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Preliminary investigation on coconut oil-based self-emulsifying formulation for the delivery of Metronidazole

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ABSTRACT

The aim of this study was to perform a preliminary evaluation of the suitability of natural coconut oil in the formulation of a self-emulsifying drug delivery system (SEDDS) for metronidazole. Different SEDDS mixtures comprising of different proportions of coconut oil, cremophor EL (surfactant) and PEG-400 (co-surfactant) of varying mass ratios were prepared. A pseudo-ternary phase diagram was generated from the phase titration studies between water and the different SEDDS mixtures at room temperature which facilitated the selection of a stable SEDDS. The stable SEDDS was then loaded with the metronidazole powder and characterized with respect to globule size, polydispersity index (PDI), emulsification time, stability, post-dilution drug precipitation and *in vitro* release studies. It was also converted to powder by adsorption on cabosil® at 1:2 ($^{w}/_{w}$) ratio and evaluated. The formulation consisted of 20 % coconut oil, 60 % cremophor EL, 20 % PEG-400 and demonstrated stability against phase separation. Also, it exhibited an emulsification time of 15.0 s, a mean globule size of 18.95 nm, a polydispersity index (PDI) of 0.238 and released over 90 % of the drug within 30 min. The powdered formulation demonstrated acceptable flow properties, contains particles that are irregular and free from rough edges. At 20 min, the percentage of drug released from the metronidazole-SEDDS powder during the *in vitro* release studies was 37 % lower than the liquid metronidazole-SEDDS, indicating that solidification of lipid-based formulations through adsorption onto a carrier may limit drug release. The experimental results from this preliminary investigation were satisfactory, indicating that natural coconut oil can serve as a promising alternative colloidal drug carrier in the field of novel drug delivery systems.

Keywords: coconut oil, preliminary studies, microemulsion, droplet size

INTRODUCTION

A self-emulsifying drug delivery system (SEDDS) is a homogeneous mixture of natural or synthetic oils, surfactants and co-surfactants which form fine oil-in-water emulsion or microemulsion with a particle size of less than 50 nm when exposed to aqueous media under conditions of gentle agitation or digestive motility that would be encountered in the gastrointestinal (GI) tract (Barakat, 2010; Kim *et al*., 2019). This property renders SEDDS a good candidate for the oral delivery of hydrophobic drugs. The spontaneous formation of microemulsion presents the drug in a dissolved state, and the attendant small droplet size provides a large interfacial surface area for drug release and absorption. Also, the specific components of SEDDS promote the intestinal lymphatic transport of drugs. Main mechanisms include increasing membrane fluidity to facilitate transcellular absorption, opening tight junction to allow paracellular transport, inhibiting permeability glycoproteins (P-gp) and/or cytochromes P450 (CYP450) to increase intracellular concentration and residence time by surfactants, and stimulating lipoprotein/chylomicron production by lipid (Wu *et al*., 2006). Oral absorption of several drugs has been enhanced by SEDDS employing single or combined mechanism (Wu *et al*., 2006; Yahaya *et al.*, 2019; Kim *et al*., 2019). The objective of this study was to evaluate the suitability of natural oil obtained from pressed coconut fruit in the formulation of a self-emulsifying drug delivery system containing metronidazole. This will provide a cheap and readily available alternative to the costly starting materials, and also cut down on processing stages. Metronidazole, a poorly water-soluble drug was used in this study as the model drug to improve its aqueous solubility by incorporating it into the natural coconut oil-based self-emulsifying drug delivery system (SEDDS).

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MATERIALS AND METHODS

Materials

Metronidazole powder from Loba chemie laboratory (Mubai, India), Coliphor HS-15 (polyoxyl-15-hydroxy stearate/macrogol-15-hydroxystearate) from BASF AG, (Ludwigshafen, Germany), Cremophor EL (polyoxyl-35-castor oil) from BASF, (Mumbai, India), Labrafac CC (caprylic/capric glycerides) from Gaefosse, (Lyon, France), Polyethylene Glycol 400 (PEG-400) from BDH Chemicals Ltd, (Poole England), Cabosil® (silicon dioxide) from Evonik Industries AG, (Essen, Germany), Malvern Zetasizer ZS90 (M/s Malvern Instruments, Worcestershire, UK). WWR UV-6300PC double beam spectrophotometer from Sargent Welch, (New York, United States of America). The natural coconut oil was obtained from a batch processed in our laboratory. All other materials and chemicals used were of analytical grade and were used as received.

Methods

Construction of pseudo ternary phase diagram

The pseudo ternary phase diagram of coconut oil, surfactant-cosurfactant mixture (cremophor EL® + PEG-400® at the ratio of 3:1) and distilled water was plotted using SigmaPlot 13.0 software following the water titration method described by Tripathi *et al.*, (2016). Briefly, varying mass ratios ($^{w}/_{w}$) of oil to surfactant-co-surfactants mixture (S_{mix}) from 1:9 to 3:1 were prepared in pre-weighed test tubes. To the resultant mixtures, water was added drop-wise, mildly agitated and the different phases exhibited by the system during the titration were visually observed, i.e., micro/nanoemulsion, microgel, emulsion and emulgel, respectively. A completely transparent appearance (depicting miscibility) of the liquid system was taken up as the micro/nanoemulsion, while its semisolid gel-like consistency was taken up as the micro/nanogel. Likewise, a liquid with milky appearance (depicting coacervate colloidal droplets) was treated as an emulsion, while its semisolid form with gel-like consistency was taken up as emulgel. The amount of water at which transparency-to-turbidity transition occurs was derived from the weight measurements. The proportions of oil, surfactant and cosurfactant ($\%^{w}/_{w}$) that yielded a miscible system were selected from the highlighted miscibility region of the phase diagram and used for the preparation of metronidazole loaded SEDDS.

Formulation of metronidazole-SEDDS

Prior to the formulation, the required volumes of the liquid excipients were converted to weights using their densities for easy measurement. The density of coconut oil was determined using a density bottle. Coconut oil (20%), cremophor EL (47.5%), PEG-400 (15.8%) and metronidazole (16.7%) were accurately weighed and transferred into a borosilicate glass vial. Using a magnetic stirrer, the ingredients were mixed for 15 min at 60 - 65 ^{o}C until a yellowish transparent formulation was attained. The metronidazole-SEDDS formulation was then allowed to cool to room temperature before they were used in subsequent studies.

Fourier transform-infrared spectroscopy (FTIR) characterization

The FT-IR spectra of metronidazole and the metronidazole-SEDDS were obtained over the range 650 - 4,000 cm^{-1}.

Emulsification time test of metronidazole-SEDDS

A 0.5 g quantity of the formulation was introduced into a beaker containing 250 ml of 0.1 N HCl, maintained at 37 ± 2 °C under continuous stirring at 50 rpm. The time required to obtain an entirely uniform cloudy dispersion was recorded as the emulsification time.

Post emulsification stability test of metronidazole-SEDDS

A 0.5 g quantity of the formulation was emulsified as above and the emulsion stored at ambient temperature (28 ± 2 °C) for 24 h. Thereafter, it was observed for phase separation and the presence of drug precipitates. Measurement of globule size and polydispersity index of emulsified metronidazole-SEDDS. The mean globule size of the metronidazole-SEDDS was measured by diluting 0.5 g of the formulation to 100 mL with distilled water and gently mixed. The resultant mixture was then subjected to globule size (Z) and polydispersity index (PDI) analysis by dynamic light scattering technique using Malvern Zetasizer.

Adsorption of metronidazole-SEDDS on silicon dioxide

Prior to adsorption, Cabosil® was dried at 60 °C for 2 h to remove physically adsorbed water. The required mass of metronidazole-SEDDS was pipetted and added in increments onto cabosil® and blended in a mortar until a homogeneous, dry powder was obtained. The ratio of metronidazole-SEDDS:Cabosil® was 1:2 ($^{w}/_{w}$). The flow behavior of metronidazole-SEDDS powder was then analyzed and its surface morphology examined using a Scanning Electron Microscope (SEM)

.

Release Profile of metronidazole-SEDDS

The United State Pharmacopoeia (1995) paddle method was employed as follows: a volume of the metronidazole-SEDDS containing 200 mg of metronidazole was transferred into a dialysis bag securely tied at both ends and placed in the chamber of the release apparatus containing 500 mL of the dissolution medium (pH 1.2) buffer maintained at 37 ± 0.5 °C and stirred continuously at a rate of 100 rpm. At predetermined time intervals, 1 mL portions of the dissolution medium were withdrawn appropriately diluted and assayed in a spectrophotometer at λ_{max} of 277 nm. The volume of the dissolution medium was kept constant by replacing it with 1 mL of fresh buffer solution after each withdrawal. The experiment was similarly repeated using an equivalent amount of pure metronidazole and metronidazole-SEDDS powder.

Statistical analysis

The data sets generated were analyzed using SPSS 20.0 software (SPSS, Chicago, IL, USA) and are presented descriptively in charts. The differences between the data sets were determined using *T*-test and $p < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Pseudo-ternary phase diagram

The phase diagram of the system containing coconut oil, cremophor EL® combined with PEG-400® (at the ratio of 3:1) and distilled water is shown in Figure 1. The marked area in the phase diagram indicates the miscibility region. As seen from the pseudo-ternary phase diagram, the combination produced a small miscibility region, indicating that the ratios of coconut oil, cremophor EL® and PEG-400 that will combine with distilled water to yield miscible systems are few. Long chain triglycerides (LCT) oils are not usually miscible with hydrophilic surfactants (Pouton and Porter, 2008), so in practice, it is often necessary to blend these materials with a cosurfactant to promote mutual miscibility. The result of this investigation shows that PEG-400 was only able to promote mutual miscibility in this case to a lower extent. Mutual miscibility of excipients is necessary to produce a clear, stable, liquid formulation. Each spot in the marked area depicts the optimum proportion of components (oil and surfactant-cosolvent mixture) that can result in spontaneous emulsification to produce a stable micro/nanoemulsion. The proportions of coconut oil, cremophor EL® and PEG-400® ($\%^{w}/_{w}$) used in formulating the metronidazole-SEDDS were selected from the highlighted miscibility region of the phase diagram.

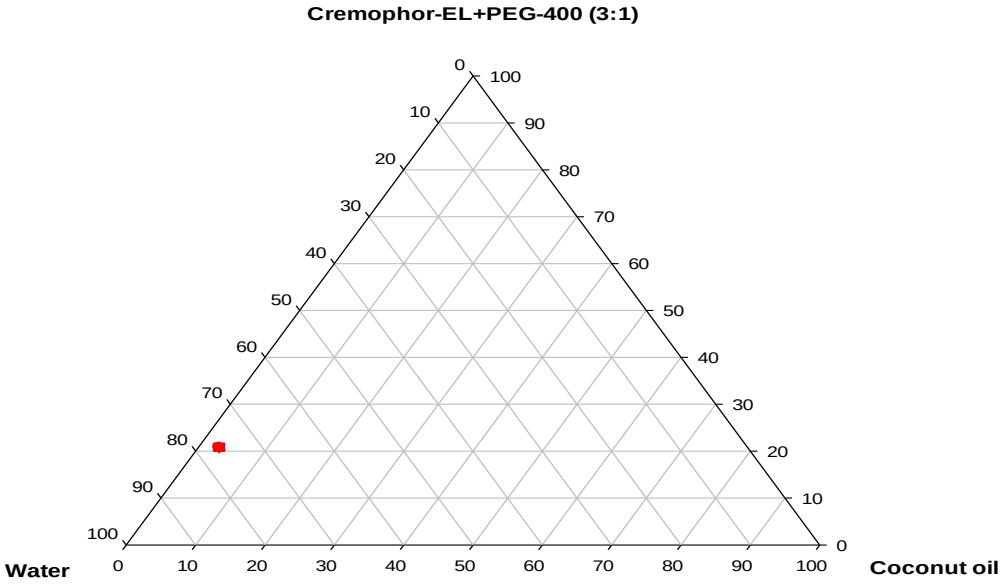

Figure 1: Pseudo-ternary phase diagram for cremphor EL-35/PEG-400 (3:1), coconut oil and water

FTIR characterization

The FTIR spectra of metronidazole and metronidazole-SEDDS are as presented in Figures 2 and 3. Metronidazole produced characteristics peaks at 1265 cm^{-1} (C–O stretch), 2879 cm^{-1} (Aliphatic C-H stretch), 1624 cm^{-1} (C=C stretch) and 1357 cm^{-1} (C–N vibration) as reported by Hani *et al*., (2011), which were also present in the metronidazole-SEDDS. The FT-IR spectra of the pure drug and the metronidazole-SEDDS indicated that characteristics peaks of metronidazole were not altered in their position after successful loading in the SEDDS, indicating no chemical interactions between the drug and delivery system. FT-IR spectra of the metronidazole-SEDDS showed all the metronidazole characteristics absorption bands suggesting the absence of interactions between the drug and the other components of the formulations. These results indicate that the method used in preparing the formulation did not affect the physicochemical properties of the drug.

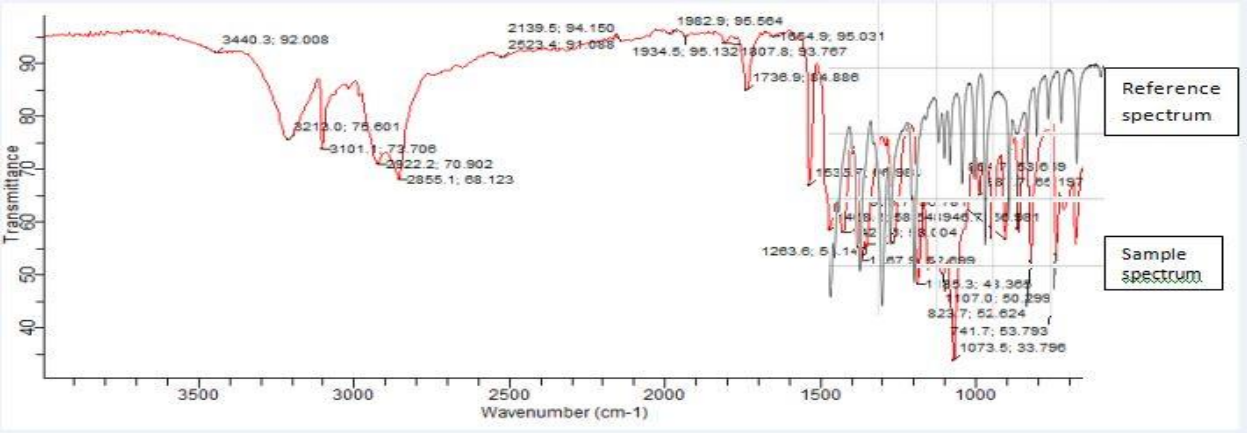

Figure 2: Superimposed reference and obtained FTIR spectra of pure metronidazole

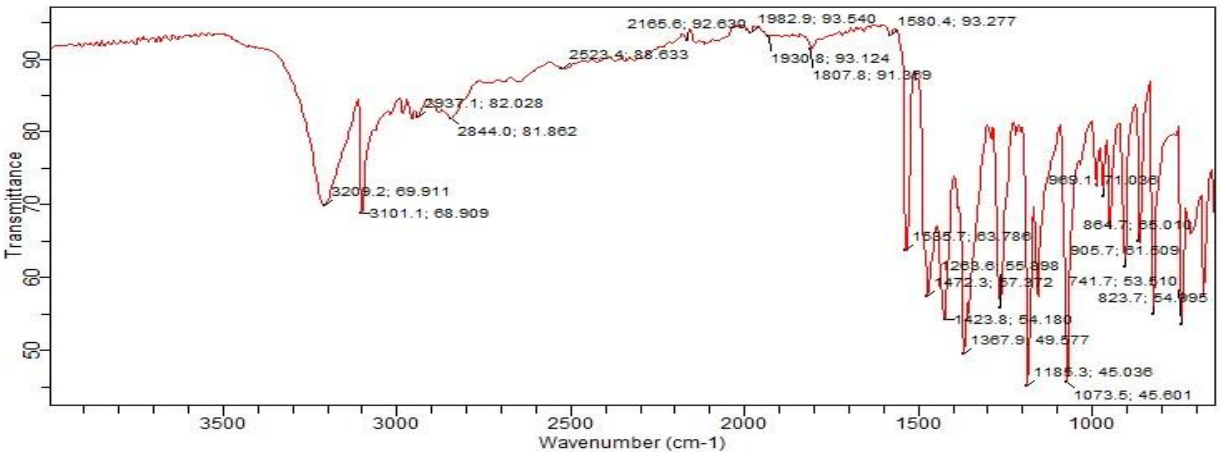

Figure 3: FTIR spectrum of the metronidazole-SEDDS

Results of emulsification time, post emulsification stability and mean globule size of metronidazole-SEDDS

The formulation gave an emulsification time of 15 s, exhibited no phase separation or drug precipitation 24 h post emulsification. Figure 4 provides a graphical presentation of the results of globule size (Z) and polydispersity index (PDI) determination. In SEDDS, visual observation is the primary method for judging self-emulsification (Kim *et al*., 2019). The efficiency of self-emulsification can be evaluated by measuring the rate of emulsification and the emulsion globule

size (Barakat, 2010). The formation of micro/nanoemulsion from self-emulsifying formulations is a spontaneous process. An emulsification time of 15 s suggests that the formulation will rapidly form fine oil-in-water emulsions when dispersed in aqueous media under mild agitation. Emulsions should possess considerable stability against creaming, cracking and precipitations which are massive threats to its stability (Porter, 2013). The metronidazole-SEDDS exhibited no phase separation or drug precipitation, indicating a high degree of physical stability. Globule size distribution following self-emulsification is a critical factor to evaluate a self-emulsifying system. It shows the quality of the emulsion formed (Barakat, 2010). Globule size is thought to have an effect on drug absorption, the smaller the globule size, the larger the interfacial area for drug absorption (Gershanik and Benita, 2000; Pouton, 2006; Zhang *et al.*, 2008). Polydispersity is the ratio of the standard deviation to the mean globule size. The polydispersity index describes the degree of uniformity in globule size within a formulation. The higher the polydispersity index (PDI), the lower the uniformity of the globule sizes in the formulation (Dixit *et al.*, 2010). An emulsification time of 15 s, mean globule size of 18.95 nm and a PDI of 0.238, therefore, suggest that the coconut oil-based metronidazole-SEDDS exhibited efficient emulsification to produced quality emulsions.

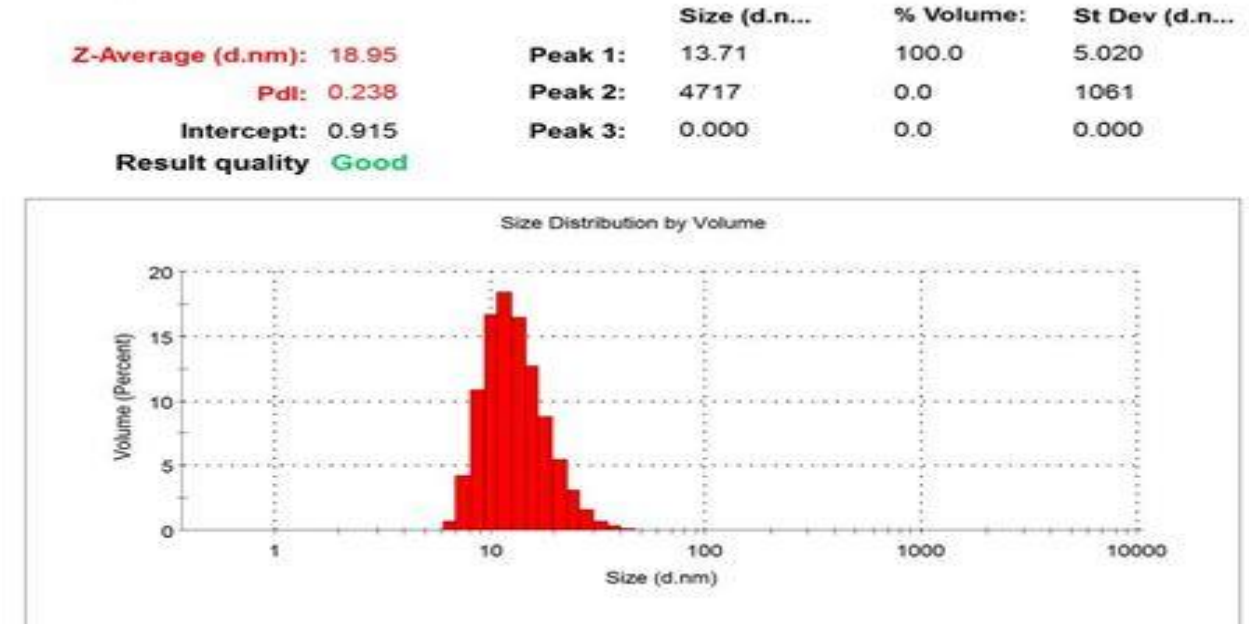

Figure 4: Result of mean globule size and PDI of metronidazole-SEDDS

Flow properties of metronidazole-SEDDS powder

Adsorption of metronidazole-SEDDS onto carbosil® at 1:2 $^{w}/_{w}$ ratio resulted in dry powders. However, it is essential that the powders have good flow because free-flowing powders give uniform tablets and capsule weight and content uniformity. The powder had a Carr's index of 16 %, a Hausner's ratio of 1.18 and an angle of repose of 28.0 ^{0}. The Carr's Index (CI) and the Hausner ratio (HR), which is essentially the ratio of the bulk density to the tap density of powders, are interrelated according to HR=100/ (100 - CI), and they usually reflect how easily the arch formed by powders on the hopper of a tablet press could be broken. The values of these two indices are indirectly influenced by bulk density, tap density, size, shape, moisture content and cohesiveness of the materials and, therefore, serve as useful tools to assess powder properties. In the present investigation, the CI and HR values of the powder were 'good' base on the United States Pharmacopoeia (1995) specification, indicating that there would not be issues with the flow of powder from hoppers. The angle of repose is an old and simple technique that also gives a general idea of the cohesivity and flow properties of powder; however, it is heavily affected by the methodology of the test and may not be highly reproducible (Shah *et al*., 2008; Gumaste *et al*., 2013). Nonetheless, the metronidazole-powder equally exhibited 'good' angles of repose as per the USP guideline. It was observed from the result of SEM studies as presented in Figure 5 that the metronidazole-SEDDS powder contains particles that are irregular and free from rough edges. Such particles tend to flow freely since they avoid inter-particulate entanglement caused by rough edges.

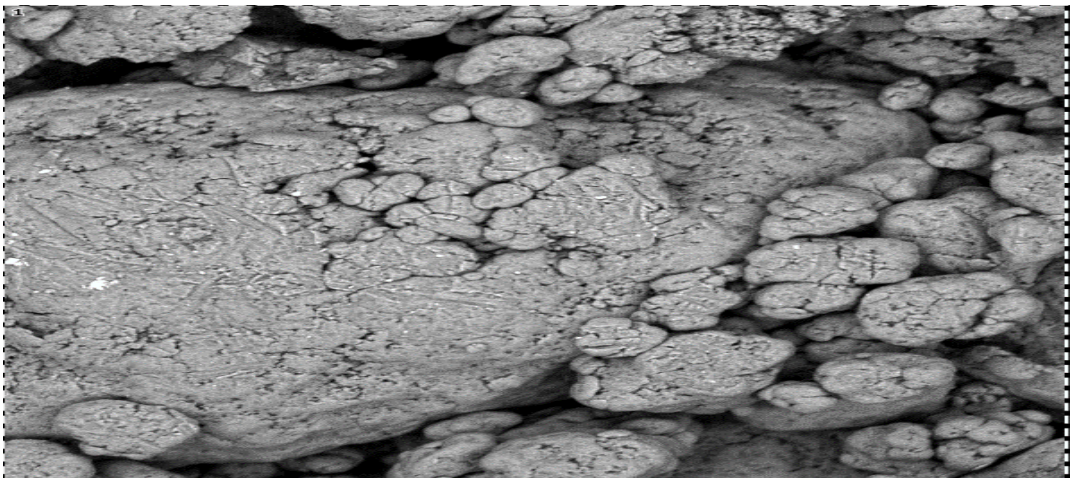

Figure 5: SEM photomicrograph of metronidazole-SEDDS powder (1000x)

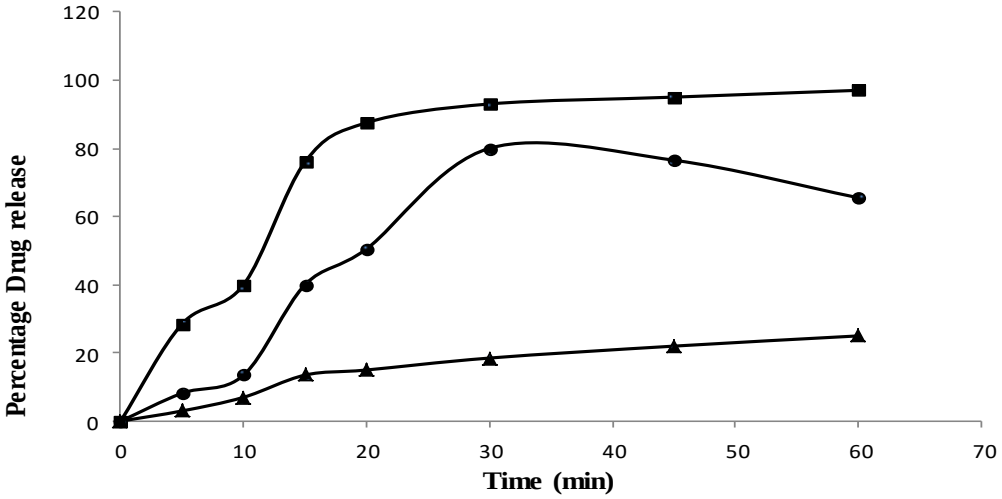

Figure 6: Release profiles of (▲) pure metronidazole, (■) metronidazole-SEDDS and (●) metronidazole-SEDDS powder

***In-vitro* release profile**

Figure 6 provides the release profiles of pure metronidazole, metronidazole-SEDDS and metronidazole-SEDDS powder. As shown in the figure, the average cumulative percentages of metronidazole released from the pure metronidazole, metronidazole-SEDDS and metronidazole-SEDDS powder in 30 min are 18.5, 93.0 and 80.0 % respectively, representing over 4-folds increase in drug release by both the metronidazole-SEDDS and metronidazole-SEDDS powder compared to the pure metronidazole. The total drug release at the end of the release test (after 60 min) was 25, 97 and 80% for pure metronidazole, metronidazole-SEDDS and metronidazole-SEDDS powder respectively. Metronidazole-SEDDS powder released 17% lower metronidazole compared to the liquid metronidazole-SEDDS, this suggests that the presence of carbosil® indirectly altered (reduced) the release of metronidazole most likely via incomplete desorption from the formulation as a result of physical retention of the formulation in the adsorbent pores, a similar observation was reported by Speybroeck *et al*., (2012). These, therefore, illustrate the need for caution when using adsorbents to facilitate the "solidification" of lipid formulations.

CONCLUSION

Coconut oil, cremophor EL and PEG-400 admixtures have been formulated to show the potential

applications of natural coconut oil in the development of a self-emulsifying formulation for the delivery of a known poorly water-soluble drug, metronidazole. The emulsion droplet size was generally small, indicating that the formulation has efficient self-emulsification ability. The formulated metronidazole-SEDDS enhanced the release of metronidazole. The results of the release study, however, indicate that solidification of formulation through adsorption onto a high surface area carrier may reduce the pharmaceutical performance of the adsorbed formulation by limiting the formulation (and drug) release which could have a negative impact on oral bioavailability.

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