Original Research Article

DEVELOPMENT AND CHARACTERIZATION OF SELF-NANO EMULSIFYING DRUG DELIVERY SYSTEM OF IBUPROFEN

ABSTRACT

Aim: The aim of this work was to formulate self-nano emulsifying drug delivery systems (SNEDDS) for augmenting the biopharmaceutical performance of ibuprofen, a poorly water soluble drug and subsequently evaluate its anti-inflammatory activity.

Methodology: Pseudoternary phase diagram studies facilitated selection of caprylic/capric glycerides as the oily phase, cremophor EL as surfactants, and polyethylene glycol-400 as the cosurfactant for formulating the SNEDDS. A stable combinations from the phase diagram consisting of 27 % *caprylic/capric* glycerides, 58 % cremophor EL and 15 % polyethylene glycol-400 was loaded with ibuprofen and characterized with respect to globule size, polydispersity index (PDI), stability, emulsification time, % drug loading efficiency (DLE), *in vitro* drug release, infinite aqueous dilution, post-dilution drug precipitation and *in vivo* anti-inflammatory tests.

Results: The optimized ibuprofen SNEDDS (ibu-SNEDDS) had a mean globule size of 25.23nm, PDI of 0.093, showed excellent emulsification time of 5.0 s, released > 94 % of the drug within 15 min while the pure drug showed only 8.8 % drug release over a period of 1 h, exhibited no phase separation and demonstrated significantly ($P < 0.05$) higher anti-inflammatory effect than the reference drug.

Conclusion: Our study illustrated the potential use of SNEDDS as a promising nano drug carrier for the efficient delivery of ibuprofen that may solve the low bioavailability, high intra- and intersubject variability frequently associated with the oral delivery of the drug.

Keywords: ibuprofen, anti-inflammatory, self-nano-emulsifying drug delivery system (SNEDDS), solubility

INTRODUCTION

Drug bioavailability from an oral formulation in the gastrointestinal tract (GIT) is heavily reliant on favorable physiochemical characteristics, including adequate solubility and permeability and resistance to first pass metabolism [1]. A large majority of the newly discovered chemical entities and many existing drug molecules do not meet these criteria [1, 2]. Of these limiting factors to oral drug delivery, low water solubility is perhaps the most amenable to a resolution based on the use of enabling formulation approaches [3, 4]. In contrast, formulation approaches that markedly enhance intestinal permeability or reduce first pass metabolism, are much less common. Permeation enhancement for oral delivery has met with some moderate successes in early clinical development as described in a recent review by Feeney *et al*. [4]. In the case of highly (first pass) metabolized compounds, strategies such as prodrugs, co-administration with inhibitors, or alternative routes of absorption, e.g., pulmonary, nasal and buccal administration are more commonly employed [5]. However, for many compounds with significant permeability or metabolic liabilities, parenteral administration is often required for efficient delivery. For drugs where low aqueous solubility limits absorption, several formulation strategies have been developed and applied to support increases in dissolution rate and/or apparent solubility in the gastrointestinal tract (GIT). These include particle size reduction and nano milling, salt formation, isolation as a cocrystal or high energy polymorph, the use of surfactants, cyclodextrins, generation of solid dispersions, and formulation in lipid-based formulations (LBFs) [2, 4, 6]. Self-nano emulsifying drug delivery system (SNEDDS) is an oral lipid-based formulation. It is a mixture of oil, surfactant and cosurfactant which on gentle agitation in an aqueous medium undergo self-emulsification to yield oil-in-water emulsions with droplet sizes of less than or equal to 100 nm [7]. The major advantage of lipid-based formulations (LBF), has been in

increasing apparent gastrointestinal solubility, it is also becoming increasingly clear that they may provide advantages in permeability and, under some circumstances, in avoiding first-pass metabolism [4].

LBF confer a range of biopharmaceutical, pharmaceutical and commercial advantages. Pharmaceutically, the ability to process LBF as solutions provides an advantage for drugs with inherently low melting points (where solid dosage forms may be impractical), for low dose compounds with potential content uniformity issues and for irritant and toxic compounds where dust control is a challenge. Commercially, LBF provides additional patient preference opportunities and in combination with a range of different finished dose forms (softgels, hard capsules or lipid multiparticulates) [4]. Lipids and many of the other common components of LBF (surfactants and cosolvents) have been described to impact intestinal permeability, both via changes to passive permeability and via inhibition of efflux transporters. Presystemic drug metabolism is also avoided by drugs that are trafficked to the systemic circulation via the intestinal lymph - a process that is supported by coadministration with lipids. Finally, and perhaps most importantly, lipids and LBF significantly enhance the intestinal solubilization of lipophilic poorly water-soluble drugs. This increases exposure and in most cases also attenuates the large positive food effect commonly seen for poorly water-soluble drugs after oral administration. These effects stem from the integration of poorly water-soluble drugs into the lipid digestion/absorption cascade. [8-14].

Ibuprofen, a propionic acid derivative, is a non-steroidal anti-inflammatory drug (NSAID). It is used in the management of mild to moderate pain and inflammation in conditions such as dysmenorrhoea, headache including migraine, postoperative pain, dental pain, musculoskeletal and joint disorders such as ankylosing spondylitis, osteoarthritis, and rheumatoid arthritis including juvenile idiopathic arthritis, peri-articular disorders such as bursitis and tenosynovitis, and soft-tissue disorders such as sprains and strains. It is also used

to reduce fever. Ibuprofen is also used as an alternative to indomethacin in the treatment of patent ductus arteriosus. The drug is practically insoluble in water and poorly absorbed from the gastrointestinal tract following oral administration leading to correspondingly low bioavailability [15, 16]. The aim of this study is to develop and characterize SNEDDS that will have ibuprofen intact in a solubilized form thereby culminating in invents that may maintain lumen solubility and enhanced consistent absorption profile. The presence of surfactants in the formulation may additionally provide a permeability-enhancement effect in the gut lumen.

MATERIAL AND METHODS

Material

Ibuprofen (ibu) was kindly provided as a gift sample by Pal Pharmaceutical Nigeria Ltd, Cremophor EL (PEG-35-castor oil) by Gattefosse, France. Sesame oil (from Sigma Chemical Co., USA) Caprylic/Capric Triglyceride (GTCC) (from Aeco Group Limited, China), polyethylene glycol-400 (BDH Chemicals Ltd Poole England) were used as procured. Malvern Zetasizer ZS90 (M/s Malvern Instruments, Worcestershire, UK). All other reagents and solvents were of analytical grade.

Methods

Solubility studies

The solubility of ibuprofen in the oil, the various surfactants and co-surfactants was determined. Briefly, an excess quantity of ibuprofen was added to the oil, various surfactants and co-surfactants respectively and vortex-mixed for 15 min. Each suspension was subsequently centrifuged. The resulting supernatant was filtered through a membrane filter, diluted appropriately with simulated intestinal fluid without enzyme (SIF). The solubilized

fraction of ibuprofen in the solubility samples was assayed by the spectrophotometric method at the wavelength of 221 nm.

Construction of pseudoternary phase diagrams

Sesame oil or labrafac CC was the oil phase, Cremophor EL was the surfactant and polyethylene glycol-400 was the co-surfactant. The phase titration studies were carried out by water titration method for constructing the pseudoternary phase diagrams employing lipid and surfactant/co-surfactant mixtures (Smix) in the ratios ranging between 1:9 and 4:1. The Smix ratios of 1:0, 1:1, 2:1, 3:1 and 4:1 were explored to delineate the boundaries of the nanoemulsion region [17, 20]. At each ratio, the mixtures were visually observed for different phases, i.e., micro/nanoemulsion, micro/nanogel, emulsion and emulgel, respectively. A completely transparent appearance 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 was treated as an emulsion, while its semisolid form with gel-like consistency was taken up as emulgel [20, 21]. The amount of water at which transparency-to-turbidity transition occurs was derived from the weight measurements. The results were then plotted on a pseudo-ternary phase diagram using SigmaPlot 13.0 software to demarcate the nano emulsification region. No attempts were made to completely identify the other regions of the phase diagrams. Based on the results, the appropriate percentage of oil, surfactant and co-surfactant was selected, correlated in the phase diagram and were used for the preparation of SNEDDS containing ibuprofen.

Formulation of ibuprofen SNEDDS

Based on the stable batches obtained from the demarcated nano-emulsifying region, appropriate oil, surfactant and cosurfactant were selected and used in the preparation of nano-emulsifying drug delivery system containing ibuprofen. The required volumes of the liquid excipients were converted to weights using their densities for easy measurement. The density

of sesame oil was determined using a density bottle. Ibuprofen was dissolved in the appropriate oil in a water bath at 50 ^{o}C ± 5 ^{o}C with frequent shaking. After complete dissolution, the surfactant and cosurfactant were added and vortexed. The resultant Ibuprofen SNEDDS (ibu-SNEDDS) formulations were stored for further studies. Placebo formulations were also prepared in a similar manner without the addition of ibuprofen. The compositions of the developed ibu-SNEDDS are shown in Table 1**.**

Table 1: Composition of the developed ibu-SNEDDS

	Composition (mg)			
Components	**A1**	**A2**	**A3**	**A4**
Ibuprofen	400	400	400	400
Sesame oil	216	238.4	-	-
Labrafac CC	-	-	216	238.4
Cremophor EL	464	449.6	464	449.6
PEG-400	120	112	120	112

Characterization of the Ibuprofen-SNEDDS

Phase separation and drug precipitation

Two (2) mL samples of each of the formulation were diluted to 10 mL and 100 mL with distilled water respectively at room temperature (28 ± 3 ^{o}C), stored for a period of 24 h and observed afterward for phase separation and drug precipitation.

Assessment of emulsification time

Aliquot (1) mL portion of each formulation was introduced into a beaker containing 250 mL of distilled water, maintained at 37 ± 1 ^{o}C under continuous stirring at 50 rpm. The time required to obtain a completely uniform cloudy/turbid dispersion was recorded as the emulsification time.

142 The tendency to form an emulsion was judged as 'good' when droplets spread easily in water
143 and formed fine cloudy/turbid/milky dispersion, and it was judged 'bad' when there was poor
144 or no dispersion with immediate coalescence of oil droplets, especially when stirring was
145 stopped [22].

146 ***Centrifugation studies***

147 After 100-fold dilution with distilled water, 5 mL sample of each formulation was transferred
148 into a glass test tube and centrifuged at 4,000 rpm for 5 min in a laboratory centrifuge.
149 Thereafter, the samples were checked for physical instability, such as phase separation and
150 drug precipitation.

151 ***Loading efficiency***

152 About 1 g of each formulation was dissolved in 100 mL of 0.1N NaOH and filtered via a
153 Whatman filter paper. The filtered solution was appropriately diluted and assayed for drug
154 content by the spectrophotometric method at λ_{max} of 221 nm.

155 ***Globule size determination***

156 An aliquot (1 mL) of each formulation (batches which did not exhibit phase separation or
157 drug precipitation, i.e., A1, A3 and A4) was diluted 100-fold in distilled water, followed by
158 gentle mixing. The resultant mixture was then subjected to globule size analysis and
159 polydispersity index (P.I.) using a Malvern Zetasizer ZS90 (M/s Malvern Instruments,
160 Worcestershire, UK).

161 ***Release rate determination***

162 A drug release study was carried out on the selected formulation (batch A3). The studies were
163 performed by dialysis bag method [20] in 500 mL of simulated gastric fluid (SGF) without
164 pepsin (pH 1.2) for 1 h. A formulation containing 400 mg of ibuprofen was filled into dialysis
165 bags and subjected to drug release studies. The drug release studies were also carried out for
166 the pure drug for comparative evaluation of the dissolution performance. The dissolution

medium temperature was maintained at 37 °C ± 1 °C while the rotation speed was set at 100 rpm. Aliquots (5 mL) were withdrawn at a predetermined time interval, namely 5, 10, 15, 20, 30, 40, 50 and 60 min, followed by replenishment with an equal volume of fresh dissolution medium. The drug content was analyzed by the spectrophotometric method at λ_{max} of 221 nm.

Stability studies

The selected formulation (batch A 3) was stored for 6 weeks under refrigeration (4 - 8 ± 2 °C), ambient room temperature (27 - 30 ± 2 °C) and high temperature (45 ± 2 °C) and evaluated for pH, drug content, drug precipitation and emulsification time.

Anti-inflammatory studies

The anti-inflammatory activity of the selected ibu-loaded SSEDDS (batch A3) was carried out using the rat paw edema test method [23]. All experimental protocols were in accordance with the Ahmadu Bello University Zaria Committee on Animal Use and Care. The phlogistic agent employed in the study was fresh undiluted egg albumin [22]. Adult Wistar rats of either sex (weighing between 180 to 200 g) randomly divided into various groups (n = 5 per group) as depicted in Table 4 were used for the study. The rats were fasted and deprived of water for 12 h before the experiment. The deprivation of water was to ensure uniform hydration and to minimize variability in edematous response [23]. Group 1 was administered distilled water and served as control. Group 2 was administered pure sample of ibuprofen (6 mg/kg) dispersed in distilled water. Group 3 received placebo SNEDDS while group 4 was administered ibu-SNEDDS (batch A3) with the equivalent of 6 mg/kg ibuprofen orally using a 1 mL syringe. Thirty minutes post-treatment edema was induced by injection of 0.1 ml of fresh undiluted egg-albumin into the subplantar region of the left hind paw of each rat. The paw diameter was measured with the aid of a Vernier caliper 1, 2, 3, 4, 5 h after the injection

of the egg albumin. The percentage inhibition of paw edema was calculated by the formula [24].

$$\% \text{ inhibition of paw oedema} = \frac{Vc - Vt}{Vc} X\ 100 \quad . \quad . \quad . \quad (1)$$

Vc = Mean volume of paw edema in the control group of animals

Vt = Mean volume of paw edema in the drug-treated group of animals

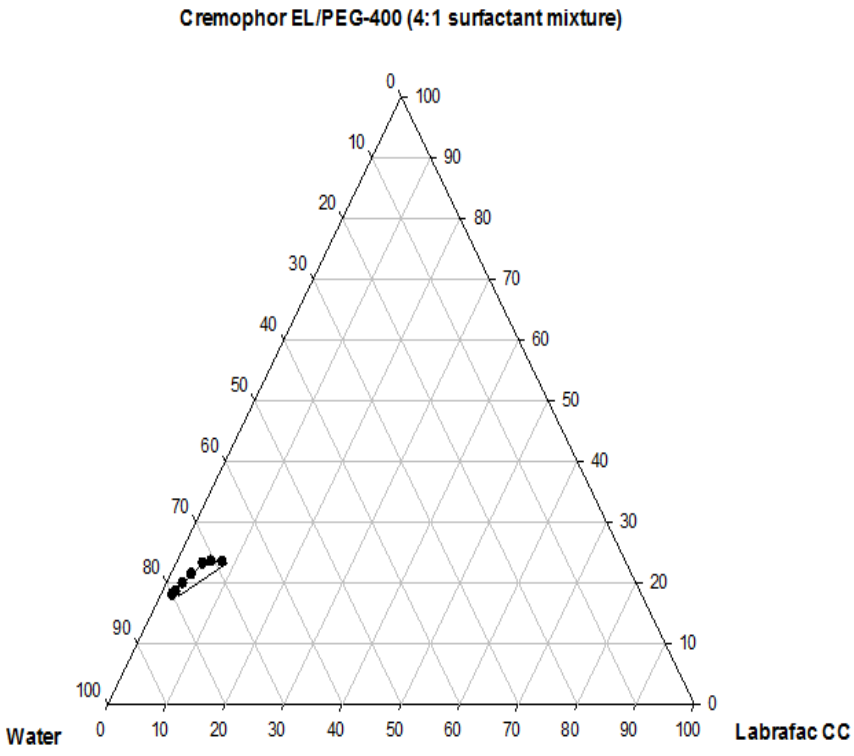
Statistical analysis

The data generated from the various determinations were analyzed using SPSS 20.0 software (SPSS, Chicago, IL, USA) and are presented as the mean ± standard deviation (SD). The differences between the data sets were determined using T-test and $p < 0.05$ was considered statistically significant.

RESULTS

Pseudo-ternary phase diagram

Mixtures that exhibited phase separation or could not form transparent systems were discarded. On the other hand, those mixtures that produced transparent systems were noted and a pseudo-ternary phase diagram plotted. The area of nanoemulsion existence is depicted in Figure 1 and 2 with the delineated outline. The maximum field of self-microemulsion was obtained with a surfactant - cosurfactant mixture ratio of 4:1.



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210 **Fig. 1: Pseudo-ternary phase diagram for cremophor EL/PEG-400 (4:1), labrafac CC**

211 **and water**

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213

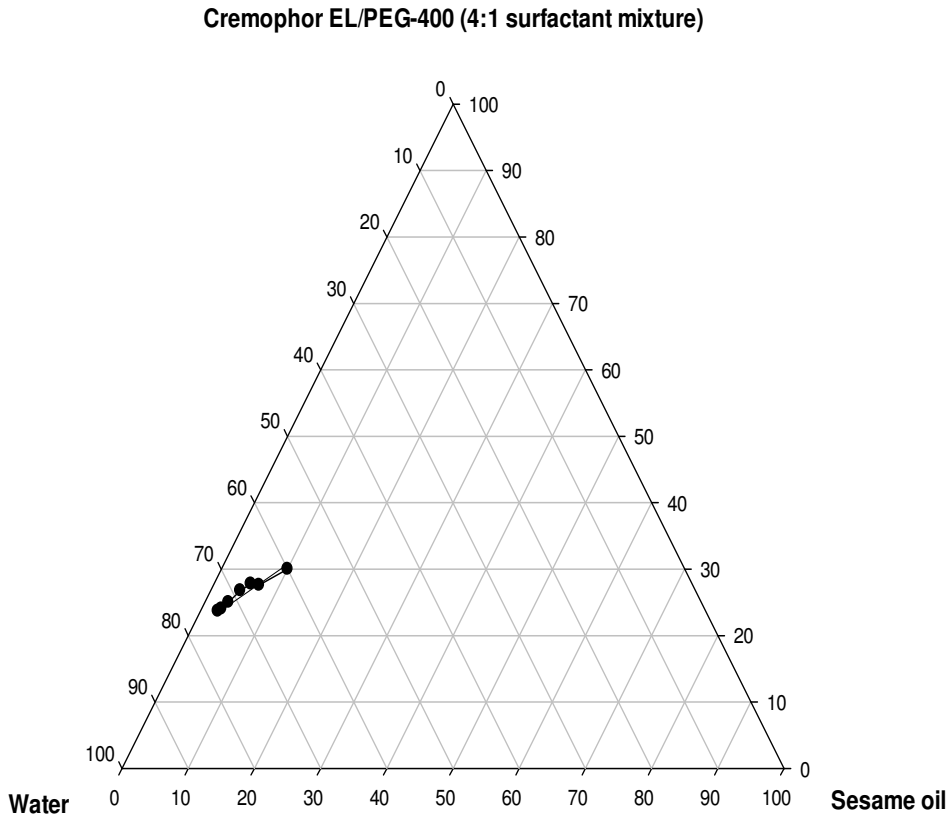


Fig. 2: Pseudo-ternary phase diagram for cremophor EL/PEG-400 (4:1), sesame oil and water

Emulsification time, phase separation, drug precipitation and loading efficiency

Batch A2 exhibited drug precipitation upon storage for three (3) months and was therefore dropped. It also exhibited phase separation, batch A1, A3 and A4 however past both tests. They all had emulsification time less than 10 s. The loading efficiency was between 96-98 %. The results are as presented in Table 2.

Table 2: Results of emulsification time, phase separation, drug precipitation and loading efficiency assessment of the developed ibu-SNEDDS

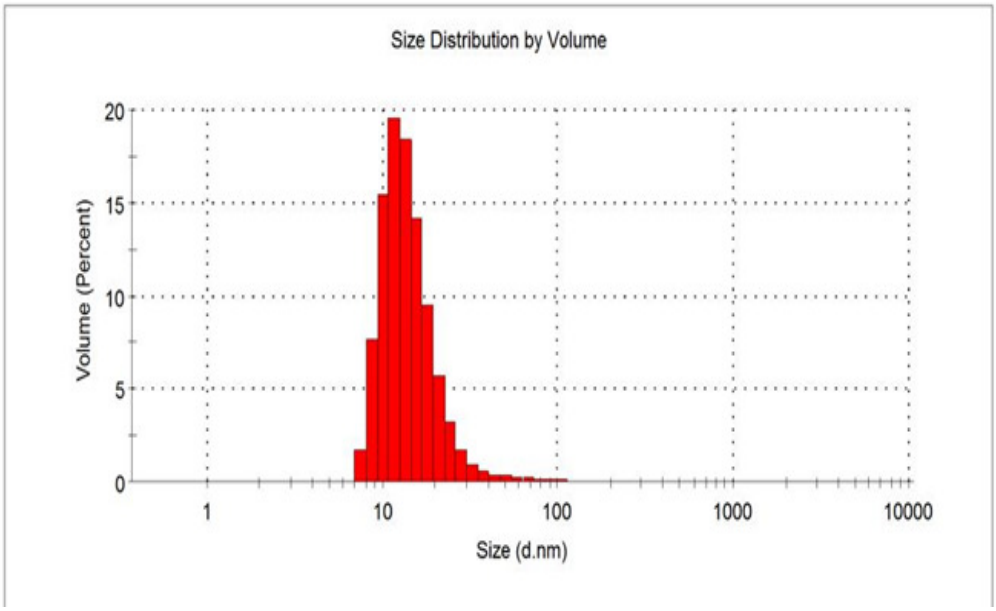
Sample	Emulsification time (sec)	Phase separation	Drug precipitation	Loading efficiency
A1	8.0±0.04	No	No	97.0±0.32
A2	8.5±0.01	Yes	Yes	96.0±0.00
A3	5.0±0.05	No	No	96.0±0.18
A4	7.0±0.03	No	No	98.0±0.41

Mean globule size determination and polydispersity index

Figure 3, 4 and 5 provide a graphical presentation of the results of mean globule size (Z) and polydispersity index (PDI) of the formulation. The mean globule size of batch A1, A3 and A4 were found to be 40.36, 25.23 and 22.18 nm respectively, all less than 100 nm, typical of SNEDDS. The polydispersity index which describes the degree of uniformity in droplet size was 0.495, 0.093 and 0.143 respectively.

Results

		Size (d.n...	% Volume:	St Dev (d.n...
Z-Average (d.nm): 40.36	Peak 1:	1527	0.0	276.9
PdI: 0.495	Peak 2:	15.26	99.9	11.16
Intercept: 0.948	Peak 3:	4402	0.1	1210
Result quality Good				



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236 **Figure 3: Graphical presentation of globule size (Z) and polydispersity index (PDI) of**

237 **batch A1**

Results

		Size (d.n...	% Volume:	St Dev (d.n...
Z-Average (d.nm): 25.23	Peak 1:	21.31	100.0	6.740
PdI: 0.093	Peak 2:	0.000	0.0	0.000
Intercept: 0.956	Peak 3:	0.000	0.0	0.000
Result quality Good				

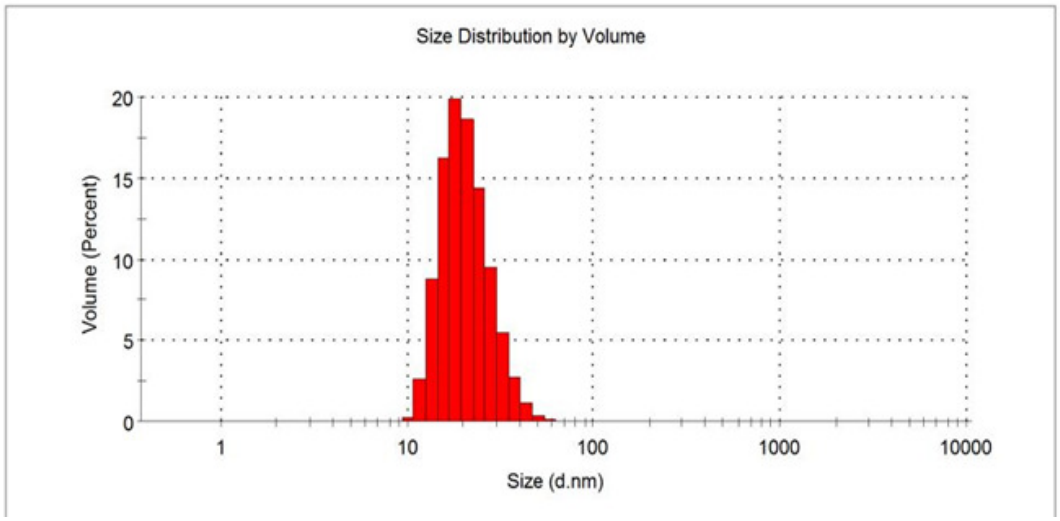

Figure 4: Graphical presentation of globule size (Z) and polydispersity index (PDI) of batch A3

Results

		Size (d.n...	% Volume:	St Dev (d.n...
Z-Average (d.nm): 22.18	Peak 1:	17.50	100.0	6.254
PdI: 0.143	Peak 2:	4948	0.0	906.5
Intercept: 0.966	Peak 3:	0.000	0.0	0.000
Result quality Good				

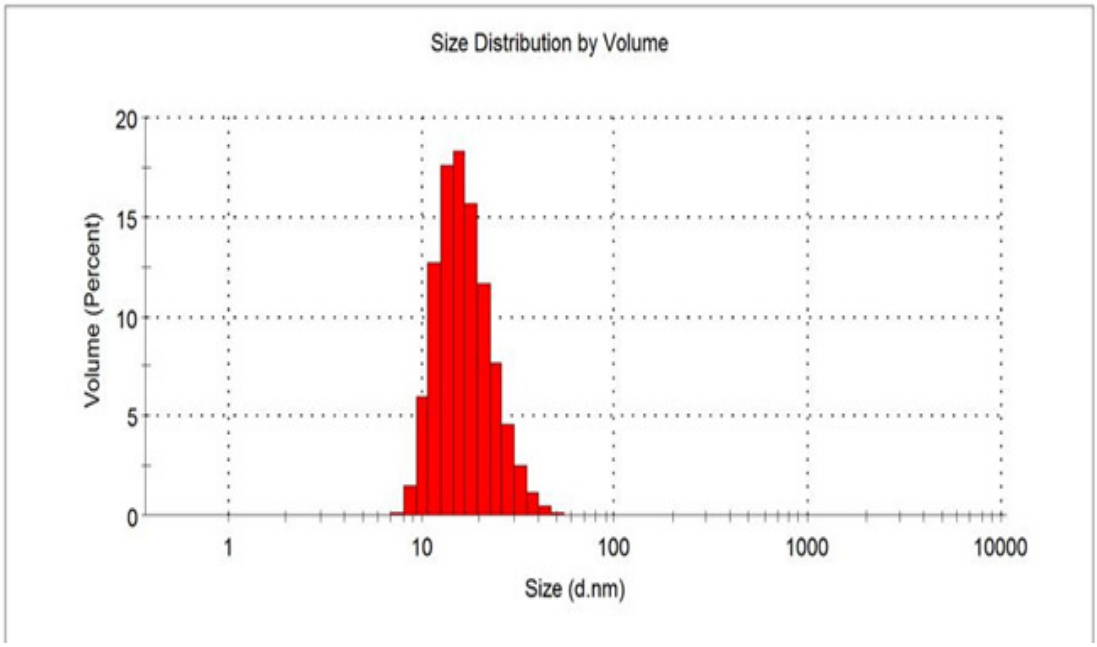

Figure 5: Graphical presentation of globule size (Z) and polydispersity index (PDI) of batch A4

Release rate determination

The ibu-SNEDDS formulation showed marked improvement in the drug release rate compared to the pure drugs as shown in Figure 6. The pure drug showed only 8.8 % release over a period of 60 min while about 94 % of the drug was released from the developed ibu-SNEDDS within 15 min.

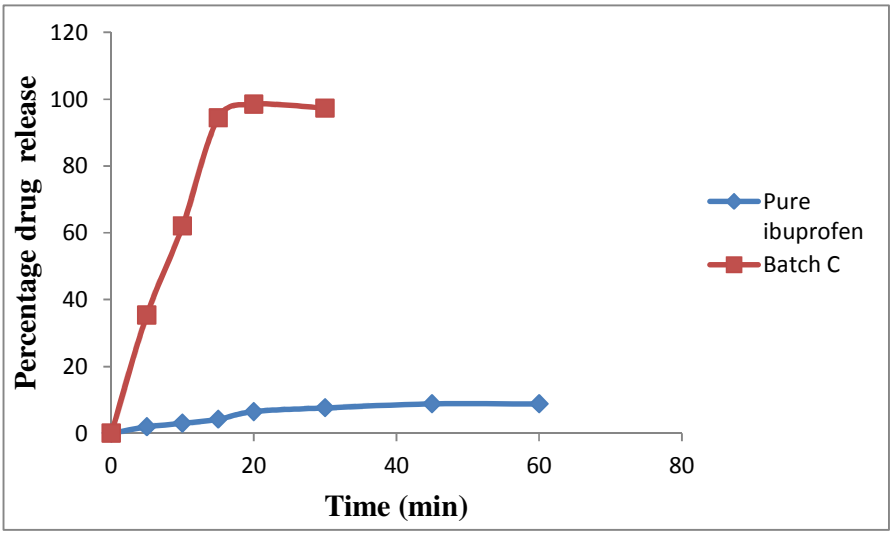

Figure 6: Plot representing percent drug released from pure ibuprofen and Batch A3

Stability studies

During the 12 weeks of stability study, none of the stored batch samples showed any change in color or appearance under all storage conditions. No significant difference was found between the emulsification time of freshly prepared and stored samples. No drug precipitation was observed with any batch under all storage condition. However, there was a decrease in drug content by 3.3 % between samples stored at a refrigerated or ambient temperature and elevated temperature as presented in Table 3.

Table 3: Results of drug content, emulsification time, phase separation and drug precipitation assessment of the six (6) weeks old loaded SEDDS at stored under refrigeration, ambient temperature and elevated temperature

Storage condition	Sample	Drug content (%)	Emulsification time (sec)	Phase separation	Drug precipitation
Refrigeration	A3	95.7±0.01	5.0±0.02	No	No
Ambient temperature (27-30±2 °C)	A3	96.0±0.11	5.0±0.30	No	No
Elevated temperature (45 ± 2 °C)	A3	92.7±0.07	6.0±0.90	No	No

Anti-inflammatory studies

The results of anti-inflammatory studies are as shown in Table 4. Results showed that the developed ibu-SNEDDS exerted significantly ($P < 0.05$) higher anti-inflammatory activity than the reference ibuprofen powder ($P = 0.01$) and blank formulation (placebo) ($P = 0.00$).

Table 4: Anti-inflammatory properties of ibu-SNEDDS

S/No	Treatment	Percentage decrease in paw edema					
		1 h	2 h	3 h	4 h	5 h	Mean
1	Aqueous artemether dispersion	2.8±0.01	16.0±0.10	17.2±0.05	18.6±0.02	23.6±0.15	15.6±0.13
2	Placebo SNEEDS	0.19±0.02	0.03±0.00	0.19±0.30	0.13±0.07	0.17±0.08	0.14±0.14
3	Ibu-SNEDDS	36.6±0.21	43.7±0.00	48.0±0.11	51.7±0.13	59.1±0.00	47.8±0.09
Aqueous artemether and ibu-SNEDDS		T-Test Statistic 7.443			*P*-value 0.01***		
Placebo SNEDDS and ibu-SNEDDS		T-Test Statistic 18.938			*P*-value 0.00***		

***indicates a significant difference at 1% level of error

DISCUSSION

Pseudo-ternary phase diagram

This present study involved the use of pre-concentrates consisting oil, surfactants and cosurfactants and the pseudo-ternary diagram was only used to select the appropriate oil, surfactant and co-surfactant mixtures. Phase diagram makes it easy to find out the concentration range of components for the existence range of nanoemulsions. The integral properties of the oil and surfactants largely determined the nature of the plot [7]. The largest

282 field of SNEDDS was obtained when labrafac CC was used as the oil phase. The delineated
283 area in the phase diagram indicates the nanoemulsion existence region. The compositions of
284 the developed ibu-SNEDDS were selected from within the delineated area. The selected
285 SNEDDS yielded nanoemulsion that could withstand accelerated stress tests such as storage
286 at elevated temperature, refrigeration and centrifugation at 4000 rpm. This preconcentrate
287 would readily form a microemulsion in the body on dilution with physiological fluids. These
288 systems often require high surfactant concentrations in order to provide very low interfacial
289 tension (≤ 10-3 mN/m) and sufficient interfacial coverage to microemulsify entire oil and
290 water phases [25, 26]. The ease and degree of surface tension lowering were increased at high
291 Smix content. In order to reduce the interfacial tension to significantly low levels, a co-
292 surfactant was combined with the surfactant.

293 ***Emulsification time, phase separation, drug precipitation and loading efficiency***

294 The rate of emulsification is an important index for the assessment of the efficacy of
295 emulsification. The importance of this is that the formulation should disperse completely and
296 quickly when subjected to aqueous dilution under mild agitation [24, 27, 28]. All the batches
297 exhibited prompt and fast emulsification with the highest been 8.5 s. This indicates that the
298 formulations will disperse promptly upon contact with an aqueous medium under mild
299 agitation. Phase separation and drug precipitation is a huge threat to the stability of the
300 formulations [7]. Since the formation of nanoemulsion from SNEEDS is a spontaneous
301 process, the formulation should possess considerable stability against creaming, cracking and
302 precipitation. All except batch A2 demonstrated stability (absence of phase separation and
303 drug precipitation) after storage for 48 h and after appropriate dilutions. In addition, the
304 absence of drug precipitation or phase separation upon centrifugation further confirmed
305 stability. This confirmed the high degree of physical stability and robust nature of the
306 prepared formulations. The observed drug precipitation in batch A2 indicates that the

formulation has low drug loading capability. The batch contained a relatively high percentage of oil and sesame oil as it oily phase, this may be responsible for it low drug loading capability. The batches had loading efficiency values that fell within 96 to 98 %. This means that the drug was well encapsulated within the oil droplets.

Mean globule size determination and polydispersity index

All the formulations exhibited globule size in the nanometric range. The batch with labrafac CC had smaller globule size (25.23 nm and 22.18 nm) and PDI (0.093 and 0.143 respectively) than the formulation containing sesame oil as the oily phase which had a droplet size of 40.36 nm and a PDI of 0.495. This result is in consonant with the report that labrafac CC has a relatively shorter triglyceride chain, which is the reason behind the smaller mean droplet size of microemulsions formulated with it [29, 30]. Oils consisting of long chain triglycerides have higher viscosity, this impact on the emulsification process which in turn have a strong effect on the emulsion globule size [31]. Droplet size distribution following self-nano emulsification is a critical factor to evaluate a self-nanoemulsion system. Droplet size is thought to have an effect on drug absorption. The smaller the droplet size, the larger the interfacial surface area will be provided for drug absorption [32 - 35]. Besides, larger sizes may be predisposed to early drug precipitation prior to absorption. Polydispersity is the ratio of standard deviation to the mean droplet size and is inversely proportional to droplet size uniformity; the higher the polydispersity the lower the uniformity of droplet size [7].

Based on the results of the above investigations, batch A3 was chosen as the optimum formulation on the basis of possession of minimal globule size and emulsification time (i.e., necessary for faster solubilization and absorption of drugs) [20].

Release rate determination

In vitro release studies are performed to determine the rate at which the drug in a formulation is released into the dissolution medium and to also have an idea about the self-emulsification

efficiency of the developed system. There was a marked improvement in the drug release rate from the optimized ibu-SNEDDS as compared to the pure drug. This confirmed that the optimized formulation is markedly better than the pure drug. Over 94 % of the drug was released within 15 min for the optimized formulations, while the pure drug showed only 8.8 % release over a time period of 1 h. The slow release exhibited by the pure drug is as a result of it limited aqueous solubility, release rate describes the process by which the drug particles dissolve or become solubilized by the dissolution fluid [36]. At 20 min, 98.5 % of the drug was released from the ibu-SNEDDS while the pure drug only showed 6.5 % drug release representing an about 15-fold increase over the pure sample. Significant improvement in dissolution rate indicated improved solubilization of the drug in the aqueous media ostensibly owing to spontaneous emulsification of the lipidic and emulsifying agents to produce the ultrafine emulsions by micellar solubilization [20, 37]. The developed SNEDDS is expected to quickly present ibuprofen in a solubilized form in gastric fluids after ingestion and would provide a large interfacial area for ibuprofen absorption.

Stability studies

During the 12 weeks of stability study, there was no change in any of the physical parameters - phase separation, drug precipitation, appearance and smell of the developed ibu-SNEDDS under all storage conditions. This indicates the stability of the formulation. Also, there was no significant difference in the ibuprofen content at zero time and through the 12 - week stability study period under refrigeration and ambient storage conditions. This indicates that ibuprofen is chemically and physically stable in the formulation. For samples stored at elevated temperature, there was about 3.3 % decrease in drug content when compared with the drug content at time zero, this is expected since the rate of degradation is markedly influenced by

355 temperature. At high temperatures, reactions may take place which is not significant at
356 normal temperatures [38].

357 ***Anti-inflammatory studies***

358 Ibuprofen is a known poorly soluble drug that may suffer from inconsistent bioavailability
359 owing to inconsistent dissolution and absorption. It is well established that dissolution is the
360 rate-limiting step to absorption [24]. The improved aqueous solubility was a key factor to the
361 improved bioavailability and consequently anti-inflammatory activity. Poor drug dissolution
362 in the gastrointestinal tract (GIT) was probably responsible for the observed relatively low
363 anti-inflammatory activity of the reference drug. Other than poor water solubility, some drugs
364 are known to be susceptible to the degradation effect of stomach acid [7, 39]. SNEDDS
365 emulsify into nano droplets that offer gastro-protection to the entrapped drug solution and
366 thus prevent contact between the drug and stomach acid. This may also have contributed to
367 the observed increased in the anti-inflammatory activity of the ibu-SNEDDS. Lipid-based
368 formulations have been widely reported to promote lymphatic drug transport. Drug transport
369 via the lymphatic system avoids first pass effect and may consequently result in increased
370 plasma concentration and faster onset of action [2, 4, 6]. The higher anti-inflammatory
371 activity of ibu-SNEDDS is a combined result of the nanosize of the nanoemulsion, increased
372 dissolution rate of ibuprofen which would ease prompt absorption likely enhancement in
373 bioavailability due to the lipidic nature of the formulation, protection of the drug from the
374 acidic environment of the stomach.

375 **Conclusions**

376 SNEDDS containing the poorly water-soluble drug, ibuprofen, was prepared and optimized
377 by using *in vitro* parameters like globule size, polydispersity index and emulsification time.
378 The components and their ratio ranges for the formulation were determined by pseudo-

ternary phase diagram construction. The optimum formulation contains Labrafac CC as the oil phase, Cremophor EL as a surfactant, and PEG-400 as cosurfactant. The formulation consisted of 27 % caprylic/capric glycerides, 58 % cremophor EL and 15 % polyethylene glycol-400, yielded SNEDDS with a globule size of 25.23 and a PDI 0.093, and had sufficient drug loading and rapid self-emulsification in aqueous media. This optimized SNEDDS showed good *in vitro* release with about 15-fold increase over the pure ibuprofen sample. *In vivo* anti-inflammatory efficacy results showed that the developed ibu-SNEDDS exerted significantly ($P < 0.05$) higher anti-inflammatory activity than the reference ibuprofen powder. Our study illustrated the potential use of SNEDDS as a promising nano drug carrier for the efficient delivery of ibuprofen.

ETHICAL APPROVAL

The authors declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed. All experiments have been examined and approved by the Ahmadu Bello University Zaria, Nigeria, Committee on Animal Use and Care.

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