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Research Article

Stability Study of Microemulsion and Their Use in Formulation of Pellets with Enhanced Solubility and Dissolution Efficiency of Nevirapine

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ABSTRACT

This investigation explores use of Oleic acid, Tween 80 and Transcutol HP in self emulsifying pellets of nevirapine with enhanced solubility and dissolution efficiency. Liquid SEDDS was prepared and characterized by self emulsification time, precipitation, thermodynamic stability, globule size, PDI, zeta potential. Self emulsifying pellets were characterized by surface topography, flow properties, disintegration time, *in vitro* drug release and stability. Optimized SEP6 had disintegration time (7 ± 2 min) and % dissolution efficiency about 86.22 ± 1.25 for 1 h. In conclusion, self emulsifying pellets of nevirapine could be demonstrated as new approach to enhance its solubility and dissolution efficiency.

INTRODUCTION

The most frequent causes of low oral bioavailability are attributed to low solubility and low permeability. Low water soluble drugs often require high doses in order to reach therapeutic plasma concentrations after oral administration. Low aqueous solubility is the major problem encountered with formulation development of new chemical entities as well as generic formulation development. Oral delivery of hydrophobic drugs presents major challenge due to its low aqueous solubility. Various methods are used to enhance solubility of drug like pH adjustment, self emulsifying drug delivery system, conversion of crystalline to amorphous form, micro emulsion and nano-suspension, supercritical fluid process, inclusion complexation, co solvency, micellar solubilisation, hydrotrophy, solid dispersions, floating granules, cryogenic techniques [1-5].

To overcome the problems associated with the development of poorly-soluble drugs and new chemical entities, self-emulsifying drug delivery systems (SEDDS) have gained attention of researcher in the last two decade. Self-emulsifying drug delivery system is a lipid based formulation which consists of isotropic mixtures of oils, surfactants and co-surfactants.

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It can conveniently develop the emulsion on gentle agitation and offers a considerable surface area for interaction between the SEDDS formulation and the aqueous gastrointestinal fluid. This may lead to enhanced bioavailability of hydrophobic agents [6-9].

The self-emulsification process is specific to the nature of the oil/surfactant pair, surfactant concentration, oil/surfactant ratio and temperature at which self-emulsification occurs. Some parameters have been proposed to characterize the self-emulsifying performance; it include rate of emulsification, microemulsion size distribution and the charge on resulting droplets. Among them, microemulsion globule size is considered a decisive factor in self-emulsification/ dispersion performance since it determines the rate and extent of drug release and absorption [10-12].

The key step for SEDDS formulation is to find a suitable oil surfactant mixture that can dissolve the drug within the required therapeutic concentration. Liquid SEDDS can then be used to fill either soft or hard gelatin capsules. The drawback of this system includes GI tract irritation due to high surfactant concentrations, high manufacturing costs, interaction of the fill with the capsule shell, as well as problems due to storage temperature. These inconveniences can be avoided by preparing solid self-emulsifying

drug delivery systems. The conversion of the liquid SEDDS into the solid SEDDS by extrusion/ spheronization technique eliminates the demerits of the liquid SEDDS and simultaneously attains the merits of the solid SEDDS [13].

Solid SEDDS provides several advantages for pellets, eliciting great interest in its development due to easy to scale up. As pellets disperse freely in the GIT, drug absorption is maximized and diminution in peek plasma fluctuations occurs, thereby minimizing the potential side effects without lowering drug bioavailability. The method of choice in preparing the pellets dosage form is extrusion/ spheronization since it provides much more benefits than other methods including large scale manufacturing, reproducibility, spherical shape, narrow size distribution, good flow properties, low friability, and uniform packing characteristics. It is therefore suitable to combine the advantages of self-emulsifying drug delivery systems with those of pellets [14-16].

This self emulsifying system was added to MCC and K-carrageenan, water was added for wet mass, and then it was made suitable to form pellets. Surfactant non-ionic surfactants with high HLB values are used in the formulation of SEP. (e.g., Tween, cremophore, labrasol, etc.) To form a suitable SEP, the strength of a surfactant should vary between 30% and 60% w/w of the formulation. The large quantities of surfactants used in SEP preparation might irritate the GIT, which leads to the possible consideration of using non-ionic surfactants over ionic ones. Amphiphilic surfactants can dissolve relatively high amounts of hydrophobic drugs, thereby preventing the precipitation of drugs within the GI lumen [17-20].

Nevirapine is a non-nucleoside reverse transcriptase inhibitor and antiretroviral used in the treatment of human immunodeficiency virus type 1 (HIV-1) infections. Nevirapine binds directly to reverse transcriptase and blocks the RNA-dependent and DNA-dependent DNA polymerase activities by causing a disruption of the enzyme's catalytic site. It is highly lipophilic and very slightly soluble in water (0.0007 mg/ml) and Log P value 1.75 which gives rise to difficulties in the formulation of dosage forms and leads to variable dissolution rates with a resultant decrease in bioavailability.

In the present investigation the solubility enhancement of nevirapine is of prime importance that ultimately increases dissolution rate**.** We prepared a liquid SEDDS formulation containing nevirapine and it was incorporated into spherical pellets produced by the extrusion spheronization technique. The prepared liquid SEDDS was characterized by self emulsification time, precipitation analysis, thermodynamic stability studies, globule size, shape, PDI and zeta potential. The prepared self emulsifying pellets were characterized by DSC, FTIR, XRPD, surface morphology, particle size, shape, flow properties, sphericity studies, percent production yield, friability, disintegration time, percent drug content, *in vitro* drug release and stability studies. The novelty of the current work is that, first time attempt have been made for enhancing the solubility and dissolution efficiency of Nevirapine by self emulsifying pellets. In addition, the pellets of nevirapine are never formulated and marketed using the proposed approach.

MATERIALS AND METHODS

MATERIALS

Nevirapine was received as a gift sample from Au robindo Pharmaceuticals Ltd (Hyderabad, India) k-Carrageenan, Microcrystalline cellulose (Avicel pH101) was provided by FMC Biopolymer (Philadelphia, PA, USA). Oleic acid (OA), Transcutol HP, Tween 80, mannitol and sodium starch glycholate were purchased from Signet Chem. Ltd (Bangalore, India).

Solubility Study

Nevirapine solubility was determined in various oils, surfactant and co-surfactant by adding an excess amount of drug into 5 ml of glass vials containing 3 mL of different vehicle. Mixture were mixed continuously for 24 h at 37 ºC in environmental orbital shaking incubator Remi Instruments Ltd. (Bombay, India) and then centrifuged at 5000 rpm for 15 minutes to separate the undissolved drug. Supernatant liquid were diluted with methanol and Nevirapine content was estimated by the UV-VIS Spectrophotometer (UV 1700, Shimadzu, Tokyo, Japan).

Emulsification Studies

Emulsification studies were conducted to select the optimum oil, surfactant and co-surfactant ratio (Table 1.) The mixtures with different concentrations were homogenized with the aid of gentle heat (60-70 ºC) and vortexed for 5 min in a vortex mixer. 1 mL solution was added to USP Type II Dissolution apparatus containing 900 mL 0.1 N HCL (pH 1.2) at 100 rpm.

Table 1: Emulsification studies of liquid self emulsifying drug delivery systems

Liquid SEDDSs	Oil (% w/w)	Surfactant and Co-surfactant mixture	Transmittance (%)	Observation	Dispersion time (Sec)
A1	20	80	42.126	Turbid	45-60
A2	25	75	31.778	Transparent	40-70
A3	30	70	25.342	Transparent	70-90
A4	35	65	36.682	Transparent	30-50
A5	40	60	60.881	Transparent	15-30
A6	45	55	52.621	Transparent	30-45

Table2: Thermodynamic stability and precipitation studies of liquid self emulsifying drug delivery systems

Liquid SEDDS	Globule size (nm)	Centrifugation	Freez thaw cycles	Precipitation after 1h	Precipitation after 6h	PDI	Zeta Potential (mV)
A1	536.6	No Phase Separation	Phase Separation	Clear	Clear	0.72	29.9
A2	618.7	No Phase Separation	No Phase Separation	Clear	Clear	0.68	27.3
A3	498.2	No Phase Separation	Phase Separation	Clear	Turbid	0.53	25.8
A4	612.4	No Phase Separation	No Phase Separation	Clear	Clear	0.81	26.4
A5	523.9	No Phase Separation	No Phase Separation	Clear	Clear	0.35	18.9
A6	551.7	No Phase Separation	Phase Separation	Clear	Clear	0.79	23.5

Table 3: Compositions for different formulations of self emulsifying pellets

Composition (%)	SEP1	SEP2	SEP3	SEP4	SEP5	SEP6	SEP7	SEP8	SEP9
Liquid SEDDS	18	18	18	18	18	18	18	18	18
K-Carrageenan	30	40	50	30	40	50	---	---	---
MCC	50	40	30	---	---	---	30	40	50
Pectin	---	---	---	50	40	30	50	40	30
Mannitol	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
SSG	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5

Table 4: Flow properties for different formulations of self emulsifying pellets

Formulation code	Bulk density ±SD	Tapped density ±SD	Angle of repose ±SD	Hausner's ratio ±SD	Carr's index ±SD
SEP1	0.23±0.11	0.26±0.13	27.75±0.23	1.97±0.03	12.56±1.23
SEP2	0.18±0.12	0.23±0.14	37.07±0.56	0.95±0.05	14.21±1.43
SEP3	0.21±0.10	0.24±0.15	27.43±0.21	1.16±0.08	8.69±1.56
SEP4	0.16±0.15	0.19±0.11	36.87±1.02	0.91±0.02	15.18±1.78
SEP5	0.22±0.18	0.27±0.17	32.45±0.56	0.90±0.05	8.97±1.16
SEP6	0.14±0.14	0.17±0.10	38.67±0.37	1.27±0.04	9.93±1.72
SEP7	0.21±0.13	0.23±0.11	42.41±1.12	1.10±0.09	15.28±1.17
SEP8	0.22±0.12	0.28±0.12	25.98±0.82	0.84±0.03	16.67±1.15
SEP9	0.27±0.17	0.33±0.16	35.42±0.45	0.87±0.08	13.23±1.98

Table 5: Physicochemical properties for different formulations of Self emulsifying pellets

Formulation code	Granule size (µm) ± SD	Abrasion resistance (%) ±SD	Production yield (%) ± SD	Drug content (%) ± SD	Disintegration Time (min) ± SD	Dissolution efficiency (%) ± SD
SEP1	980.8 ± 30.91	1.28±0.34	57±0.89	97.66± 0.88	9 ± 2	80.37±0.43
SEP2	1106 ± 48.62	0.25±0.19	42±0.76	97.68±0.85	11±1	84.80±0.34
SEP3	950 ± 49.69	0.35±0.27	55± 0.41	98.22± 0.31	12±1	88.22±0.71
SEP4	1121 ± 21.23	0.72±0.56	47±0.83	95.78±0.54	10±2	87.20±0.46
SEP5	1025.5± 51.61	1.20±0.41	52±0.96	93.56±0.68	12±1	76.43±0.56
SEP6	1124.5± 37.47	0.98±0.63	51±0.28	97.43±0.82	7±2	75.67±0.36
SEP7	1103.1±42.08	1.41±0.32	40±0.33	91.58±0.92	14±1	81.19±0.45
SEP8	1056.2±38.06	1.58±0.61	43±0.67	96.78±0.46	18±2	86.55±0.72
SEP9	1106.7±21.41.	0.88±0.57	45±0.18	95.22±0.76	17±2	83.19±0.53

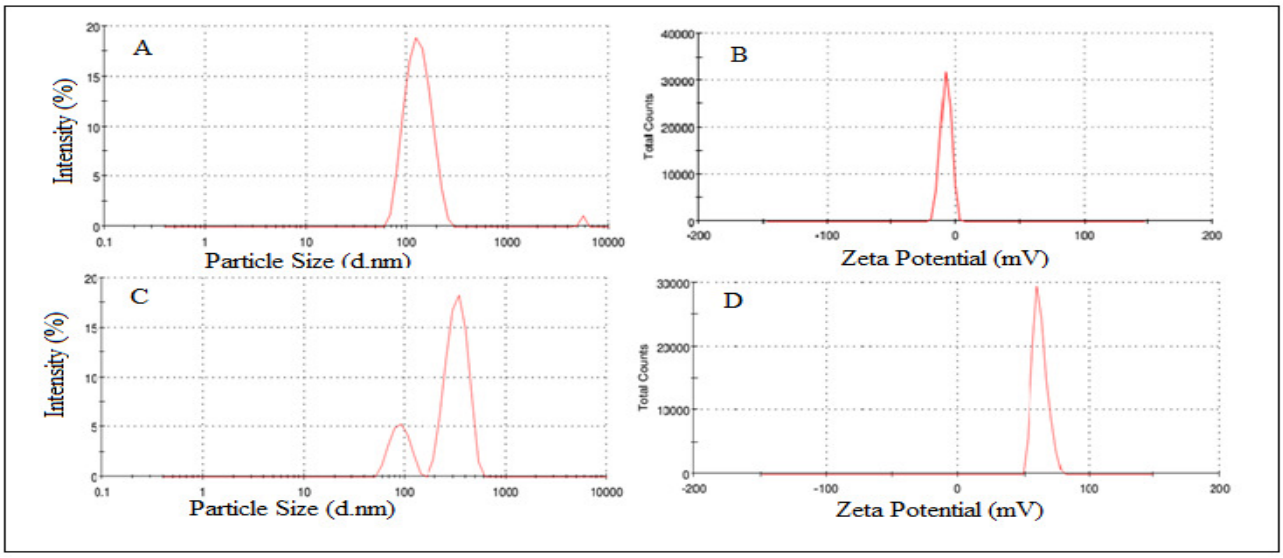

Figure 2: Particle size (Globule size) of pure drug-A and liquid SEDDS-C; Zeta potential of pure drug- B and liquid SEDDS-D

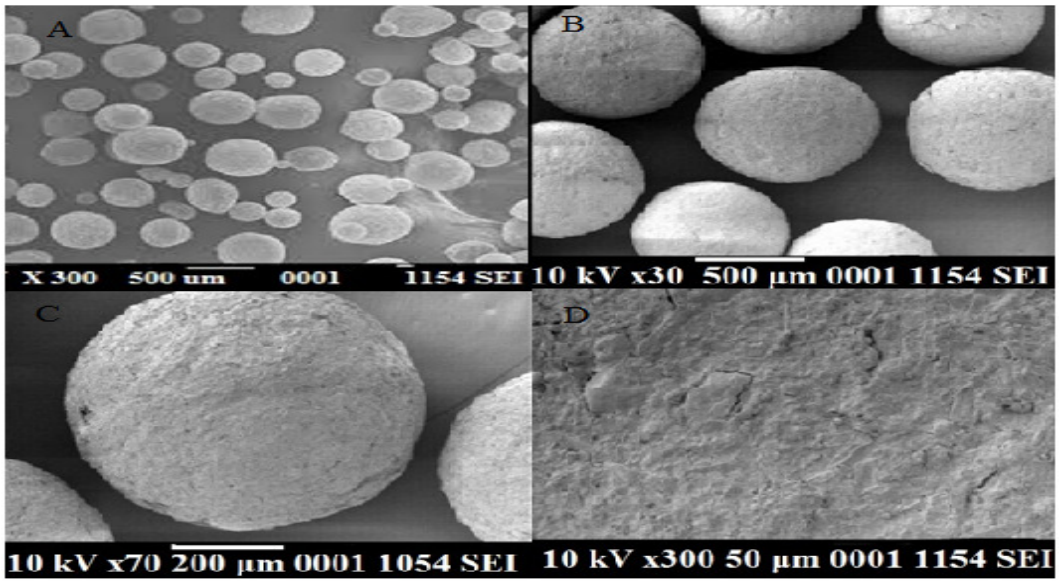

Figure 3: Photomicrographs of optimized liquid SEDDS and self emulsifying pellets of formulation code SEP6 (A-Globules of optimized liquid SEDDS and B, C, D-optimized SEP6 at different magnifications

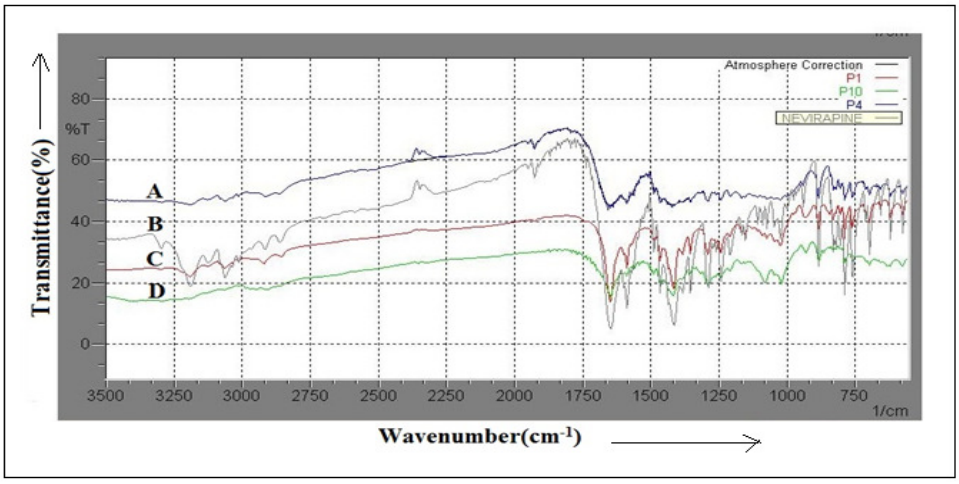

Figure 5: FTIR Spectra of drug with K- carrageenan (A), Pure drug –Nevirapine (B), drug with MCC (Avicel PH-101) (C), and optimized self emulsifying pellets SEP6 (D)

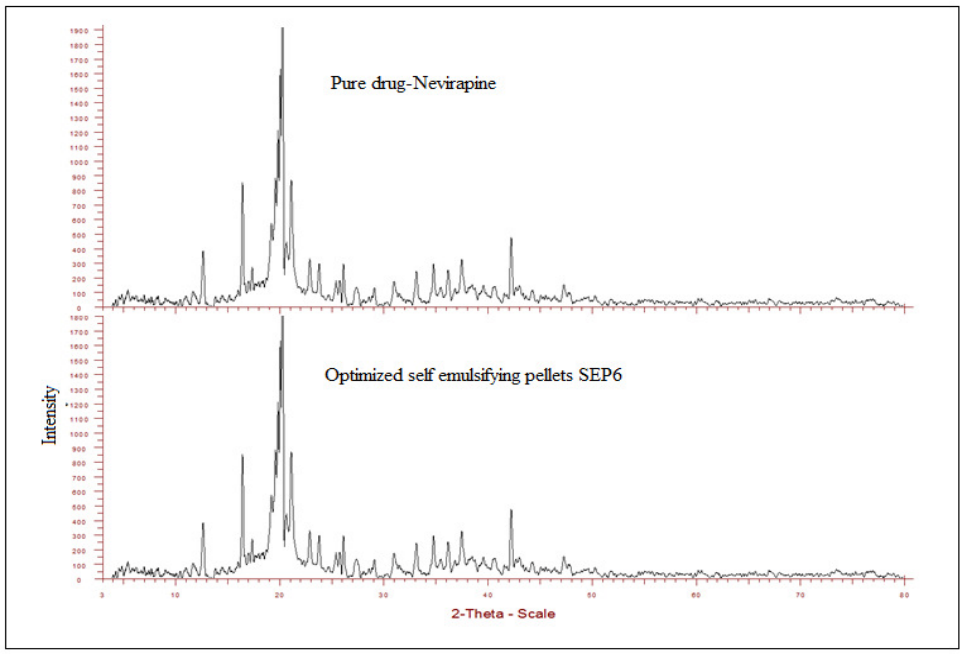

Figure 6: X-ray diffraction pattern Nevirapine and optimized self emulsifying pellets SEP6

Time required to disperse the formulation was noted. The resulting emulsions were observed visually for relative turbidity. The resultant microemulsion were allowed to stand for 2 h and their % transmittance was measured at 296 nm by UV-VIS Spectrophotometer using 0.1 N HCL (pH 1.2) as a blank. Preparation which dispersed within 1 min and formed transparent or translucent emulsion, were considered for further development.

Saturated Solubility Study for Liquid SEDDS

Excess amount of the drug was added to 1 mL of each of the clear ratios (microemulsion) in the selected system and mixed using a vortex mixer, moved to a thermodynamic water bath shaker for 72 h at room temperature and then centrifuged by Ultracentrifuge, Bachman Coulter (Saint Lucia, USA) at 5000 rpm for 20 min. The supernatant was diluted with 0.1 N HCl (pH 1.2) using a magnetic stirrer at 500 rpm for 2 min at room temperature. The amount of dissolved drug was determined using UV visible spectrophotometer at 296 nm.

Preparation of liquid SEDDS (Microemulsion)

Based on the pilot studies (equilibrium solubility and super saturation studies), a liquid SEDDS containing of nevirapine (1%, w/w) was prepared, it could be diluted with 0.1N HCL solution (1:100) without precipitation within 2h. The optimized blank liquid SEDDS consisted of oleic acid (as the oil phase, 40%, w/w), Tween 80 (as the surfactant, 45%, w/w) and Transcutol HP

(as the co-surfactant. 15%, w/w). Formulation was prepared by mixing of Oleic acid, Tween 80, and Transcutol HP at 70 ºC with magnetic stirrer. Nevirapine was dissolved in the blank SEDDS and resultant mixture was vortexed until a clear solution was obtained. The formulation was examined for signs of turbidity or phase separation prior to self emulsification and particle size studies.

Preparation of Self Emulsifying Pellets

Immediate release pellets containing nevirapine were prepared by extrusion-spheronization technique. K-carrageenan (50%), MCC (30%), mannitol (5%) and sodium starch glycholate (5%) were subjected to dry mixing in a double cone blender at 50 rpm for 20 min. The dry mixture was mixed in mortar by liquid SEDDS (18%) as a binder solution to achieve a consistency of the damp mass. The prepared damp mass was immediately passed through radial basket extruder, UICE-LAB, Umang Pharmatech Pvt Ltd (Ahmadabad, India) using 1.0 mm diameter screen (Table 3). The extrudates were produced at speed of 50 rpm in 15 min. The extrudates were then rolled in a spheronizer with friction plate of regular crosshatch geometry for 15 min at a rotation speed of 1300 rpm with a constant compressed air supply of 0.5–1.5 bar. The resultant pellets were dried in a hot air oven at 40 ± 5ºC for 20 min in dark area protected from light and screened to achieve the final product.

Characterization of liquid SEDDS

Determination of Self Emulsification Time

The self emulsification time indicates rate of formation of emulsion that is the time taken to form a homogenous dispersion was determined for the prepared formulation using USP Type II (paddle type) Dissolution apparatus by visual observation. Each formulation (1gm) was added in drop wise manner into 900 mL distilled water at 37 ±0.5ºC and stirred at agitation speed of 50 rpm. Emulsification time was assessed by visual inspection.

Thermodynamic Stability Studies

The objective of the thermodynamic stability was to evaluate the effect of temperature variation on the liquid SEDDS formulation. Prepared liquid formulations were centrifuged by Ultracentrifuge, Bachman Coulter at 15,000 rpm for 15 min and the formulations were observed visually for phase separation. The formulations were subjected to freeze- thaw cycles (-5ºC for 2 days followed by 40ºC for 2 days) the samples were observed visually after freeze-thaw cycles.

Precipitation Analysis

The prepared liquid SEDDS were diluted with 0.1 N HCL (pH 1.2) up to 250 times. The diluted microemulsion was observed at 1 and 6 h for any sign of phase separation or drug precipitation.

Globule Size, PDI, Zeta Potential and Shape Analysis

The mean globule size, zeta potential and polydispersity index (PDI) of emulsion formed by the addition of the liquid SEDDS (0.3 gm) into 30 ml of 0.1N HCL solution were determined by photon correlation spectroscopy (PCS) with a Malvern Zetasizer Nano ZS90, Malvern Ltd (Worcestershire, UK) The measurement using PCS is based on the light scattering phenomena in which the statistical intensity fluctuations of the scattered light from the particles in the measuring cell are measured. The morphological characteristic that is surface topography and shape of the liquid SEDDS was observed by using a SEM microscope. Each sample was coated with gold or palladium under an argon atmosphere using a sputter coater Bal-Tec SCD. The photomicrographs were taken at an acceleration voltage of 25 kV.

Characterization of self emulsifying pellets

DSC, FTIR and XRPD analysis

Each sample (2 mg) of pure drug-Nevirapine, pectin, sodium starch glycholate, K-carrageenan, MCC, physical mixture and optimized formulation SEP6 were analyzed using a Mettler Toledo DSC with star "e' software (Model 822c). The thermographs were obtained at a heating rate 10◦C/min over a temperature range of 30-300 °C under an inert atmosphere flushed with nitrogen at a rate of 30 mL/ min.

The each sample of pure drug, drug with K-carrageenan, drug with MCC and optimized self emulsifying pellets SEP6 were mixed with KBr of IR grade in the ratio of 1:100 and compressed using motorized pellet press at 10-12 tons pressure. The pellets were then scanned using FTIR spectrophotometer. The FTIR spectra of pellets were compared with that of the FTIR Spectra of pure drug, to confirm any changes occur or not in the principle peak.

XRPD patterns of pure drug and optimized self emulsifying pellets SEP6 was carried out using diffractometer with a copper tube anode over 1–40° 2θ range.

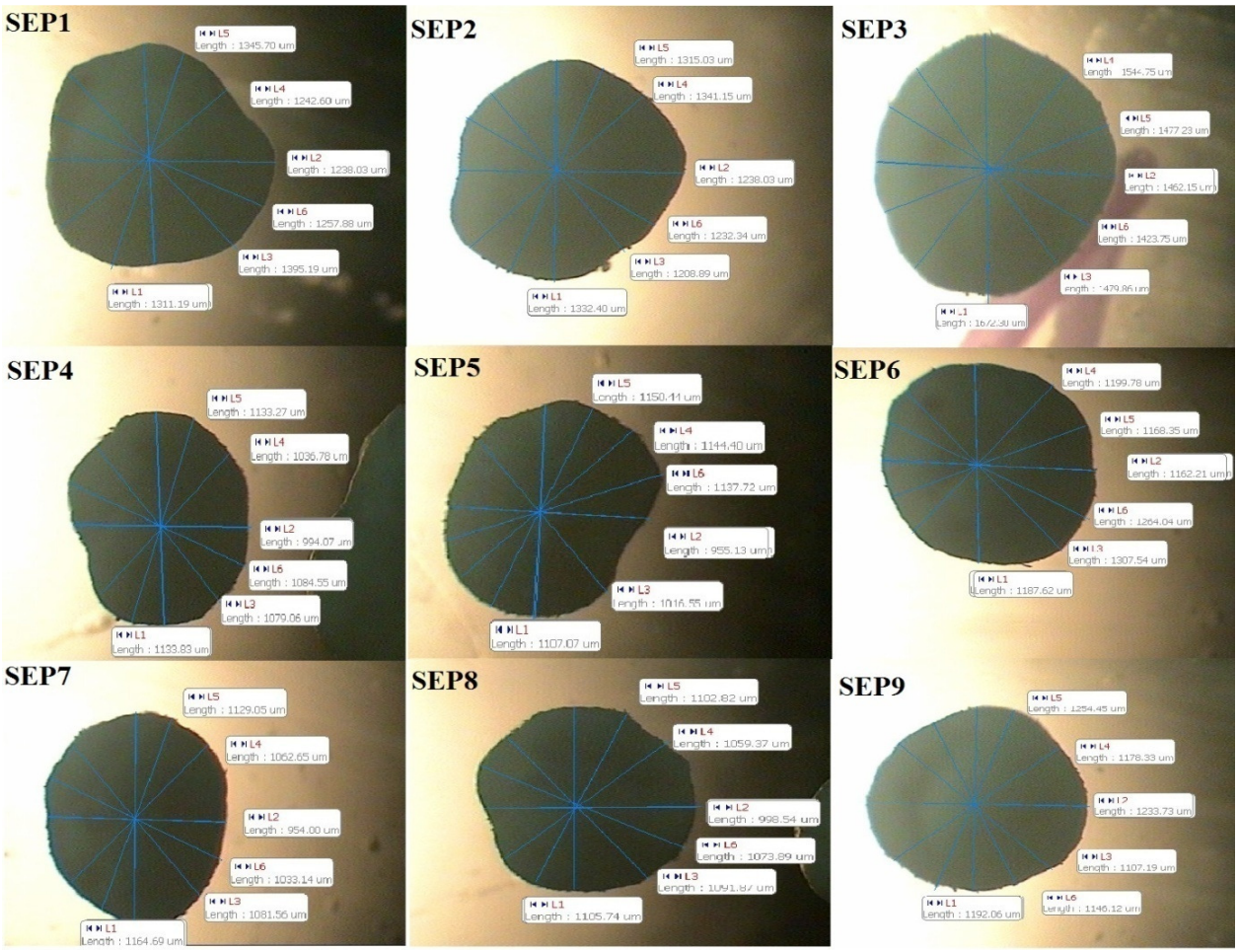

Figure1: Microscopic images for determination of aspect ratio, pellips, circularity, roundness to assess the sphericity of different formulation of pellets

The sample analysis was carried out at generator tension (voltage) 45 kV; generator current 40 mA; scan step time 9 sand scan step size of 0.008° (2θ).

Flow Properties

Pellets were characterized for flow properties such as angle of repose, bulk density, tapped density, Hausner's ratio, Carr's and compressibility index and flow behaviour was studied. The results were expressed as mean values of three determinations.

Sphericity Studies

The shape and the area of pellets were investigated by optical microscopic image analysis. Pellet size and shape was measured using an optical DMW2-223 digital microscope (Motic Instruments (Toronto, Canada) equipped with a 1/3'' CCD camera imaging accessory and computer-controlled image analysis software (Motic Images 2000, 1.3 version). 50 pellets from each batch were selected and analyzed by microscopic image analysis technique and variety of parameters like aspect ratio, roundness, circularity and pellips have been used to assess the shape of the pellets [21, 22].

Friability and Disintegration Time

500 mg of self emulsifying pellets were placed in the Roche friabilator, Electrolab (Bombay, India) together with 500 mg of glass spheres, and rotated for 15 min at 25 rpm. The self emulsifying was collected, weighed and percent friable amount was calculated. 200 mg pellet samples from each formulation were tested (n=3) in 900 mL distilled water at 37ºC ± 2 ◦C using a USP disintegration apparatus and the end point was taken at which no obvious particles were remained on the sieve in each disintegration basket.

Drug Content

Drug content of self emulsifying pellets was determined by extraction with 100 mL of 0.1 N HCl (pH1.2). Accurately weighed 1.0 g of pellets was placed in a mortar and then grinded into fine powder using pestle and transferred into a 100

mL volumetric flask containing 100 mL of 0.1 N HCl (pH1.2) and the mixture was stirred overnight for complete extraction of drug. The solution was filtered through a 0.45 µm filter paper (Millipore), diluted with appropriate amount of of 0.1 N HCl and assayed spectrophotometrically at 296 nm. The results were expressed as mean values of three determinations.

***In vitro* dissolution**

1gm of self emulsifying pellet was placed into USP apparatus 2 at 37±0.5ºC at 100 RPM in 900 mL 0.1 N HCL (pH 1.2). At predetermined intervals, 5 mL of the medium was withdrawn and filtered through the Millipore filter paper. The resulting solution was diluted and analyzed by UV Spectrophotometer at 296 nm. At each sampling point 5 mL of fresh media was added. The dissolution efficiency was calculated by equation (1)

$$DE = \frac{\int_{t1}^{t2} y - dt}{(t2 - t1)\, y_{100}} \times 100 \qquad (1)$$

Where y_{100} is maximum percent of the drug dissolved in the media, t_1 and t_2 is time points and y is percent of the drug dissolved in the media,

The results were expressed as mean values of five determinations.

Production yield

The size distribution of pellets was determined by passing through 16/25 mesh sieves of Tylor's standard using a sieve shaker (Electromagnetic sieve shaker, EMS8, Electrolab (Bombay, India) for 5 min at a frequency of 50 Hz with amplitude of 1 mm. A fraction of pellets between 710 and 1,190 µm sizes was selected as the final product [23, 24]. The percent production yield was calculated by equation (2)

$$\%\ \text{Production Yield} = \frac{Pm}{Tm} \times 100 \qquad (2)$$

Where, Pm and Tm are practical and theoretical weights of the pellets, respectively. The results were expressed as mean values of three determinations.

Stability studies

To check the effect of environmental condition or storage conditions on formulation, optimized batch SEP6 was kept in environmental stability chamber (CHM-10S, REMI Instruments, Bombay, India) for accelerated stability condition at 40 ºC ± 2 ºC temperature and 75 ± 5 % relative humidity for a period of 3 months. Drug content and *in vitro* drug release were measured at 0, 30, 60 and 90 days. To confirm the similarity of drug release profiles before and after stability studies, a model independent statistical tool for comparison of dissolution profile dissimilarity factor (F1) and similarity factor (F2) was calculated by equation (3) and (4).

$$F1 = \left\{\frac{[\sum_{t=1}^{n}(Rt - Tt)]}{[\sum_{t=1}^{n} Rt]}\right\} \times 100 \qquad (3)$$

$$F2 = 50 \log\left\{\left[1 + \frac{1}{n}\sum_{t=1}^{n} wt(Rt - Tt)^2\right]^{-0.5}\right\} \times 100 \qquad (4)$$

Where, F1 indicates dissimilarity factor (0-15) and F2 indicates similarity factor, n is the number of observations, wt is optional weight, Rt is percentage drug dissolved from reference formulation (before stability studies) and Tt is percentage drug dissolved from test formulation at (after completion of 90 days in stability chamber). In general, F2 values higher than 50 (50 – 100) show similarities of the dissolution profiles. The results were expressed as mean values of three determinations ± S.D.

Statistical analysis

The experimental results are expressed as mean ± S.D. Statistical evaluation of the data was done using ANOVA. The evaluation of data was used to assess the significance of differences. Statistically significant difference between the means of formulation batches were defined as $p < 0.05$.

RESULT AND DISCUSSION

Solubility plays an important role in drug disposition, since the maximum rate of passive drug transport across a biological membrane, is the product of permeability and solubility. According to the biopharmaceutical classification system (BCS), aqueous solubility and permeability are the most important parameters affecting drug bioavailability. Retrospective studies show that greater than 60% of drug failures in development can be traced to poor biopharmaceutical properties, mainly due to poor dissolution or poor permeability. Thus improvement of aqueous solubility in such case

is valuable goal to effectively formulate them into bioavailable dosage forms.

Nevirapine is a weak base (pKa 2.8) with low intrinsic water solubility which gives rise to difficulties in the formulation of dosage forms and leads to variable dissolution rates with a resultant decrease in bioavailability. In the initial phase of spheronization, the long cylindrical extrudates broke into shorter cylinders and were plastically transformed to spherical pellets due to incorporation of liquid SEDDS. The 0.5 % of SSG as disintigrant was optimum to get the very thin consistency. This was indicated that, it has sufficiently increased hydrophilic nature the prepared pellets and their swelling capacity. In addition, hydrophilic surfactant Tween 80 showed highest solubility as compared to other surfactant, therefore, SSG and Tween 80 at optimum concentration showed the hydrophilic nature of the self emulsifying pellets. SSG is used as superdisintigrant. In addition, to self emulsifying ability, SSG might increase the disintegration that may reduce the disintegration time of self emulsifying pellets.

On the basis of desired flow properties, physical properties, % production yield, % drug content, *in vitro* drug release pattern formulation code SEP6 was selected as the optimized formulations. It was most essential, in preparing pellets for filling into the hard gelatine capsules, to avoid compression, coating drying and dose dumping of tablets, which may have led to deformation or structural damage during the process. The optimized formulation SEP6 had an optimum polymer ratio suitable to produce solid, discrete, spherical, free flowing pellets with sufficient mechanical strength. In addition, it had desirable disintegration time and *in vitro* drug release for immediate release pellets. The extrudates were formed at a speed of 50 rpm for 15 min. If the speed was increased above this, the extrudates break and if the speed was kept below 50 rpm, it takes more time for the extrudates to form. The spheronization speed of 1300 rpm was chosen to be optimum as increasing the speed resulted in powder formation and decreasing the speed forms rod and dumbbell shaped pellets.

Solubility Studies

The equilibrium solubility of nevirapine in various oil, surfactant, and co-surfactant are shown in figure 1. SEDDS point of view, solubility in various excipients is an important criteria. In SEDDS the drug was soluble in the oil phase and/or was present at the interface. It depends on the hydrophobicity of the drug and the HLB value of the system. Oleic acid (liquid lipid) showed higher solubility than other oils. In order to prevent precipitation of drug on dilution it is necessary to select oil which showed higher solubility. Tween 80 had higher solubility compared to Tween 20 and polaxamer 188. Drug showed highest solubility in co-surfactant Transcutol HP.

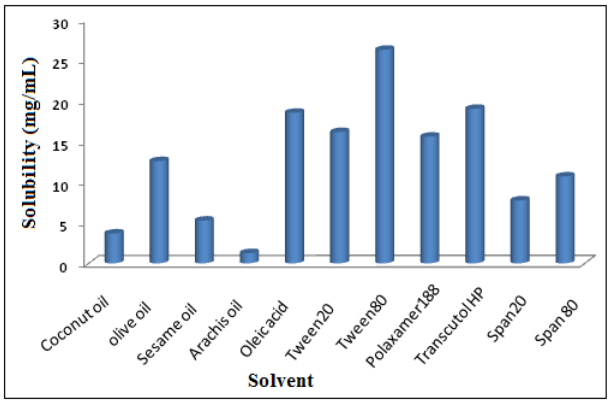

Figure 7: Comparative solubility profile of Nevirapine in various oil phases, surfactants, and co-surfactants.

Emulsification Studies

Quick emulsification of the preconcentrate is necessary for the proper functioning of self emulsifying system, therefore emulsification studies were performed to evaluate the ability of selected surfactants to emulsify maximum amount of selected oils which yields transparent or translucent emulsion in less than 2 min. Emulsification studies were conducted in order to select proper ratio of oil, surfactant and co-surfactant [39]. Six different ratios of oil: surfactant were selected. Emulsification studies were performed to evaluate ability of surfactant and co-surfactant to emulsify oil phase (Table 1). 40% oleic acid and 60% surfactant and co-surfactant ratio produced a clear emulsion in less than 30 sec with transmittance 60.881 %.

Thermodynamic Stability and Precipitation Studies

Thermodynamic stability studies illustrated the ability of the tested formulations to withstand wide range of changes in temperature conditions and centrifugal stress without any phase separation and precipitation of drug/excipients. A stable microemulsion formulation should not lose its ability of spontaneous emulsification upon dilution. Out of all liquid formulations, batch A2, A4 and A5 were found to be stable in

the centrifugation test and in freeze thaw cycles there was no sign of phase separation (Table 2). Out of six different formulations, the formulation A3 was precipitated on dilution with 0.1 N HCL (pH 1.2) at 250 times after 6 h, although, there was no sign of precipitation up to six hours in all other formulations.

Globule Size and Shape Analysis

Globule size analysis of microemulsion is a critical step in pathway of enhancing drug dissolution. Globule size and PDI of pure drug was found to be 140.5 d nm and 0.27, respectively. The liquid SEDDS globule size and PDI was found to be 536.6 and 0.720, respectively, although, value of globule size and PDI changed for liquid and its corresponding solid SEDDS (Figure 2). The zeta potential represents the electrical charge to the globule surface. Zeta potential is important and useful indicator to predict and control the stability of emulsions. The measurement of zeta potential is the key to understanding the dispersion and aggregation processes. The greater the zeta potential value, more likely the suspension is to be stable because the charged particles repel one another and thus overcome the natural tendency to aggregate. It is currently admitted that higher zeta potential values, either positively or negatively charged, mean that dispersion will have greater long-term stability. The zeta potential of pure drug and liquid SEDDS was found to be -29.1 and -29.9 mV, respectively.

SEM Analysis

Photomicrographs of the reconstituted liquid SEDDS A5 showed well dispersed spherical globules without any agglomeration of droplets (Figure 3). Surface topography of optimized self emulsifying pellets SEP6 was studied using SEM (Figure 4). While, the pellets of formulation SEP6 were observed to be fairly porous and spherical with nearly regular surface and the pellets of remaining batches were fairly porous and spherical with irregular shapes.

DSC, FTIR and XRPD Analysis

The DSC thermographs were recorded for pure drug, pectin, sodium starch glycholate, K-carrageenan, MCC, physical mixture and optimized self emulsifying pellets (Figure 4). Pure drug has showed sharp endothermic peak at about 243.91°C with crystallinity about 8552.18 % indicated that, it is having highly crystalline in nature. The optimized batch SEP6 exhibited a sharp endothermic peak at 236.16 °C which is corresponds to the drug. In addition, the glass transition temperature (Tg) of optimized batch SEP6 was found to be 270.49 °C with crystallinity about 913.13 %. Therefore, there was significant decrease in crystallinity of drug in the optimized self emulsifying pellet was found. In physical mixture there was significant backward shift of endotherm while in the optimized formulation SEP6 no significant shift was observed. This thermal behaviour revealed that, the optimized formulation converted the highly crystalline drug into the amorphous nature. In addition, based on thermal behaviour it is conformed that, there was no any interaction between drug and excipient.

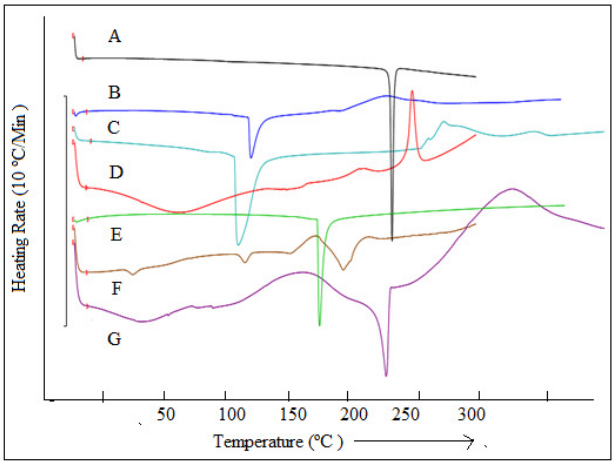

Figure 4: Thermographs of Pure Drug (A), Pectin (B), Sodium Starch Glycholate (C), K-carrageenan (D), MCC (E), physical mixture (F) and Optimized batch SEP6 (G)

To evaluate the compatibility of drug with excipient and the physical state of drug in pure form and formulation, we studied the crystalline or amorphous behaviour of pure drug and drug containing pellets (Figure 5). The most interesting bands of drug in the FTIR spectra are the C–H stretching vibrations, N–H stretching vibrations and the C=O stretch in aromatic groups. In the crystalline drug these bands are narrow at 3,331 cm-1 and at 1,688 cm-1, while in the formulation blend SEP6 bands were appeared as broad it clearly revealed its amorphous nature and its compatibility with excipient.

X-ray powder diffraction studies have been most valuable in elucidation of phase behaviour, arrangement and crystal order of molecules. The XRPD pattern of self emulsifying pellets are shown in figure 6, the internal state of drug in the self emulsifying pellet SEP6 was further

verified. The δ value and intensity of each peak in the respective diffract graph of pure drug and drug in the optimized batch SEP6, revealed the conversion of drug from crystalline to amorphous properties.

Flow Properties and Physicochemical Properties of SEP Pellets

The various batches of formulations were subjected to the characterization of pre-compression parameters for the determination of their flow behaviour [36, 37]. Angle of repose, Carr's index, Hausner's ratio of the SE pellets of different batches is shown in Table 4. Granule size, drug content and production yield of various formulation batches are shown in Table 6. The production yield was found to be in the range of 40–57 %. Mean granule size was found to be within a narrow range of 0.9500–1.124 mm (Figure 3). The abrasion resistance was found to be in the range of 0.2–1.5 %. The shape of the pellets has enormous effect on the properties of final formulation and their processing for further formulation development. Hence, it is revealed that the shape of the pellets determines the quality and durability of the formulation.

Sphericity Studies

Pellets with the spericity value of 1 are considered to be exactly spherical and pellets with sphericity value near to 1 are considered nearly spherical. The spericity value for the optimised formulation SEP6 was found to be 0.997 ± 0.011. In addition, pellets of all remaining batches were nearly spherical in shape.

Friability and Disintegration time

Percent abrasion resistance for all the batches of self emulsifying pellets was found to be in the official limit. Out of all the formulated batches, disintegration time for batch SEP 6 was found to be 7 ± 2 min (Table 6). The concentration of carrageenan used in optimized batch SEP6 was 50% and it was highest as compared to all other batches that attributed to faster disintegration of pellets in 0.1 N HCl (pH1.2).

Drug Content

Drug content of self emulsifying pellets of different batches were found to be in between 91.58 ± 0.92 % to 98.22 ± 0.31 %. All of the batches showed drug content are within pharmacopoeias limits (95-105%) except formulation batch SEP5 and SEP7.

***In vitro* Dissolution**

In vitro dissolution profile of different pellets formulations are shown in figure 8. The pure drug showed that only 26% was released within 120 min (Figure 6). *In vitro* release profile of batch SEP6 in 0.1 N HCl (pH 1.2) was found to be 86.22 ± 1.25 for 1 h at 37 ± 0.5 ºC and the desired dissolution efficiency of various formulations were found (Figure 7). It might be due to quick emulsification properties of SEDDS and its ability to keep drug in solubilised state. This indicated that solid SEDDS also able to disperse spontaneously and can release drug.

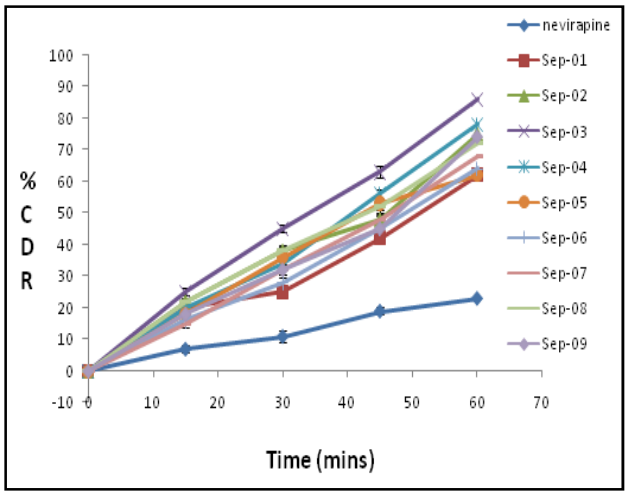

Figure 8: *In vitro* drug release profile of various batches of self emulsifying pellets in 0.1 N HCl (pH 1.2) media at (37 ± 0.5 °C)

Stability Studies

Optimized formulation SEP6 was subjected to stability studies at accelerated conditions for a period of three months. After 90 days the percentage drug content and % CDR were found to be 97.02 ± 0.43 and 84.32 ± 0.93, respectively. The F1 value (n = 3; mean ± SD) was found to be 1 and F2 value (n = 3; mean ± SD) was found to be 64 ± 6. It was revealed that the % CDR pattern after stability studies was nearly the same as before with little difference. The results of stability studies showed no significant change in % drug content and % CDR. Hence, this indicated that optimized batch SEP6 was stable for 90 days at 40 ± 2 ◦C and 75 % ± 5 % RH.

CONCLUSION

Liquid SEDDS and their self emulsifying pellets containing nevirapine were prepared successfully. Liquid SEDDS containing 40% w/w oleic acid as the oil phase, 45% w/w tween 80 as the surfactant, and 15%, w/w Transcutol HP as the co-surfactant, showed spontaneous

emulsification properties and good thermodynamic stability. SEM, IR, XRD and DSC results confirmed that drug was present in an amorphous state in solid SEDDS and there was no compatibility issue in drug and excipient. The optimized pellets SEP6 exhibited good flow behaviour, uniform size and shape with porous surface. The disintegration time and percent dissolution efficiency of optimized self emulsifying pellets SEP6 was found to be 7 ± 2 min and 86.22 ± 1.25 for 1 h, respectively. It can be concluded that, self emulsifying pellets had promising potential to improve solubility and dissolution rate of nevirapine.

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