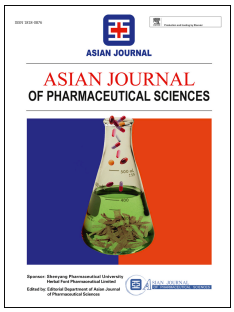
Accepted Manuscript



Self nano-emulsifying drug delivery system for Embelin: Design, characterization and *in-vitro* studies

Komal Parmar, Jayvadan Patel, Navin Sheth

PII: S1818-0876(15)00041-0

DOI: 10.1016/j.ajps.2015.04.006

Reference: AJPS 131

To appear in: *Asian Journal of Pharmaceutical Sciences*

Received Date: 12 February 2015

Revised Date: 22 April 2015

Accepted Date: 27 April 2015

Please cite this article as: Parmar K, Patel J, Sheth N, Self nano-emulsifying drug delivery system for Embelin: Design, characterization and *in-vitro* studies, *Asian Journal of Pharmaceutical Sciences* (2015), doi: 10.1016/j.ajps.2015.04.006.

Graphical abstract

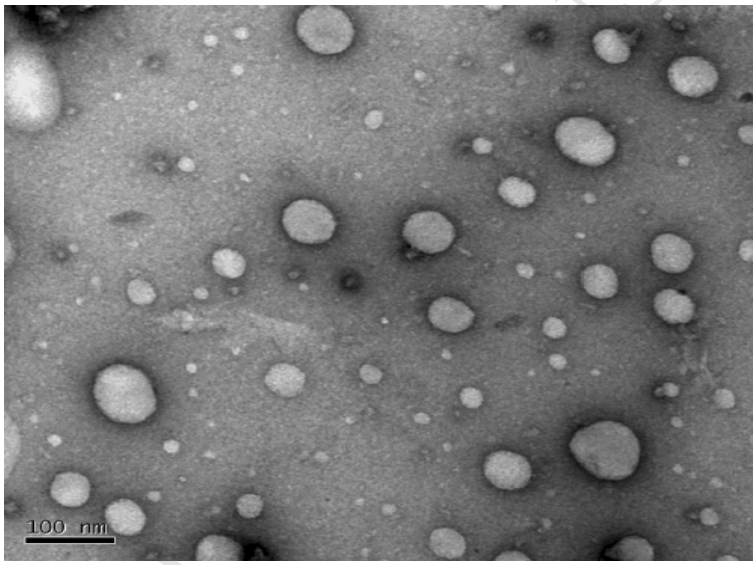
Self nano-emulsifying drug delivery system for Embelin: Design, characterization and *in-vitro* studies

Komal Parmar[a], Jayvadan Patel[b], Navin Sheth[a]

a Department of Pharmaceutical Sciences, Saurashtra University, Rajkot, Gujarat-360005

b Nootan Pharmacy College, Visnagar, Gujarat-384315

Spherical globules of SNEDDS of Embelin can be seen in TEM micrographs. Embelin was encapsulated in the globules and dissolution properties of poorly water soluble drug were improved.



Title Page:

Self nano-emulsifying drug delivery system for Embelin: Design, characterization and *in-vitro* studies

Komal Parmar[a*], Jayvadan Patel[b], Navin Sheth[a]

[a] Department of Pharmaceutical Sciences, Saurashtra University, Rajkot, Gujarat-360005

[b] Nootan Pharmacy College, Visnagar, Gujarat-384315

Ccorresponding Author: Komal Parmar*

Mailing address of the corresponding author: Department of Pharmaceutical Sciences, Saurashtra University, Rajkot 360 005, Gujarat, India.

Telephone number of the corresponding author: + 91-281-2578501

Telefax number of corresponding author: +91-281-2585083

Email of the corresponding author: komal.parmar2385@gmail.com

Abstract

The objective of the present study was to prepare solid self-nanoemulsifying drug delivery system (S-SNEDDS) containing Capryol-90 as oil phase for the delivery of Embelin, a poorly water soluble herbal active ingredient. Box-Behnken experimental design was employed to optimise the formulation variables, *X1* (amount of oil; Capryol 90), *X2* (amount of surfactant; Acrysol EL 135) and *X3* (amount of co-surfactant; PEG 400). Systems were appraised for visual characteristics for self emulsifying time, globule size and drug release. Optimised liquid formulations were formulated into free flowing granules (S-SNEDDS) by adsorption on the porous materials like Aerosil 200 and Neusilin and thereby compressed into tablet. In vitro dissolution studies of SNEDDS revealed increased in the dissolution rate of the drug. FT-IR data revealed no physicochemical interaction between drug and excipients. Solid state characterization of S-SNEDDS by DSC and Powder XRD confirmed reduction in drug crystallinity which further supports the results of dissolution studies. TEM analysis exhibited spherical globules. Further, the accelerated stability studies for 6 months revealed that S-SNEDDS of Embelin are found to be stable without any significant change in physicochemical properties. Thus, the present studies demonstrated dissolution enhancement potential of porous carrier based S-SNEDDS for poorly water soluble herbal active ingredient, Embelin.

Keywords: Embelin, SNEDDS, Dissolution enhancement, Box-Behnken design, Characterization

1. Introduction

Embelin, (EMN) a benzoquinone found in Vidanga, is poorly water soluble drug which possess wide range of medicinal properties. Embelin exhibits antibacterial, antifertility and antioxidant properties [1, 2, 3]. EMN plays an important role in diabetes by counteracting high levels of glucose in the blood [4]. EMN demonstrates cytotoxic effect and inhibits cell proliferation in various cancer cell types [5]. However, the bioavailability of EMN is extremely erratic due to inherent poor aqueous solubility and dissolution properties [6]. With the objective of improving the poor solubility and dissolution properties of drug, advanced drug delivery systems are requisite.

Oral bioavailability of poorly water soluble drugs may be enhanced if formulated with lipids. One of the accepted approaches is the exploitation of lipid and surfactant based drug delivery systems (SNEDDS) [7]. SNEDDS are the isotropic mixtures of drug, lipid and surfactants, usually with one or more hydrophilic co-solvents or co-emulsifiers which upon mild agitation generates ultrafine droplets of oil in water nano emulsion [8]. The free energy required for self nano emulsification process is low. Thus, the process will occur spontaneously [9]. The enhanced interfacial area of micronized globules will facilitate the dissolution of drug thereby improving the bioavailability and enhance permeability through biological membranes due to presence of lipid and surfactant [10]. Therefore, solid SNEDDS is highly coveted due to its scalability and lustiness along with all advantages of liquid system.

Therefore, in light of this, the present investigation was carried out to improve dissolution characteristics of EMN by preparing SNEDDS. Box Behnken Design was applied to optimize SNEDDS containing EMN. Box Behnken Design is an independent, rotatable, or nearly rotatable quadratic design (contains no embedded factorial or fractional factorial design), in which the treatment points are the end of the edges of the process space and at the centre

[11]. Among all the Response surface methodology designs, Box-Behnken design requires fewer runs (13 runs) in a 3-factor experimental design [12]. Hence, the Box-Behnken Design was applied to optimize EMN-SNEDDS. Independent variables (factors) selected were amount of oil (Capryol 90) (*X1*), amount of surfactant (Acrysol EL 135) (*X2*) and amount of co-surfactant (PEG 400) (*X3*). The depended variables included globule size in nanometer (*Y1*) and self emulsification time in sec (*Y2*). To optimise EMN SNEDDS, mathematical model equations were derived by computer simulation programming Design Expert 8.0.5 software (State-Ease Inc. Mineapolis, USA). Physicochemical characterization was done by Fourier Transform Infrared Spectroscopy, Differential Scanning Calorimetry and Powder X-Ray Diffraction studies. To understand the morphology, diluted SNEDDS of EMN were subjected to Transmission Electron Microscopy studies.

2. Materials and Methods

2.1 Materials

EMN was a gift sample from BR Nahta College of Pharmacy, Mandsaur, India. Capryol 90, Labrafil and Paceol were gifted from Gattefosse, Mumbai, India. Oleic acid and Isopropyl myristate were purchased from Loba Chemie, Mumbai, India. Acrysol EL 135 and Acrysol K 140 were gift sample from Corel Pharma, Ahmedabad, India. Gelucire 44/14 was provided from Gatteffose India Pvt Ltd, Mumbai, India. Labrasol and Cremophore RH 40 were gifted from BASF, Mumbai, India. PEG 200, PEG 400 and Propylene glycol were purchased from Hi Media Labs, Mumbai, India. Aerosil 200 and Neusilin US2 were obtained from Gangwal Chemicals Ltd, Mumbai, India. All other ingredients used were of analytical grade.

2.2 Solubility studies

Solubility studies were conducted by adding an excess amount of EMN to 2 ml of oils, surfactants or co-surfactants. The mixtures were then swirled and sonicated for 30 min, respectively, followed by shaking at 37^{o}C for 48 h using Water Bath Shaker (Remi, Mumbai, India). The mixtures were kept at room temperature for 24 h and centrifuged using 12C-micro centrifuge (Remi, Mumbai, India) at 3000 rpm for 15 min. The separated supernatant fraction was suitably diluted with methanol and analysed for drug concentration spectrophotometrically at λmax 291 nm using UV-Visible spectrophotometer (Shimadzu 1800, Japan).

2.3 Construction of Ternary Phase diagram

Various mixtures with varying concentration of surfactant, co-surfactant and oil were prepared. Ternary diagrams of surfactant, co-surfactant and oil were plotted. The levels of surfactant, co-surfactant and oil were varied from 40 % to 90 % (w/w), 0% to 30 % (w/w) and 10 % to 60 % (w/w), respectively. All compositions were examined for nanoemulsion formation after diluting each of the mixtures 200 times with distilled water. Thereafter,

transmittance and globule size of the resulting dispersions were measured using UV-Visible Spectrophotometer (Shimadzu 1800, Japan) at 650 nm and Particle size analyser (Malvern Instruments, UK) respectively. Dispersions having globule size 200 nm or below were considered acceptable.

2.4 Formulation and Optimization of SNEDDS using Box-Behnken Design

Three factors, three level (3^3) and 13 runs Box-Behnken Experimental Design using Design Expert 8.0.5 software (State-Ease Inc. Mineapolis, USA) was employed to formulate liquid SNEDDS. The concentration of Capryol 90 (*X1*), Acrysol EL 135 (*X2*) and PEG 400 (*X3*) were selected as independent variables, while globule size in nanometer (*Y1*) and self emulsification time in sec (*Y2*) were selected as responses. Response surface analysis was carried out to study the effect of different independent variables on the observed responses. The independent and dependent variables are listed in Table 1. Weighed quantity of EMN (100 mg) was mixed with oil, surfactant and co-surfactant with continuous stirring to obtain a homogeneous mixture. The prepared liquid SNEDDS were stored in sealed transparent glass bottles at room temperature until used. The formulations were recorded for any changes in turbidity or phase separation.

2.5 Characterization of SNEDDS

2.5.1 Dispersibility studies

Self emulsification time was assessed by dispersibility studies. One ml of SNEDDS was added drop wise to 500 ml of 0.1N HCl with gentle agitation using USP Type II (paddle) dissolution apparatus rotating at 50 rpm at temperature 37 ± 0.5 °C. The process of self emulsification was visually monitored. Precipitation was assessed by visual monitoring of the resultant emulsion after 24 h storage at room temperature.

2.5.2 Globule size, size distribution and Zeta Potential

Malvern Zetasizer (Malvern Instruments, UK) was employed to determine globule size, polydispersity index and zeta potential. Liquid SNEDDS or Solid SNEDDS were diluted 1000 times with distilled water and shaken gently to form a fine emulsion and the resultant emulsion was utilised for the further study. The values of z-average diameters were used.

2.5.3 Comparative In Vitro Drug release studies

In vitro drug release studies of EMN drug loaded liquid SNEDDS, Solid-SNEDDS, Formulation based tablet and pure drug were carried out in USP type II dissolution apparatus (Model Disso 2000, Lab India) using 900 ml of phosphate buffer (pH 7.4) as dissolution media, at 50 rpm and temperature 37 ± 0.5 ^{o}C. Aliquots of 5 ml was withdrawn at suitable time interval (5, 10, 15, 20, 30 45, 60 and 90 min), filtered, appropriately diluted and analysed spectrophotometrically at λmax of 291 nm by UV/Visible spectrophotometer (UV-1800 Shimadzu, Japan).

2.5.4 Refractive index measurement

The optical clarity of the optimized liquid SNEDDS formulation was determined in terms of refractive index using Abbe's refractrometer (Mettler Toledo, Mumbai, India).

2.5.5 Transmission Electron Microscopy

For visual observation optimized batch of SNEDDS was subjected to transmission electron microscopy (TEM) (H-7000, Hitachi, Japan). Briefly, a drop of diluted SNEDDS was placed on a copper grid stained with 1% w/v phosphotungistic acid solution for 5 min at room temperature. The image was taken with transmission electron microscope at an accelerated voltage of 100 kV.

2.6 Preparation of Solid-SNEDDS

The optimised liquid SNEDDS formulation was transformed into solid free flowing granules using Neusilin US2 and Aerosil 200 as adsorbent material. Appropriate quantities of

adsorbent materials required to convert a given amount of liquid SNEDDS into an acceptably flowing and compressible system were determined. The solid formulations obtained were passed through sieve number 22 to achieve uniformly free flowing self nanoemulsifying granules (SNEG's). The final blend of optimized batch obtained was compressed into tablet using Rotary tablet compression machine (Hardik, Tablet Press, Ahmedabad, India); flat faced and die sized 9 mm punch were used. For comparison other tablets were prepared by directly compressing powder mixture of EMN with Neusilin US2, Aerosil 200 and sodium starch glycolate.

2.7 Characterization of Solid-SNEDDS

The prepared S-SNEDDS were evaluated for various micromeritic properties viz. Bulk density, tapped density, angle of repose, Carr's Index and Hausner's ratio. *In vitro* drug release studies of S-SNEDDS and the tablet prepared were determined using dissolution studies.

2.7.1 Drug content estimation

Liquid SNEDDS, S-SNEDDS and tablet containing EMN, each equivalent to 100 mg of drug was dispersed into appropriate quantity of methanol, stirred sufficiently to dissolve the drug, and centrifuged at 3000 rpm for 15 min. The supernatant was duly diluted and analysed spectrophotometrically at λmax of 291 nm by UV/visible spectrophotometer (UV-1800 Shimadzu, Japan).

2.7.2 Fourier Transformed Infrared Spectroscopy

Fourier Transformed Infrared Spectroscopy (FT-IR) of pure drug, optimized Solid-SNEDDS, Physical mixture and blank Solid-SNEDDS were carried out using KBr disc. The spectra were recorded using Fourier transform infrared spectrophotometer (Shimadzu 8400, Japan). Each KBr disc was scanned at 4 mm/s at a resolution of 2 cm over a wave number region of 4000–400 cm^{-1}. An average of 20 scans was taken.

2.7.3 Differential scanning calorimetry studies

Thermal analysis of final formulation of pure drug, optimized Solid-SNEDDS, Physical mixture and blank Solid-SNEDDS were carried out using Differential scanning calorimeter (DSC) (Shimadzu, DSC 60 TSW 60, Japan). Study was carried out between the range of 50–200ºC, at a scanning rate of 10°C/min. An empty pan was used as a reference.

2.7.4 Powder X-ray Diffraction Studies (PXRD)

To observe crystallographic pattern of pure drug, optimized Solid-SNEDDS, Physical mixture and blank Solid-SNEDDS were carried out using Powder X-ray diffractometer (Phillips X-Pert MPD, The Netherlands) using a voltage of 45 kV, generator current 40 mA, scan step time 9 sec^{-1} and scan step size of 0.008° (2θ). The scanning rate employed was maintained over the interval 1–50° $2\theta^{-1}$.

2.7.5 Accelerated stability studies

The optimised S-SNEDDS based tablets were stored at 40˚ ± 5˚C/75 ± 5% RH for 6 months. Samples were withdrawn at definite time interval (0, 1, 2, 3 and 6 months) and analysed for self emulsification time, globule size and drug release at 15 min.

3. Results and Discussion

3.1 Solubility studies

Comparative study of solubility of EMN in various liquids is given in the Table 2. Solubility was found to be highest in Capryol 90, Acrysol EL 135 and PEG 400 among the oils, surfactants and co-surfactants respectively. They were further utilized for the construction of ternary phase diagram.

3.2 Construction of Ternary Phase Diagrams

Fig. 1 depicts the phase diagram of the systems containing Capryol 90 as oil, Acrysol EL 135 as surfactant and PEG 400 as co-surfactant. The ternary phase diagrams were constructed to determine the concentration range of components for the formation of nanoemulsion. All the components were converted into weight/weight percent before construction of the phase diagram. The darker region in the phase diagram represents the self emulsification area. It was observed that the efficiency of emulsification was improved by the aid of surfactant-cosurfactant addition due to their higher hydrophilicity properties [13]. Stable systems were observed with concentration of oil upto 45% w/w in the system. It was observed that addition of co-surfactant, PEG 400 improved the self emulsification property of the system [14]. It was observed that spontaneous emulsion formation was not efficient with less than 45 % w/w of the surfactant in the system.

3.3 Optimization of SNEDDS

A Box-Behnken experimental design with 3 independent variables at 3 different levels was used to study the effect on dependent variables. A total of 13 formulations were prepared as per the experimental design and characterized further for responses like globule size and self emulsification time as shown in Table 3. For all the 13 batches dependent variables, globule size (*Y1*) and self emulsification time (*Y2*) demonstrated wide variations from 28.85 ± 1.82 to

99.35 ± 1.33 nm and 20.68 ± 2.49 to 113.38 ± 4.13 sec respectively indicating good influence of independent variables (*X1, X2* and *X3*) on the selected responses.

The relationship between the dependent and independent variables was further enlightened using response surface plot shown in Fig. 2. The mathematical relationships were established and coefficients of second order polynomial equation generated using multi linear regression analysis for globule size and self emulsification time. The equations were found to be quadratic in nature with interaction terms. The coefficients of the polynomials fitted well to the data, with the values of R^2, 0.9964 and 0.9978 for *Y1* and *Y2* respectively.

$$Y_o b_o + b_1X_1 + b_2X_2 + b_3X_3 + b_4X_1X_2 + b_5X_1X_3 + b_6X_2X_3 + b_7X_1^2X_{12} + b_8X_2^2X_{13} + b_9X_3^2X_{23}$$

Fig. 2a depicts an interaction effect between oil (Capryol 90) and surfactant (Acrysol EL 135) on the globule size as dependent variable. With increasing the amount of Capryol 90 and Acrysol EL 135, a linear increase in the globule size was observed. Fig. 2b shows the response surface plot, characterizing increase in the SEF with increase in the concentration of oil (Capryol 90) and co-surfactant (PEG 400). All the response surfaces were fitted with quadratic polynomial models. The ANOVA results are shown in Table 4. The F calculated value 4.16 and 3.38 is less than the table value 9.55 for *Y1* and *Y2*, respectively. Hence it is concluded that the omitted terms do not significantly contribute in predicting globule size and self emulsification time.

Using software optimisation process and response surface plots shown in Fig. 2c, level selected for *X1, X2* and *X3* were 49.50, 115.50 and 24.75 respectively, which gives theoretical values of 33.39 nm and 23.26 sec for globule size and self emulsification time respectively. Fresh formulation was prepared using the optimum levels of independent variables. The observed values of globule size and self emulsification time were found to be 30.15 ± 2.68 nm and 21.62 ± 1.19 sec, respectively, which were in close agreement with the theoretical values.

3.4 Characterization of SNEDDS

Table 3 summarised mean globule size and self emulsification time of prepared SNEDDS (E1-E13). The largest droplet size appeared in E6 of 99.35 ± 1.33 nm which could be due to presence of high amount of oil and lesser amount of surfactant-co-surfactant mixture [15]. All the liquid formulations showed good self emulsification efficiency forming nanoemulsion immediately after dilution. *In vitro* drug release study data as shown in Fig. 3, revealed dissolution enhancement with more than 80% drug release in initial 15 min while pure drug release was found to be 5.7 ± 0.30 % within 15 min. This suggested that dissolution enhancement of pure drug in formulation might be imputed to solubility enhancement property of selected excipients [16].

Zeta potential values of E1 to E13 as depicted in Table 5 were found in the range of -31.47 ± 1.76 mV to -26.02 ± 1.53 mV. Optimised formulation had zeta potential value of -28.69 ± 1.67 mV. Zeta potential values in the range of -25 mV to -30 mV in either charge signifies a stable formulation [17]. Polydispersity index is the measure of globule size homogeneity. Value closer to zero, more homogeneous are the particles. Refractive index (RI) values of SNEDDS were found to be in the range of 1.35 ± 0.03 to 1.57 ± 0.02. RI of optimized batch was found to be 1.38 ± 0.03. PI and RI values are shown in Table 5. TEM image of the optimized SNEDDS after dilution appeared as dark globules as depicted in Fig. 4.

3.5 Characterization of Solid-SNEDDS

The drug loaded S-SNEDDS were prepared choosing optimised batch as per the composition illustrated in Table 6. Tablets were prepared using 5% w/w Sodium starch glycolate as superdisintegrant. Results of micromeritic properties indicate good flow property of the formulation with values of angle of repose; 25.5, Carr's Index; 20.5 and Hausner's ratio; 1.15 . The *In-vitro* dissolution studies revealed faster drug release property with 99.60 ± 0.85 % of drug release within 15 min.

The tablet hardness was found to be 5 kg/cm^2, disintegration time less than 3 min and friability less than 1%.

3.5.1 Comparative In-vitro drug release studies

In vitro drug release studies of pure drug, optimised liquid SNEDDS, S-SNEDDS and prepared tablet were carried out using dissolution studies as shown in Fig. 5. The drug release demonstrated optimized liquid SNEDDS, S-SNEDDS and prepared tablet exhibited faster drug release within 15 min up to 99.60 ± 0.85 %, 97.80 ± 1.27 % and 96.45 ± 1.91 % respectively, in comparison to 5.7 ± 0.30 % of pure drug. The faster drug release from liquid SNEDDS might be attributed to spontaneous nanoemulsion formation [18]. Drug release from S-SNEDDS was slightly lower than liquid SNEDDS, it might be attributed to the presence of adsorbent materials while that for tablet, additional process of disintegration of tablet into granules and then desorption of liquid SNEDDS from excipients surface might be the reason.

3.5.2 Fourier Transformed Infrared (FT-IR) Spectroscopy

The FT-IR spectra, Fig. 6 (a) of drug showed bands at 3398 (-OH), 3001 (-CH) and 1776 (C=O) cm^{-1}. Drug loaded S-SNEDDS system Fig. 6 (b) showed no specific physicochemical interaction. It was observed that all important peaks due to functional group of drug were presented in physical mixture, Fig. 6 (c). There was no significant difference found in wave number (cm^{-1}) of the drug, broadening effect was observed [19].

3.5.3 Differential scanning calorimetry studies

Fig. 7 shows DSC thermograms of pure EMN (a), optimized S-SNEDDS (b), Physical mixture (c) and blank S-SNEDDS (d). The thermogram of EMN showed a single endothermic peak at 149 °C, whereas thermogram of S-SNEDDS showed dissappearance of characteristic peak of EMN, supporting molecular dispersion of drug in excipients and

conversion of crystalline form into amorphous form [20]. Physical mixture showed retention of characteristic peak of EMN.

3.5.4 Powder X-ray Diffraction Studies (PXRD)

The PXRD of EMN in Fig. 8 (a) showed sharp peaks at 13.3˚, 16.2˚, 19.0˚, 21.6˚, 23.5˚ and 25.3˚ demonstrating its crystalline nature whereas spectra of optimized S-SNEDDS Fig. 8 (b) showed disappearance of sharp peaks of EMN indicating conversion into amorphous form [21, 22]. Physical mixture, Fig. 8 (c) showed sharp peaks of ELN at 13.3˚, 19.0˚ and 21.6˚. Amorphous halo peaks were observed in the diffractogram of blank S-SNEDDS with a peak at 21.8˚ corresponding to peak of PEG 400.

3.5.5 Accelerated stability studies

Results of different parameters like self emulsification time, globule size and drug release of optimized S-SNEDDS based tablet were evaluated at specific intervals (0, 1, 2, 3 and 6 months) are depicted in Table 7. Results showed that there was no significant change in these parameters.

4. Conclusion

In the present investigation, SNEDDS formulation was proposed to enhance the dissolution properties of poorly water soluble herbal active ingredient, EMN. Liquid SNEDDS were transformed into S-SNEDDS by adsorbing onto adsorbents, Aerosil 200 and Neusilin US2. The drug release studies revealed higher and uniform release of EMN from the formulations. DSC and PXRD studies indicated incorporation of drug into SNEDDS components. EMN SNEDDS were found to be chemically and physically stable for 6 months. Thus, it can be inferred that physicochemically stable EMN SNEDDS have potential to enhance the dissolution properties of poorly water soluble drug, EMN.

Acknowledgement

The authors are thankful to Mr. Swapnil Goyal (Asst. Prof., BR Nahta College of Pharmacy, Mandsaur) for providing the gift sample of EMN.

Conflict of Interest

The authors declare that they have no competing interest.

References

1. Chitra M, Shyamala C, Sukumar E. Antibacterial activity of embelin. Fitoterapia 2003; 74: 401-403.
2. Radhakrishnan N, Alam M. Antifertility effects of embelin in albino rats. Indian J Exp Biol 1975; 13: 70-71.
3. Gupta R, Sharma A, Sharma M, et al. Antioxidant activity and protection of pancreatic β-cells by embelin in streptozotocin-induced diabetes. J Diabetes 2012; 4: 248-256.
4. Tripathi S. Screening of hypoglycemic action in certain indigenous drugs. J Res Ind Med 1979; 14: 159–169.
5. Joy B, Lakshmi S. Antiproliferative properties of embelia ribes. Open Proc Chem J 2010; 3: 17-22.
6. Pathan R, Bhandari U. Preparation and characterization of embelin-phospholipid complex as effective drug delivery tool. J Incl Phenom Macro Chem 2012; 69: 139-147.
7. Jun H, Dong H, Yu K, et al. Effects of solid carriers on the crystalline properties, dissolution and bioavailability of flurbiprofen in solid self-nanoemulsifying drug delivery system (solid SNEDDS). Eur J Pharm Biopharm 2012; 80: 289-297.

8. Nicholas C, Kenneth C, Ifeanyi T, et al. Self-Nanoemulsfiying drug delivery systems based on melon oil and its admixture with a homolipid from Bos indicus for the delivery of Indomethacin. Trop J Pharm Res 2011; 10: 299-307.
9. Porter C, Pouton C, Cuine J, et al. Enhancing intestinal drug solubilisation using lipid-based delivery systems, Adv Drug Deliv Rev 2008; 60: 673–691.
10. Wang L, Dong J. Design and optimization of a new self-nanoemulsifying drug delivery system, J Colloid Interface Sci 2009; 330: 443-448.
11. Box G, Behnken D. Some new three level designs for the study of quantitative variables. Technometrics 1960; 2: 455-475.
12. Nazzal S, Nutan M, Palamakula A, et al. Optimization of a self-nanoemulsifed tablet dosage form of ubiquinone using response surface methodology: effect of formulation ingredients. Int J Pharm 2002; 240: 103-114.
13. Rizwan M, Aqil M, Azeem A, et al. Enhanced transdermal delivery of carvedilol using nanoemulsion as a vehicle. J Exp Nanosci 2010; 5: 390-411.
14. Kommuru T, Gurley B, Khan M, et al. Self-emulsifying drug delivery systems (SEDDS) of coenzyme Q10: formulation development and bioavailability assessment. Int J Pharm 2001; 212: 233-246.
15. Gupta S, Chavhan S, Sawant K. Self-nanoemulsifying drug delivery system for adefovir dipivoxil: Design, characterization, *in vitro* and *ex vivo* evaluation. Colloids Surf A: Physicochem Eng Apects 2011; 392: 145-155
16. Beg S, Jena S, Patra C, et al. Development of solid self-nanoemulsifying granules (SSNEGs) of ondansetron hydrochloride with enhanced bioavailability potential. Colloids Surf B: Biointerfaces 2013; 101: 414-423.

17. Shakeel F, Haq N, El-Badry M, et al. Ultra fine super self- nanoemulsifying drug delivery system (SNEDDS) enhanced solubility and dissolution of indomethacin. J Mol Liq 2013; 180: 89-94.
18. Beg S, Swain S, Singh H, et al. Development, Optimization and Characterization of Solid Self-Nanoemulsifying Drug Delivery Systems of Valsartan using porous carriers. AAPS PharmSciTech 2012; 13: 1416-1427.
19. Patel M, Patel N, Patel R, et al. Formulation and evaluation of self-emulsifying drug delivery system of lovastatin. Asian J Pharm Sci 2010; 5: 266-275.
20. Shanmugam S, Baskaran R, Balakrishnan P, et al. Solid self nanoemulsifying drug delivery system (S-SNEDDS) containing phosphatidylcholine for enhanced bioavailability of highly lipophilic bioactive carotenoid lutein. Eur J Pharm Biopharm 2011; 70: 250-257.
21. Shrivastava A, Kapadia U. Design, optimization, preparation and evaluation of dispersion granules of valsartan and formulation into tablets. Curr Drug Delivery 2009; 6: 28-37.
22. Dixit R, Nagarsenkar M. Self nanoemulsifying granules of ezetimibe: design, optimization and evaluation. Eur J Pharm Sci 2008; 35: 183-192.

Figures and Tables Legends

LIST OF TABLES

Table 1 Factors investigated using Box-Behnken experimental design

	Levels (mg)		
Independent variables	Low	Medium	High
X1= Amount of oil (Capryol 90)	49.50	57.75	66.00
X2= Amount of Surfactant (Acrysol EL 135)	99.00	107.25	115.50
X3= Amount of Co-surfactant (PEG 400)	16.5	24.75	33.00

Table 2 Solubility studies of EMN in various solvents

Solvents		Solubility (mg/gm)
Oils	Capryol 90	399.41 ± 3.79
	Labrafil	77.78 ± 8.19
	Paceol	188.61 ± 4.49
	Oleic acid	77.91 ± 9.66
	Isopropyl myristate	140.93 ± 6.72
Surfactants	Acrysol EL 135	256.37 ± 1.31
	Acrysol K 140	242.88 ± 3.05
	Gelucire 44/14	101.20 ± 5.29
	Cremophore RH 40	202.77 ± 1.59
	Labrasol	37.86 ± 2.36
Co-surfactants	PEG 400	149.89 ± 1.19
	PEG 200	133.51 ± 2.35
	Propylene glycol	103.82 ± 2.17

Table 3 Box Behnken Experimental Design with measured responses

Batch no.	X1	X2	X3	Y1 (nm)	Y2 (sec)
E1	-1	-1	0	40.47 ± 2.59	27.86 ± 3.79
E2	1	-1	0	88.21 ± 2.95	113.38 ± 4.13
E3	-1	1	0	35.59 ± 3.59	22.56 ± 3.09
E4	1	1	0	71.35 ± 1.49	85.75 ± 2.11
E5	-1	0	-1	58.58 ± 2.88	39.78 ± 1.53
E6	1	0	-1	99.35 ± 1.33	127.43 ± 3.02
E7	-1	0	1	32.31 ± 1.32	20.68 ± 2.49
E8	1	0	1	80.45 ± 4.00	83.42 ± 2.85
E9	0	-1	-1	69.16 ± 1.51	75.38 ± 4.08
E10	0	1	-1	62.21 ± 0.77	70.46 ± 3.01
E11	0	-1	1	55.78 ± 1.57	58.49 ± 5.63
E12	0	1	1	28.85 ± 1.82	37.76 ± 4.28
E13	0	0	0	38.46 ± 1.08	52.23 ± 4.18

Table 4 Results of ANNOVA

Response Y1	Df (2,3)	SS	MS	F	P value	
Regression						Ftab = 9.55
FM	9	6127.33	680.81	91.23	0.0017	
RM	7	6065.24	866.46	51.28	0.0002	
Error						Fcal = 4.16
FM	3	22.39	7.46			
RM	5	84.48	16.90			
Response Y2	Df (2,3)	SS	MS	F	P value	
Regression						Ftab = 9.55
FM	9	13775.63	1530.63	153.95	0.0008	
RM	7	13708.49	1958.36	100.98	<0.0001	
Error						Fcal = 3.38
FM	3	29.83	9.94			
RM	5	96.97	19.39			

Table 5 Values of Polydispersity index, Refractive index and Zeta potential

Batches	PI	RI	Z (mV)
E1	0.17 ± 0.01	1.45 ± 0.03	-28.52 ± 1.88
E2	0.22 ± 0.02	1.36 ± 0.02	-30.07 ± 2.02
E3	0.21 ± 0.03	1.47 ± 0.01	-31.47 ± 1.76
E4	0.18 ± 0.03	1.35 ± 0.03	-28.21 ± 1.51
E5	0.17 ± 0.02	1.48 ± 0.03	-27.26 ± 2.58
E6	0.22 ± 0.02	1.52 ± 0.03	-29.04 ± 1.56
E7	0.19 ± 0.02	1.56 ± 0.04	-29.20 ± 1.59
E8	0.22 ± 0.03	1.38 ± 0.06	-29.50 ± 2.62
E9	0.23 ± 0.04	1.41 ± 0.04	-29.52 ± 1.39
E10	0.18 ± 0.02	1.48 ± 0.03	-26.02 ± 1.53
E11	0.16 ± 0.02	1.57 ± 0.02	-27.67 ± 1.41
E12	0.21 ± 0.01	1.47 ± 0.04	-27.05 ± 1.76
E13	0.15 ± 0.02	1.49 ± 0.05	-26.83 ± 0.92

Table 6 Composition of optimised batch

Components	mg/tablet	% weight (total weight 850 mg)
EMN	100.00	11.76
SNEDDS	195.68	23.02
Neusilin US2 (carrier)	437.39	51.46
Aerosil 200 (coating agent)	43.74	5.15
Sodium starch glycolate	42.50	5.00
Lactose monohydrate	30.68	3.61

Table 7 Characterization of optimised S-SNEDDS based tablet during accelerated stability studies at 40°C/75%RH

Sampling time (months)	Self emulsifying time (sec)	Globule size (nm)	% release of drug at 15 min
0	23.53 ± 1.38	30.37 ± 1.56	97.33 ± 0.89
1	23.22 ± 1.53	28.18 ± 1.18	97.99 ± 1.60
2	22.84 ± 0.58	29.48 ± 2.02	96.97 ± 1.71
3	23.19 ± 1.18	30.63 ± 0.59	96.43 ± 1.13
6	22.98 ± 1.08	30.22 ± 1.26	97.06 ± 0.91

List of figures

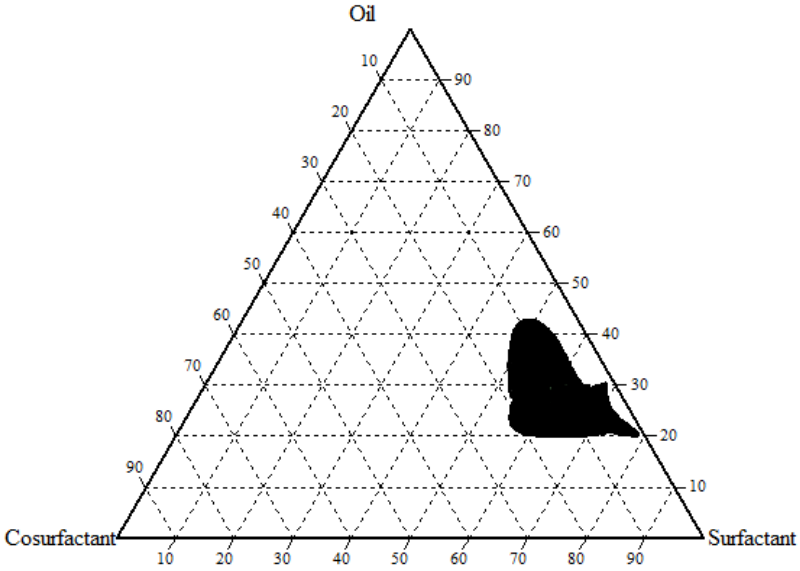
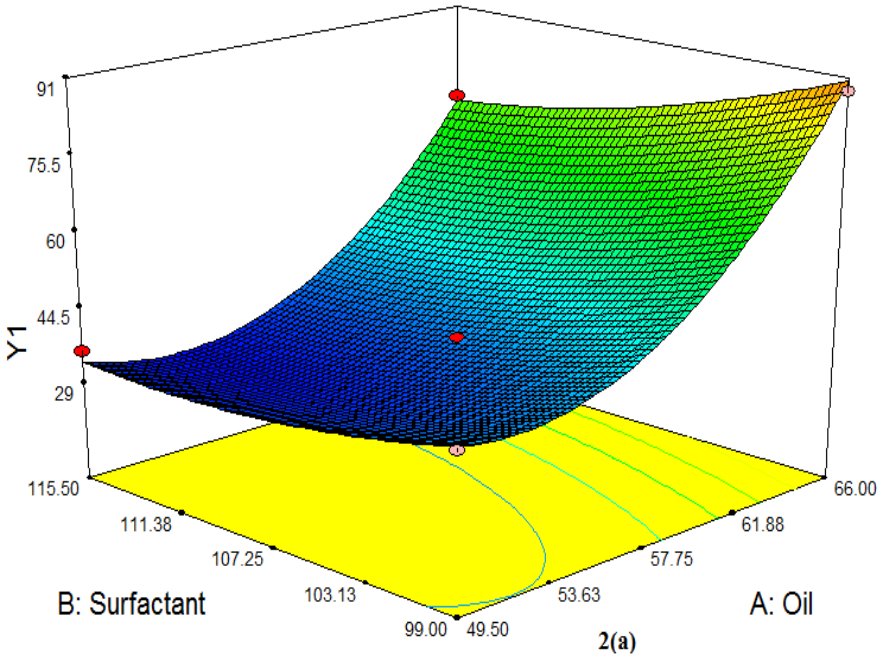
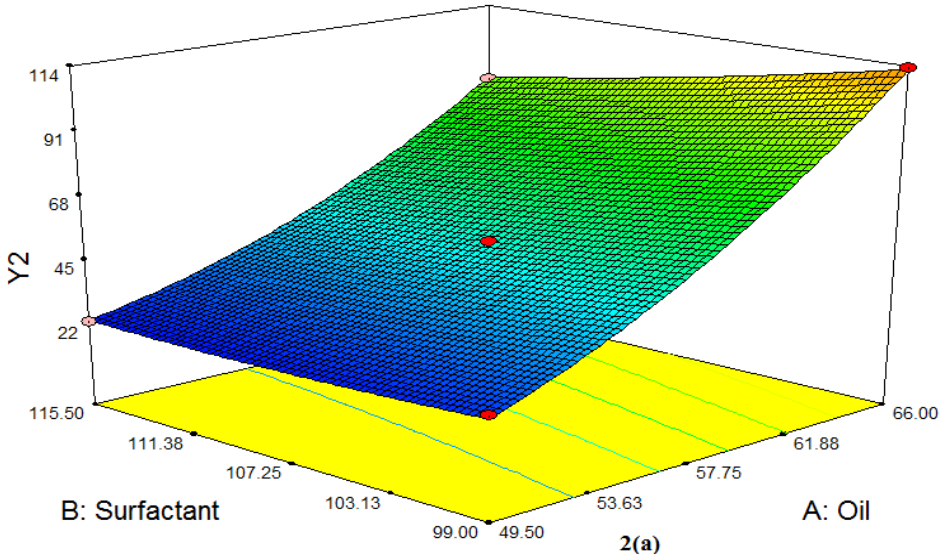


Fig. 1 Ternary phase diagram of Capryol 90, Acrysol EL 135 and PEG 400


91
75.5
60
44.5
29
Y1
115.50
111.38
107.25
103.13
99.00
B: Surfactant
49.50
53.63
57.75
61.88
66.00
A: Oil

2(a)


114
91
68
45
22
Y2
115.50
111.38
107.25
103.13
99.00
B: Surfactant
49.50
53.63
57.75
61.88
66.00
A: Oil

2(a)

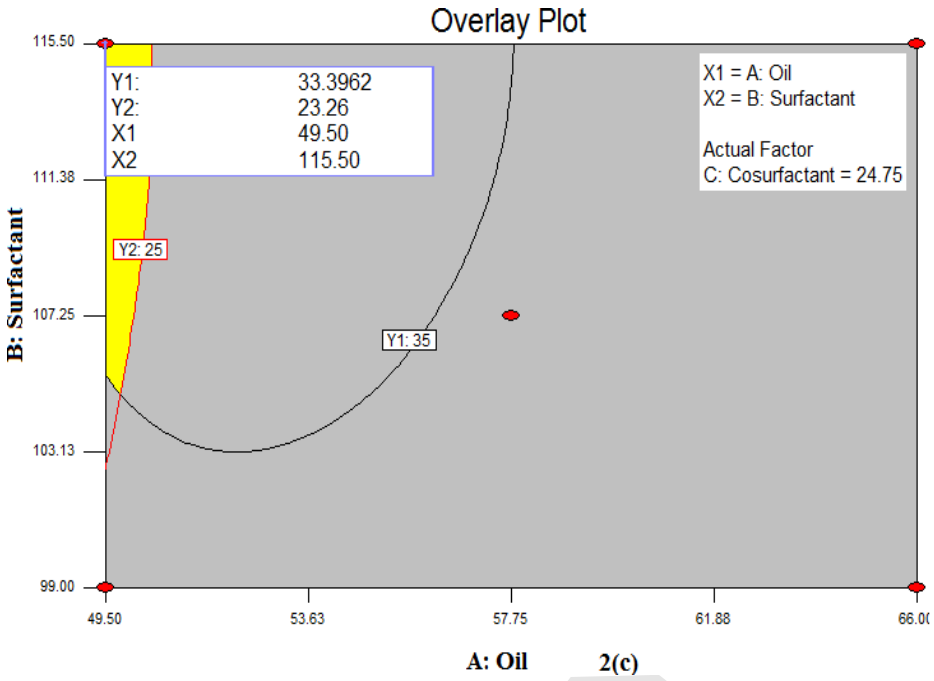


Fig. 2 Response surface plot (a) interaction between X1 and X2 onY1 (b) interaction between X1 and X3 onY2 (c) Overlay plot

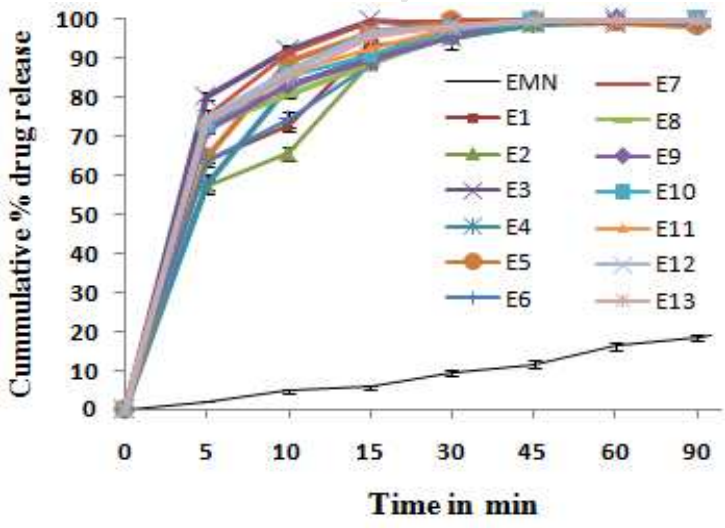


Fig. 3 In-vitro release study of EMN and EMN loaded SNEDDS (E1-E13)

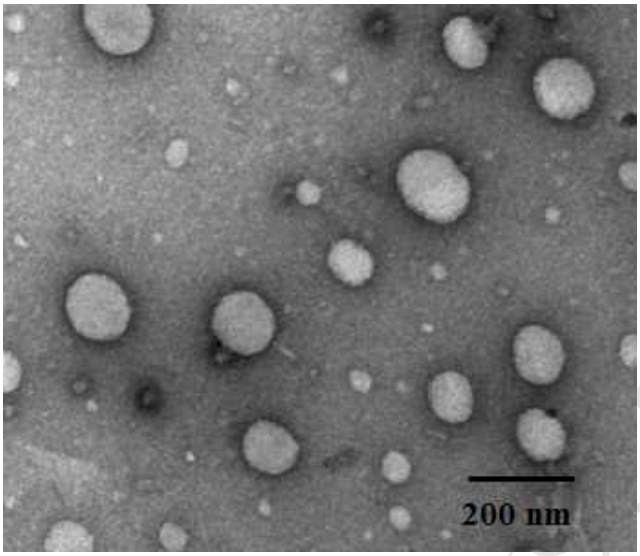


Fig. 4 Transmission electron microscopy of optimized EMN-SNEDDS

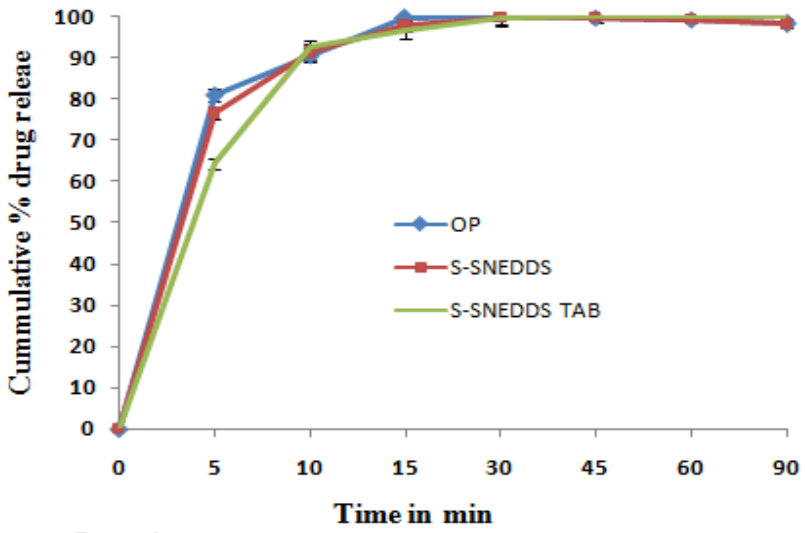


Fig. 5 In-vitro drug release study of Optimized Liquid SNEDDS, S-SNEDDS and S-SNEDDS Tablet

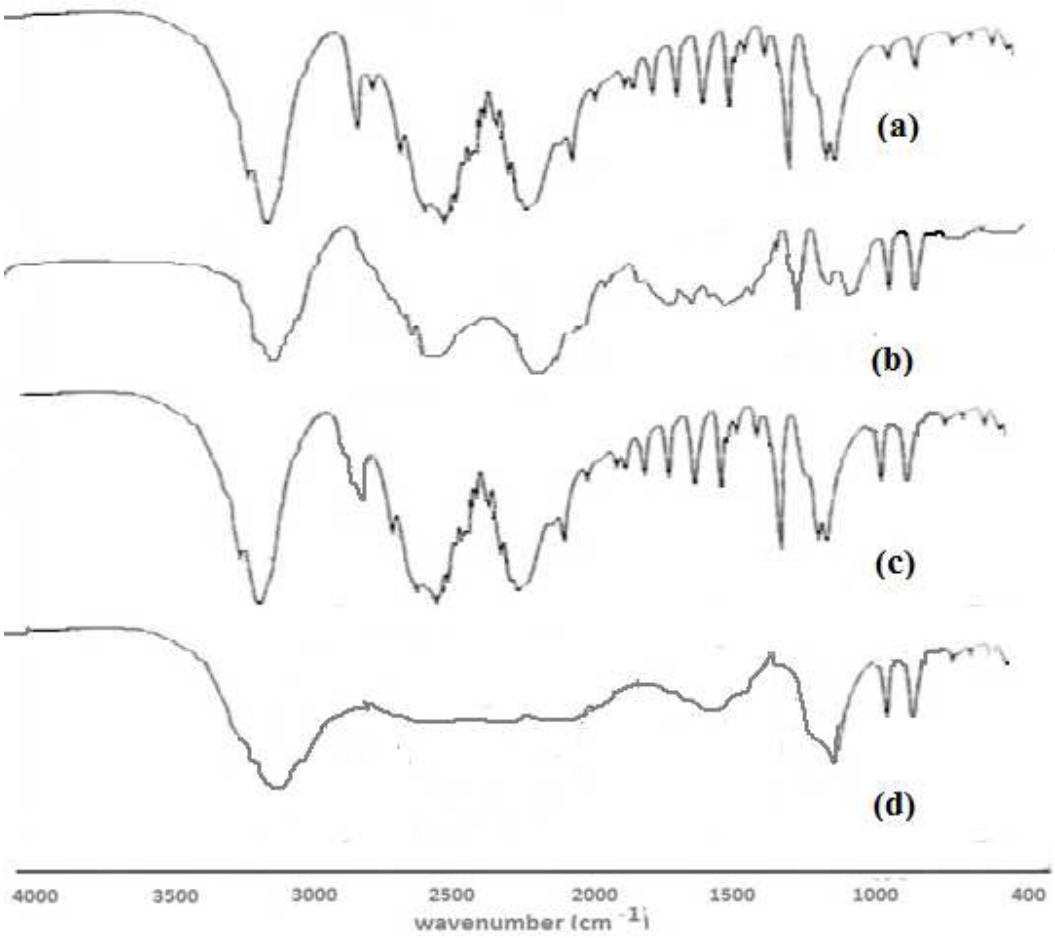


Fig. 6 FT-IR spectra of (a) EMN and (b) Optimized S-SNEDDS (c) Physical mixture (d) Blank S-SNEDDS

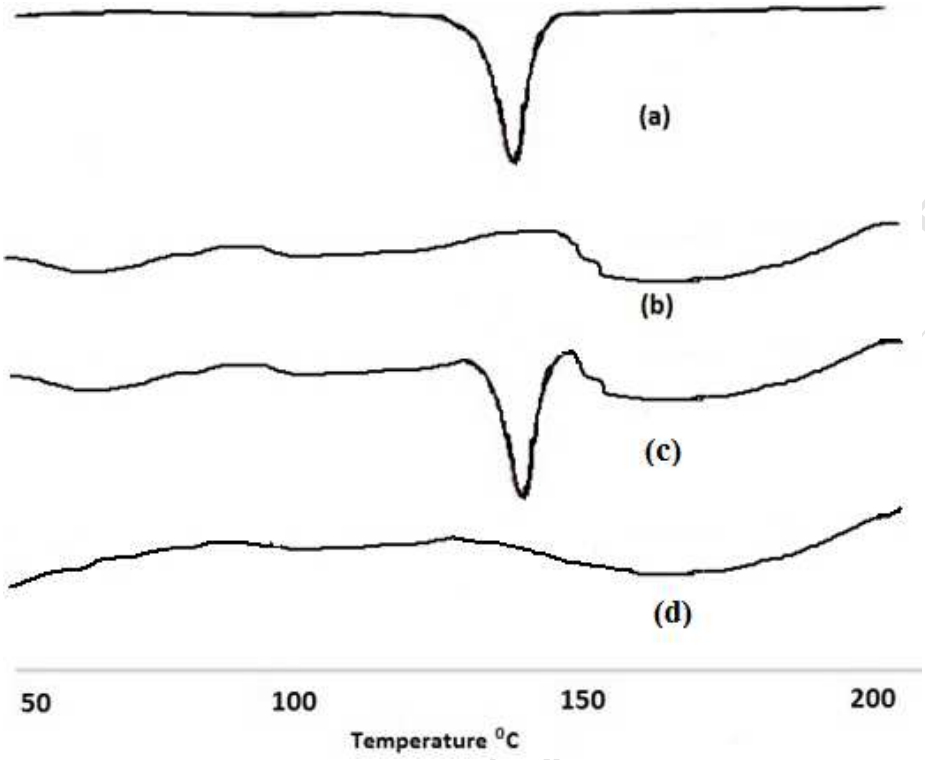


Fig. 7 DSC spectra of (a) EMN and (b) Optimized S-SNEDDS (c) Physical mixture (d) Blank S-SNEDDS

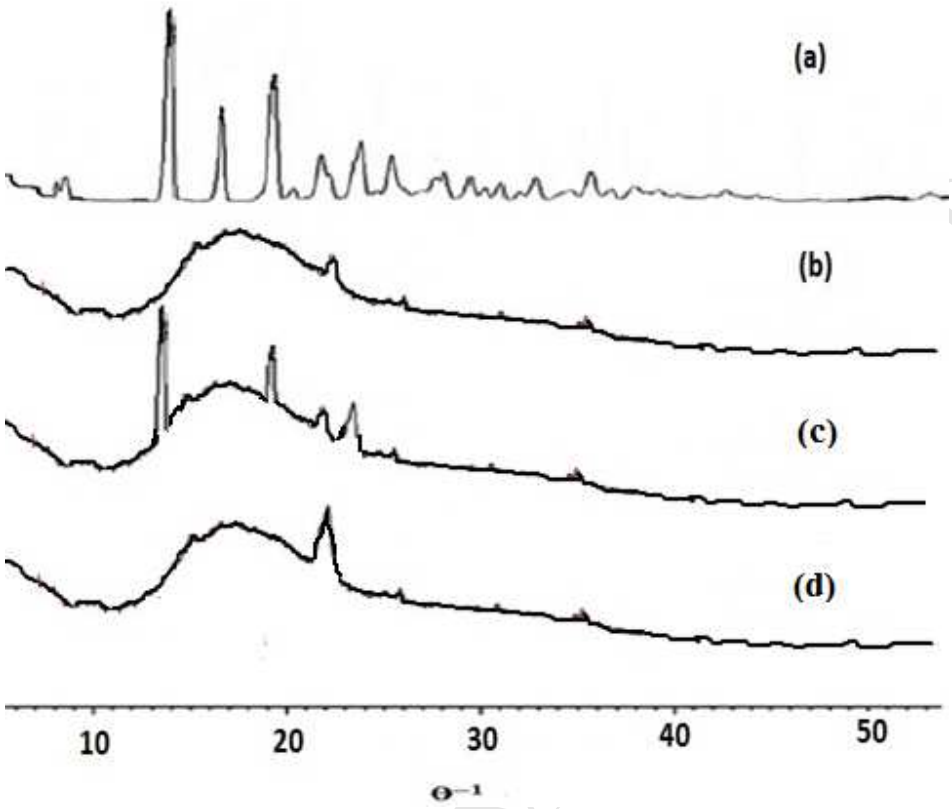


Fig. 8 PXRD spectra of (a) EMN and (b) Optimized S-SNEDDS (c) Physical mixture (d) Blank S-SNEDDS