DEVELOPMENT AND EVALUATION OF SELF EMULSIFYING DRUG DELIVERY SYSTEM OF AMLODIPINE

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ABSTRACT

Objective: The present study involves the development of SNEDDS employing essential oils for enhancing biopharmaceutical performance.

Methods: Preliminary investigations suggested the selection of cinnamon oil as an essential oil, tween 60 as a surfactant, while transcutol HP as a cosolvent for formulating SNEDDS. Formulations evaluated for stability, robustness to dilution, and emulsification time, droplet size, zeta potential (ζ), cloud point, *in vitro* drug release, drug excipient compatibility, TEM, stability assessment and *in vivo* pharmacokinetic performance in rats.

Results: All formulations were robust, stable, and revealed excellent emulsification time<40 s, with fine droplet size (11.41±2.41 nm), lower PDI (0.028-0.277

Conclusion: The study concluded that SNEDDS would be an effective formulation system in increasing the aqueous solubility and potentially bioavailability. Furthermore, it can be applied for other therapeutic categories of drugs belonging to BCS class II and IV that show comparable biopharmaceutical challenges.

Keywords: Amlodipine, essential oils, Bioavailability enhancement, Self-nano emulsifying systems, Pharmacokinetic study

INTRODUCTION

Amlodipine (AML) is a Biopharmaceutical Classification System (BCS) class II, belonging to a therapeutic category of anti-hypertensive [1]. Due to its susceptibility towards hepatic metabolism and lipophilicity, AML has poor oral bioavailability. Palpably, the oral route is preferred by its potential physiological aids, besides patient compliance. Various formulation systems were developed for enhancing AML oral bioavailability, including microparticles, solid dispersions, and nanoparticles [2, 3].

In recent times, oral lipid-based formulation systems have gained much significance in enhancing the solubility, followed by oral bioavailability of lipophilic molecules [4]. Lipid-based systems vary from simple to complex isotropic mixtures of oils, surfactants, and co-surfactant/cosolvents [5]. Apart from enhancing solubility, these systems also tend to improve absorption by several auxiliary mechanisms like inhibition of efflux transport mediated by P-glycoprotein, promoting lymphatic transport surpassing the first-pass metabolism and enhancing membrane permeability [6, 7].

Self-nano-emulsifying drug delivery system (SNEDDS) is one of the most effective approaches amongst lipid-based systems for enhancing solubility and the bioavailability of lipophilic molecules [8]. SNEDDS is a simple isotropic blend of oil, surfactant, and co-surfactant/cosolvent and drug,

MATERIALS AND METHODS

Materials

AML was a generous gift from Aurobindo Pharma Ltd., (Hyderabad, India). Caproyl , Transcutol , Labrafil M 1944 , were kindly gifted by Gattefosse India Pvt Ltd (Iraq). Clove oil, Polyethylene glycol-200 Molychem (Iraq), Anise Oil, Cinnamon Oil, Orange Oil, Lemon Oil Genuine Chemicals Co. (Iraq), Peppermint Oil Yuuca Enterprises (Iraq), Tween–60 Sisco Research Laboratory (Iraq). Dialysis membrane (MWCO 12 to 14 KD) procured from Himedia (Iraq). All other reagents and chemicals were stringently used of analytical grade and used as received.

Methods

Saturation solubility study

The saturation solubility of AML has been carried out in several oils, surfactants and co-surfactant/co-solvents using a water bath shaker [10, 11]. AML was added in an excess amount to each of closed glass vials containing about 2 ml of each vehicle. The attained mixtures were initially vortexed for 5 min to facilitate solubilization. The blends were placed in a controlled temperature of 37±0.5 ℃℃ in a water bath shaker (REMI, India) for a period of 72 h to obtain equilibrium. Then, the samples were subjected to centrifugation at 4500 rpm for 20 min to separate the undissolved drug. The supernatant was filtered through 0.45 µm (Whatman, USA) membrane filter. All filtrates were suitably diluted with methanol and analyzed spectrophotometrically at λ_{max} 238 nm by UV-vis spectrophotometer (Shimadzu, Japan).

Pseudo-ternary phase diagram study

From the results of the saturation solubility, miscibility, and emulsification efficacy, cinnamon oil as oil phase, tween 60 as surfactant and transcutol HP as co-solvent selected for SNEDDS formulation. The phase diagrams of oil, surfactant–co-solvent mixture (S_{mix}) and double distilled water plotted using a water titration method. The ratio of surfactant to co-surfactant varied from 1:3 to 3:1. For each pseudo ternary phase diagram at a specific surfactant/cosurfactant weight ratio, the blends of oil, surfactant and cosurfactant prepared

with the ratio of oil to the bends of surfactant and cosurfactant at 9:1 to 1:9 [12, 13]. All the ratios studied for visual clarity and spontaneity of emulsification. The blends considered to be good when a clear and transparent emulsion was formed spontaneously without any phase separation on standing [14]. In order to optimize the concentration selection of the nanoemulsion region form the phase diagrams was since solution remain clear regardless of infinite dilution. The phase diagrams plotted using ProSim ternary diagram software to detect nano emulsifying region.

Preparation of AML loaded SNEDDS

AML is most frequently available in the dose of 10 mg, AML loaded SNEDDS prepared in the doses of 10 mg. The preparation of SNEDDS achieved with simple blending where the AML was dissolved in an isotropic mixture of lipid, surfactant and cosolvent with vortex shaker for 5 min, to acquire a homogeneous mixture. A total of ten formulations with 10 mg of AML prepared to scrutinize through spontaneous emulsification method, which was further characterized for kinetic stability, visual observation, robustness to dilution, emulsification, droplet size, zeta potential (ζ), cloud point, *in vitro* drug release, drug excipient compatibility, TEM, stability assessment and *in vivo* pharmacokinetic performance in rats. The composition of the AML loaded SNEDDS is exhibited in (table 1).

Table 1: Formulation composition of AML loaded SNEDDS (%V/V)

Formulation code	Components (% V/V)		
	Cinnamon Oil	Tween 60	Transcutol HP
F(AML)1	7.5	69.4	23.1
F(AML)2	10	67.5	22.5
F(AML)3	12.5	65.5	22
F(AML)4	15	63.75	21.25
F(AML)5	17.5	61.9	20.6
F(AML)6	20	60	20
F(AML)7	22.5	58.14	19.36
F(AML)8	25	56.25	18.75
F(AML)9	27.5	54.38	18.12
F(AML)10	30	52.5	17.5

Kinetic stability studies

Nanoemulsions are said to be kinetically stable systems. The chosen ten formulations were imperilled to kinetic stability by employing heating and cooling cycle and centrifugation tests. Formulations which were found kinetically stable were selected for further evaluation [15].

Heating–cooling cycle

The cycles involved six cycles were the formulations were kept at refrigerator temperature, 5 and 45 for 48 h for each cycle. The formulations scrutinized for drug precipitation and phase separation. Further, all formulations diluted with distilled water (1:50), and resulting nanoemulsions examined for instability glitches — those formulations which are stable at these temperatures further subjected to centrifugation test.

Centrifugation test

In this assessment, all formulations were diluted with distilled water (1:50) and subjected to centrifugation at 3500 rpm for 30 min. Post centrifugation, the formulations were screened for instability like phase separation, creaming or cake formation. Such unstable formulations excluded from further studies, and stable formulations carried out for further parameters.

Robustness to dilution

This study involved the assessment of formulations for physical changes upon diluting by 50, 100-and 1000-fold with distilled water, 0.1 N HCl and phosphate buffer (pH 6.8), respectively. Post dilution, the visual aspect was judged after 2 h. Furthermore, the resultant

nanoemulsions were kept for 24 h and observed for any physical changes like phase separation, cloudiness or precipitation. All the formulations which yield clear nanoemulsions at different dilution pleats in examined media were said to pass this test [16].

Self-emulsification efficiency

The rate of emulsification (self-emulsification time) has been defined as the efficiency of the formulations to disperse upon aqueous dilution conferring to the method described by [17]. About 1 ml of each formulation was added dropwise to 250 ml of phosphate buffer (pH 6.8) in USP dissolution apparatus II (Electrolab TDT 06L). The paddle speed was adjusted to 50 rpm to facilitate mild agitation with the temperature-maintained 37±5 ℃ — the self-emulsification time given by the time required for the preconcentrate to yield homogeneous dispersion. Formulations passing the test carried out for further studies.

Characterization of the SNEDDS formulations

Based on the results of prior studies, six of the formulations (F(AML)1, F(AML)2, F(AML)3, F(AML)4, F(AML)5 and F(AML)6) were selected for further characterization.

Cloud point measurement

Each formulation was diluted with distilled water at a ratio of 1:50 and placed in a water bath with a slow rise in temperature (5 ℃/min). Cloud point was measured as the temperature at which the first sign of turbidity was visually observed [18].

Determination of zeta potential (ζ); mean droplet size and polydispersity index

Zeta potential means droplet size and polydispersity index of nanoemulsions were determined post-100-fold dilution of selected formulations with distilled water were determined by dynamic light scattering (DLS) technique using Malvern Zetasizer Nano (Malvern Instruments, UK).

***In vitro* drug release study**

This study involved the use of the dialysis bag method (Dialysis membrane MWCO 12 to 14 KD, Himedia., India) with some modifications [19, 20]. The drug release was estimated by diluting from each formulation (equivalent to 5 mg AML) with phosphate buffer (pH 6.8), then was loaded into the pre-soaked membrane and clamped on both sides. The release medium (500 ml of phosphate buffer pH 6.8) was added to the apparatus. The secured membrane was tied to the paddle of the apparatus USP dissolution apparatus II (Electrolab TDT 06L) and allowed to rotate freely. The experiment was thermostatically controlled at 37±0.5 ℃±0.5 ℃, and stirred 50 rpm. At predetermined time intervals, 5 ml of sample was withdrawn from the medium and immediately replenished with the same volume of fresh medium. All the samples were analysed spectro-photometrically with necessary dilutions at λ_{max} 238 nm. The release patterns compared to that of pure drug suspension (with an equivalent drug concentration of test formulations).

Thermal analysis

The endothermic melting temperature for pure AML powder, AML loaded SNEDDS was determined using DSC (DSC-TA instruments USA Q10). The pure AML and AML loaded SNEDDS were respectively packed in an aluminium pan and the thermogram was recorded with a rate of 10 ℃℃/min, over a temperature range of 50-300 ℃℃ under a nitrogen atmosphere using the empty pan as the reference.

Fourier-transform infrared (FTIR) spectroscopy

The probable interactions between the drug and employed excipients were studied by FTIR spectroscopy. The IR spectra of pure AML powder and AML loaded SNEDDS were carried out using Bruker Vertex 70 FT-IR spectrometer (Bruker, USA). The samples were prepared by KBr pellet method. The spectrum was recorded from regions of 4000 cm^{-1} to 400 cm^{-1} [21].

Transmission electron microscopy (TEM)

In this investigation, the morphology of AML loaded SNEDDS studied using transmission electron microscopy after a 1000-fold dilution with distilled water using JEM 2100 (JOEL, Japan). On a copper grid, one drop of the sample was deposited and allowed to stand for 5 min. The excess was removed with a blotting paper. The prepared grid was latter negatively stained with a drop of phosphotungstic acid (1% w/v) for 30 s. The grid was air-dried and then was observed by TEM.

***In vivo* study**

The pharmacokinetic study was carried out in male Wistar rats weighing 200-250 g.. All the rats were clinically healthy during the entire study and strictly housed under standard conditions of temperature and humidity. The *in vivo* bioavailability studies for both AML-suspension and F (AML)6 carried out in two groups (n=6). Food was withdrawn 12 h prior to the study water was provided ad libitum and no food was allowed post-dosing until the end of the study (after 8 h). Each of the animals received a single dose (1 mg/kg) of AML-suspension as a pure drug with a group I and the SNEDDS formulation F (AML) 6 with group II orally with the help of oral feeding needle. Blood samples (0.5 ml) were collected at predetermined intervals of predose 0 hr and 0.5, 1, 2, 3, 4, 6, and 8 hr post oral dose administration in pre-coated K2EDTA tubes. The samples were centrifuged at 4500 rpm for 20 min, and the separated plasma samples stored at refrigerated conditions (2-4 ℃) until analysis. AML concentrations in plasma samples were determined suitably by HPLC method [22].

RESULTS AND DISCUSSION

Saturation solubility study

The pertinent solubility of a drug substance in the excipients is categorically crucial for developing a successful formulation besides avoiding drug precipitation during *in situ* self-emulsification in the gut region. The (fig. 1) exemplifies the saturation solubility data of AML in various essential oils, surfactants and co-solvents. Among various essential oils employed, the highest solubility was observed in cinnamon oil (i.e. 281±1.07 mg/ml). Similarly, among surfactants and co-solvents employed tween 60 (152±1.22 mg/ml) and transcutol HP (i.e. 363±1.29 mg/ml) exhibited the highest solubilization capacity and spontaneity of the emulsification ability. As a result of low toxicity and higher stability to the effect of pH and ionic strength when compared to ionic and amphiphilic surfactants, nonionic surfactants are typically preferred [20]. In addition, the higher solubility of the drug in nonionic surfactants can be described as a result of higher HLB value, which enables faster solubilization of lipophilic drugs and imparts spontaneous emulsification ability [23].

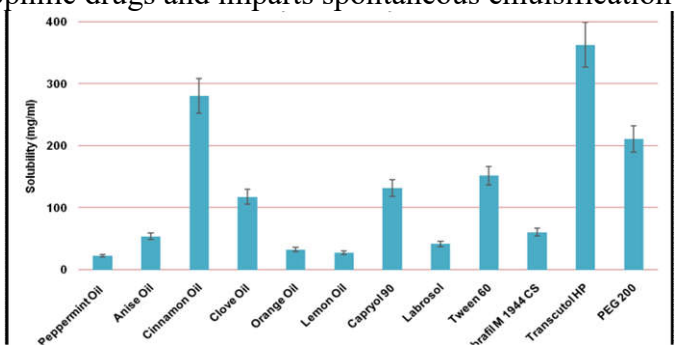

Fig. 1: Saturation solubility of AML in different vehicles (mean±SD (N=3)) Pseudo ternary phase diagram study

To identify the self-emulsifying regions, pseudo-ternary plots were constructed. Clear isotropic regions determine the self-emulsifying regions which were recognized by optical observation. Besides, it also helps in defining the appropriate excipient ratios for the

development of formulations that guarantee the spontaneity of *in situ* self-emulsification in the gut region [24].

Fig. 2(A-E) demonstrates the pseudo-ternary diagrams constructed for demarcating the suitable nanoemulsion region for AML loaded SNEDDS. The shaded region specifies the self-emulsifying region, and the higher area indicated for cinnamon oil and S_{mix} ration of 3:1 containing tween 60–transcutol HP. As it is understood, that all surfactants significantly irritate and are poorly tolerated, hence large magnitudes of surfactants may lead to irritation in the GI tract, and therefore ideally emulsifying systems with more substantial portions of essential oils are selected [25].

These mixtures were identified within the nanoemulsion region of studied pseudo-ternary plots, and the compositions of the ten formulations loaded with AML is shown in (table 1).

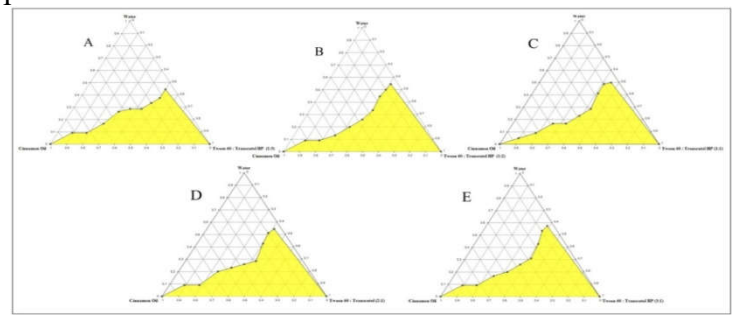

Fig. 2: Phase behaviour of nanoemulsion formed from cinnamon oil with Tween 60: Transcutol HP and water varying Tween 80: Transcutol HP ratio as (A) 1:3; (B) 1:2; (C) 1:1; (D) 2:1; (E) 3:1

Kinetic stability

As the SNEDDS forms nanoemulsion spontaneously, the formulation should possess the ability to withstand stability against creaming, precipitation and caking. The AML loaded systems portrayed excellent stability. All dispersions were clear and retained their visual facet during the study signifying their nanoemulsion nature. Accordingly, the study has not revealed any distress to the applied conditions and confirmed the stability of the prepared formulations.

Robustness to dilution

Robustness to dilution helps to understand the drug precipitation at higher dilutions, as such precipitations alter and reduce the *in vivo* fate of drug absorption. All the prepared formulations were exposed to different folds of dilutions in various media (distilled water, pH 6.8 and pH 1.2) to mimic the *in vivo* conditions and to ensure the formation of a uniform formulation. Almost all formulations exhibited to be robust to the dilutions except four formulations (F(AML) to F(AML) showed immediate milky dispersions. The higher concentrations of oil components could lead to loss of clarity. Formulations (F(AML)1 to F(AML), were determined to retain their clarity at different dilution volumes of various media thus confirming their robustness.

Self-emulsification efficiency

Post-oral administration the formulations are exposed to the *in-situ* gut conditions where the formulations experience aqueous dilution, thereby the preparations should immediately disperse under mild agitation provided by the peristaltic agitations to yield a fine nanoemulsion. Therefore, self-emulsification time is a significant assessment parameter for self-emulsification efficiency. Amid the developed formulations, six formulations (F(AML) to F(AML) were determined to be of grade A regardless of the dispersion media.

Irrespective of the dispersing medium, all formulations portrayed a short emulsification time of<40 (s) (table 2), confirming their high emulsification efficiency. Formulations with higher concentrations of essential oil required a longer time to get completely dispersed.

Subsequently, formulations exhibited increased spontaneity for nanoemulsion formation as the concentration of S_{mix} increases in the formulations.

Table 2: Self-emulsification time for selected formulations in different dispersing media

Formulation code	Self-emulsification time (s)*		
	Phosphate buffer (pH 6.8)	Distilled water	0.1N HCl (pH 1.2)
F(AML)1	8	9	11
F(AML)2	12	11	13
F(AML)3	16	15	18
F(AML)4	19	21	22
F(AML)5	24	25	28
F(AML)6	33	29	35

*Data expressed as mean of 3

Characterization of the SNEDDS formulations

Along with robustness to dilution those formulations that exhibited good kinetic stability and showed high emulsification efficiency with excellent visual grade post dilutions, namely (F(AML)1 to F(AML)6 carried for further characterization.

Cloud point measurement

The temperature above which the dispersion turns turbid is said to be cloud point, and this is due to dehydration of polyethylene oxide chains of the non-ionic surfactants leading to lowered HLB values. For the selected formulations, cloud points were (table 3) very high; hence were determined to be competent for body temperature, therefore, circumventing phase separation in the GIT. Higher cloud point temperature could be attributed to the solubility of the drug in the formulation components, with an optimized ratio of S_{mix} along with higher HLB values.

Determination of zeta potential (ζ); mean droplet size and polydispersity index

The droplet size and the interfacial area are inversely proportional; therefore, the smaller droplet size results in better diffusion and absorption owing to the increased interfacial area. Accordingly, the droplet size distribution of nanoemulsions post-self-emulsification becomes significant is assessing the *in vivo* performance of the formulation [28].

The mean droplet size of the formulations (table 3) from the study confirmed that the formulations showed droplet size in the nanometric range (fig. 3). All formulations exhibited minor PDI values indicating excellent uniformity, S_{mix} may act synergistically to lower the interfacial tension, consequently yield fine nanoemulsion. As a result of the ultrafine emulsion system, which imparts a robustness to flocculation or towards coalescence, making the system kinetically stable. The kinetic stability becomes significant in long term physical stability.

Table 3: Cloud point, droplet size, zeta potential, and PDI for selected formulations

Formulation code	Cloud point (□)	Droplet size (nm)	Zeta potential (mV)	PDI
F(AML)1	89	9.680±1.72	-3.65±1.97	0.028
F(AML)2	86	9.917±2.12	-3.68±1.49	0.067
F(AML)3	82	10.17±2.62	-5.90±1.66	0.129
F(AML)4	80	10.39±1.69	-4.84±2.32	0.182
F(AML)5	75	11.10±1.33	-6.87±1.52	0.232
F(AML)6	73	11.41±2.41	-7.22±2.81	0.277

*Data expressed as (mean±SD (N=3))

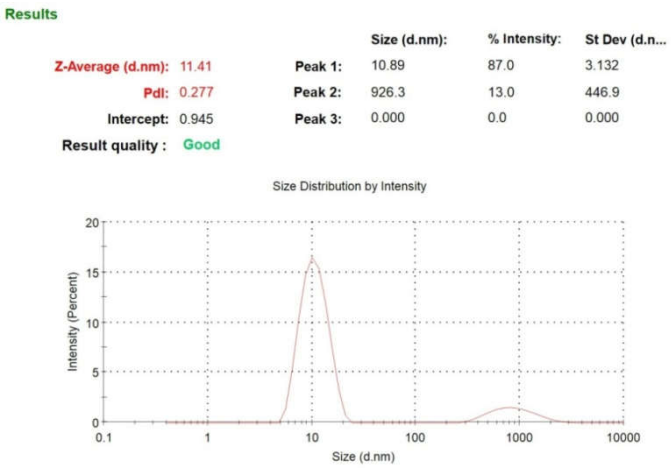

Fig. 3: Droplet size distribution of AML-loaded nanoemulsion F(AML)

Fig. 4: Zeta potential (ζ) mV of AML-loaded nano-emulsion F(AML)

The mean droplet size for formulations varied insignificantly compared to all formulations F(AML) and F(AML), exhibited slightly larger mean droplet size when compared to other formulations. Majorly due to higher essential oil concentration compared to other formulations.

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***In vitro* release study**

Only when the free drug molecules get released from the entrapped droplets of nanoemulsion system, evaluation can be achieved. Thus, the formulation system cannot be assessed by the conventional release approach. Therefore, the dialysis bag method adapted for the study . The comparative study of release profiles exhibited definite improvement in the drug release rate from the formulations when compared to the pure drug suspension. Release patterns of the formulations along with AML suspension, are shown in (fig. 5). The higher release from the formulation system may be due to the higher availability of dissolved AML from the droplets that provides a larger surface area for its release.

Drug release from F(AML) was significantly higher compared to other formulations owing to entrapment of free drug in the lipid, followed by spontaneous emulsification enabled by emulsifying agents. An almost complete release of 97.7% was achieved by the best formulation F(AML)6 within 4 h, whereas only 12% of drug release was achieved from a drug suspension. The results significantly endorse marked improvement of AML solubilization with full potential for enhancing the oral bioavailability.

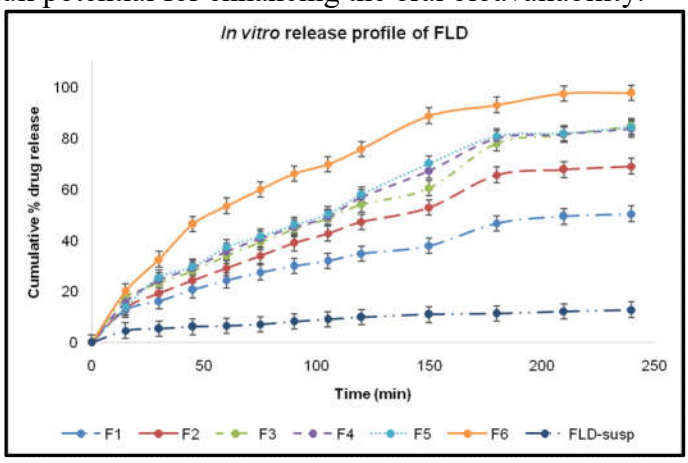

Fig. 4: *In vitro* (%) cumulative drug release profile of AML loaded SNEDDS and AML-suspension in phosphate buffer 6.8 (mean±SD (N=3))

Thermal analysis

The DSC thermograms of pure drug sample and the AML loaded-SNEDDS formulations shown in (fig.4a and 4b). Pure drug powder exhibited a sharp endothermic peak at 145.36 °C, with almost concurrent to the melting point confirming its purity, suggesting the crystallinity of drugs. Concerning to the formulation mixture, exhibited broad endothermic peak and drifted little ahead by approximately 15 °C, maybe after AML blended with the lipid excipients, it is likely that AML was in an amorphous, molecularly dispersed state in the lipid of the formulation.

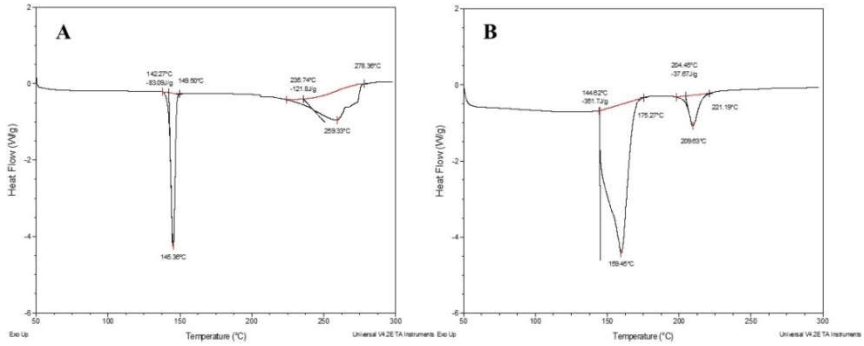

Fig. 4: (a) DSC thermogram of AML pure drug, (b) DSC thermogram of AML loaded nano-formulation mixture

Fourier-transform infrared (FTIR) spectroscopy

The FTIR spectra for AML and its SNEDDS are shown in (fig. 7a and 7b). FTIR spectra of AML exhibited characteristic peaks of C-H Strat 3523.69 cm^{-1}, Aromatic-Str at 3071.21 cm^{-1}, C=O (carboxylate) at 1620.20 cm^{-1}, C-N Strat 1099.42 cm^{-1}, Aromatic C-H bending at 616.82 cm^{-1}. Correspondingly the FTIR spectra of SNEDDS also showed all these characteristic peaks with minor shifts. These results from FTIR spectral analysis confirmed that there was no chemical interaction between drug and excipients used in the formulation.

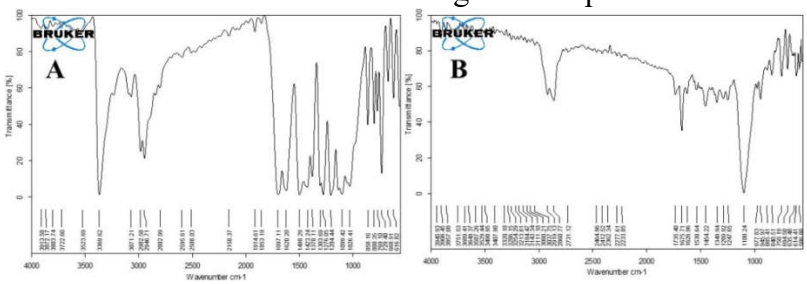

Fig.5. FTIR spectra of Amlodipine

Transmission electron microscopy (TEM)

The surface morphology of the reconstituted formulation observed using TEM (fig. 8). The TEM photograph confirms that all droplets were homogenous, spherically shaped with good integrity and these nanosized droplets had no sign of aggregation, besides photograph did not reveal any precipitation of AML.

***In vivo* study**

The *in vivo* pharmacokinetic behaviour of the AML loaded SNEDDS was assessed and compared with the pure drug suspension following oral administration to the two groups of Wistar rats (n=6). The plasma concentration versus time profiles of AML in rats for formulations system and drug suspension represented in (fig. 9). The results showed that the $[AUC]_0^{\infty}$ and C_{max} for the SNEDDS formulation found to be 1212.4 and 355.40±13.67

when compared to the drug suspension, which was found to be 518.7 and 148.93±8.92, respectively. The per cent relative bioavailability of SNEDDS, when compared with the AML suspension, was found to be 245.68, which suggests the significant improvement of the rate and extent of absorption. Besides, the Tmax was slightly longer for drug suspension when compared to the formulation system. Significant differences found between the two formulations regarding $[AUC]_0^{\infty}$ and C_{max} parameters at $p<0.05$.

CONCLUSION

The present study effectively endorsed successful development and formulation of self-nanoemulsifying system as one of the promising delivery systems for bioavailability enhancement of AML. Following evaluations, the formulation composed of cinnamon oil with 20%v/v, tween 60 with 60%v/v and transcutol HP with 20%v/v exhibited best results and no incompatibles observed. Essential oils were determined to be one of the most proficient oil phases for self-emulsifying systems and may have significant bioactive effects. The formulation enhanced the *in vitro* release approximately eight times. It provided an effective release when compared to a drug suspension.

Furthermore, *in vivo* studies revealed that best formulation exhibited a higher degree of absorption when compared to drug suspension with a relative bioavailability of 245.68%. The study concluded that SNEDDS would be an effective formulation system in increasing the aqueous solubility and potentially bioavailability. Furthermore, the formulation strategy may be applied for other therapeutic categories of drugs belonging to BCS class II and IV that show comparable biopharmaceutical challenges.

AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

The authors report no conflict of interest. This research did not receive any specific grant from any funding agencies.

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