrier 1:1-1:4 showed melting temperatures below the both partner i.e. artemisinin/nicotinamide. In these samples the physical state of drug has been changed to a high-energy state and high disorder which corresponds to decrease in melting temperature and it resulted in enhanced solubility and faster dissolution (Won *et al.*, 2005). Physical mixtures and its respective SLVPs showed gradual increase in melting temperature with rise of NA upto maximum peak temperature at drug-carrier 1:6 ratio followed by decline. Conversely enthalpy change reduced and was minimum at 1:6 ratio followed by increase at successive ratios. The ratio 1:6 showed crystallization temperature higher than ARMN which indicate higher thermal stability than all. Endothermic peak temperatures of SLVPs were slightly less than corresponding PMs and having high peak intensity indicating that many of ARMN crystals kept their crystalline nature. ΔH of SLVPs was significantly higher than respective PM especially at 1:6 ratio which is opposite to valdecoxib (Anshuman *et al*., 2004). In addition small sized endotherms were found at 1:6 ratio that indicate crystal dilution of ARMN in NA. Furthermore their crystallization temperature was much higher (200°C) than others which reflects to highest thermal stability. Dissolution profile of PMs and SLVPs agreed with its melting thermograms at drug-carrier ratio of 1:6.

FDSDs showed different DSC thermograms than respective PMs and SLVPs i.e., they showed lowest peak temperatures while exothermic peaks were weak and

broad at higher drug-carrier ratios which signified that artemisinin made solid solutions with nicotinamide in these solid dispersions due to stronger interaction as indicated by their FTIR spectra. Their endothermic temperature was enhanced with the increase of drug-carrier ratio also. These results are in accordance with their XRD and dissolution findings. Our findings are different from nifedipine where melting endothermic peak of nifedipine almost disappeared (Suzuki and Sunada, 1997). All data indicate that FDSDs are superior to their SLVPs with respect to crystallinity.

CONCLUSIONS

XRD patterns of solid dispersions by solvent evaporation method (SLVPs) exhibited more displaced angles, decreased intensity and synergistic effect at higher drug-carrier ratios compared to physical mixtures whereas in freeze dried solid dispersions (FDSDs), some peaks of artemisinin and nicotinamide were masked, showed least number of peaks having low intensity and maximum displaced angles. FTIR spectra revealed stronger interaction among N-H group of nicotinamide and C=O group of artemisinin in SLVPs compared to respective PMs. FDSDs and SLVPs imparted different kinds of bonding i.e. probably FDSDs showed London forces in addition to hydrogen bonding as exhibited by SLVPs. DSC thermograms of PMs and SLVPs showed different thermal behavior compared to FDSDs i.e. gradual increase in melting endotherms were observed upto drug-carrier ratio 1:6 followed by decline in PMs and SLVPs while melting endotherms gradually enhanced upto maximum ratio in FDSDs. In addition FDSDs showed lowest peak temperature than respective PMs and SLVPs in DSC thermograms. Furthermore thermal behavior exhibited that artemisinin was not completely soluble in nicotinamide. It was concluded that artemisinin made solid solutions with nicotinamide in FDSDs due to stronger interactions as exhibited by FTIR and XRD patterns. Phase solubility of artemisinin in SLVPs and FDSDs produced 1:1 complexes at lower ratios while high order complexes at higher ratios. FDSDs showed highest solubility and stability compared to corresponding SLVPs and PMs. Dissolution rate was highest in FDSDs at all ratios followed by SLVPs and PMs respectively that was according to DSC, FTIR, XRD and solubility results.

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