



SELF-EMULSIFYING DRUG DELIVERY SYSTEM BASED ON INDIGENOUS BREADFRUIT SEED OIL FOR ENHANCEMENT OF AQUEOUS SOLUBILITY OF ACECLOFENAC

ROMANUS CHIJIJOKE OMEH^{1,6,*}, IBEABUCHI JUDE ALI², CYRIL CHEKWUBE ADONU³, ASSUMPTA ADAOBI OMEH⁴, AUDU MUMUNI MOMOH⁵, GODSWILL CHUKWUNWEIKE ONUNKWO⁶, JACOB OKWUCHUKWU ONYECHI⁶

1. Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmaceutical Sciences, Enugu State University of Science and Technology, Enugu State, Nigeria
2. Department of Pharmaceutical Chemistry, Faculty of Pharmaceutical Sciences, Enugu State University of Science and Technology, Enugu State, Nigeria
3. Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmaceutical Sciences, Enugu State University of Science and Technology, Enugu State, Nigeria
4. Department of Biochemistry, Federal University of Technology, Owerri, Imo State, Nigeria
5. Drug Delivery Unit, Department of Pharmaceutics, University of Nigeria Nsukka, Enugu State, Nigeria
6. Department of Pharmaceutical Technology and Industrial Pharmacy, University of Nigeria Nsukka, Enugu State, Nigeria

Received 11 October, 2022; Revised 7 November, 2022; Accepted 7 January, 2023

ABSTRACT

The objective of the current work was to design and characterize aceclofenac-loaded breadfruit seed oil-based self-emulsifying drug delivery systems (SEDDS) with intent to improve the gastrointestinal fluid solubility of the drug. The breadfruit oil was extracted by the soxhlet solvent extraction technique. The oil was then characterized for acid, iodine, peroxide and saponification values. The solubility of aceclofenac in the breadfruit oils and in some selected potential components of the SEDDS was determined by the super saturation technique. Optimization of SEDDS formulation was achieved with the aid of pseudo ternary phase diagrams. The formulated SEDDS were characterized for standard SEDDS properties. The acid and iodine values of the oil were 3.40 ± 0.33 and 17.50 ± 0.05 respectively while the peroxide and saponification values were 7.20 ± 0.08 and 236.46 ± 0.02 respectively. Oil analysis revealed the presence of oleic, palmitic and linoleic acids among other constituents. Fourier transform infrared (FTIR) spectroscopy suggested no drug-excipient incompatibility. The optimized formulation exhibited average globule size of 84.67 ± 0.33 nm, zeta potential of -37.24 ± 0.04 and polydispersity index of 0.203. The SEDDS formulations showed higher in vitro drug releases than both the raw drug and a commercial aceclofenac tablet. The release data fitted more into the Hixson – Crowell model than into other release kinetic models. Based on the results, we concluded that breadfruit oil has the potential to be used as the oil component of a self-emulsifying drug delivery system formulated to enhance the aqueous/gastrointestinal fluid solubility of aceclofenac.

KEYWORDS: Breadfruit oil; Aceclofenac; Self-emulsifying drug delivery system; solid carrier; droplet size.

*Corresponding author: romanus.omeh@esut.edu.ng; +2347067175126
ajopred.com

INTRODUCTION

A self-emulsifying drug delivery system (SEDDS) is an isotropic mixture of oil (natural or synthetic), surfactant, co-surfactant and sometimes co-solvent, into which mixture, an active pharmaceutical ingredient has been dissolved and the resulting system having the unique characteristic of spontaneous formation of oil-in-water (o/w) macro, micro or nano-emulsion when introduced into an aqueous medium accompanied with mild agitation. [1, 2] For purposes of drug delivery, the drug is dissolved in the anhydrous lipid phase comprising of the oil, the surfactant and the co-surfactant prior to ingestion. When swallowed, the gastrointestinal fluid provides the required aqueous medium while the peristaltic movement of the stomach gives the gentle agitation needed for self-emulsification. The endogenous bile salts synergise with the formulation surfactants to promote faster emulsification process. The type of emulsion formed from a self-emulsification process depends on the nature and quantities of the components as well as the processing technique and environment. It is therefore, generally agreed that the tendency for spontaneous self-emulsification is highly specific to the nature of the oil/surfactant pair, surfactant concentration, oil/surfactant ratio and the subsisting formulation temperature. [3]

Self-emulsification promotes uniform dispersion of the drug-loaded oil droplets in the aqueous medium. This system is stabilized by the presence of surfactant and co-surfactant both of which reduce the interfacial tension between the water and oil phases.[4] Self-emulsifying drug delivery systems are associated with the formation of ultrafine oil droplet, high drug loading capacity, improved solubility of poorly water soluble drugs, reduced food effects and promotion of lymphatic drug delivery.[5] The system presents drugs in solubilised state to the absorption membranes thereby overcoming the challenges of poor solubility and limited absorption. The ultrafine droplet sizes also promote trans-membrane permeation, longer circulatory half-life and systemic bioavailability of drugs. SEDDS have, therefore become an attractive delivery option for poorly water soluble drugs such as aceclofenac. [6] Self-emulsifying formulations are categorized into three distinct types based on factors like, droplet sizes, transparency of the final product and the formulation components used. The droplet sizes of ordinary SEDDS range from 300 nm - 1 μ m. They exhibit white to milky appearance. Self-microemulsifying drug delivery system (SMEDDS) has droplet size in the range of 100-300 nm and

form transparent clear emulsion while self-nanoemulsifying drug delivery system (SNEDDES) has droplet sizes equal to or less than 100 nm and exhibit clear transparent appearance.[7]

Aceclofenac is the generic name of an active pharmaceutical compound chemically known as, 2-[2-[2-(2,6-dichloroanilino)phenyl]acetyl]oxyacetic acid and having the chemical formula, C₁₆H₁₃Cl₂NO₄ (US National Library). It is a non-steroidal anti-inflammatory drug (NSAID) and belongs to group II of the biopharmaceutics classification system (BCS), a group which members are reported to be poorly water soluble but highly membrane permeable and which solubility is their absorption rate limiting step. Oral delivery of this class of drugs in the conventional dosage forms presents challenges like unpredictable systemic absorption, high inter and intra subject delivery variability, lack of dose proportionality and low bioavailability. [8, 9] Self-emulsifying drug delivery systems are among some of the novel formulation interventions for overcoming the limitations of the BCS group II type of drugs.

Vegetable oils are very important components of many pharmaceutical formulations because of their natural biocompatibility, biodegradability, and low immunogenicity. They are also in the list of "Generally Regarded As Safe" (GRAS) materials. [10] In SEDDS, oils are the primary solvents for lipophilic drugs and also constitute the oil phase of the emulsion systems. Breadfruit oil is obtained from the seeds of African breadfruit (*Treculia africana*) which is reported to be native to East Indies but widely grown within the tropics. [11] The oil is reported to contain many long chain unsaturated fatty acids. [12] Aceclofenac has shown significant therapeutic and clinical usefulness in the management of many important inflammatory disorders. Its use is however limited by poor aqueous solubility, low gastrointestinal membrane absorption and unsatisfactory bioavailability. The objective of the current work was, therefore, to formulate aceclofenac in the form of self-emulsifying drug delivery system in order to enhance its solubility and systemic delivery. Some previous works had attempted this approach, but, to the best of our current knowledge no work had utilized breadfruit seed oil as the lipid component of aceclofenac based SEDDS, hence, the novelty of the current research. Probably, due to economic and developmental reasons, many nations are encouraging the exploration and use of locally available raw material for industrial applications. The challenge with this move is the poor

standardization and processing of such materials beyond their uses in crude forms. Breadfruits seeds are culturally consumed as fast food and snacks with no documented application in the pharmaceutical manufacturing sector. The current research would likely create awareness on the potentials of the breadfruit oil and equally stimulate more works on the applicability of other indigenous oils in pharmaceutical formulation. It is therefore an attempt to fill the gap between the consumption of crude local seeds and their industrial utilization. Future investigations may focus on improving the poor storage stability and low drug solvent capacity of the breadfruit oil.

METHODS

Materials

Breadfruit oil was extracted from the seeds of breadfruit heads purchased at Eke Umuitodo market in Amufie village of Enugu Ezike town in Igbo-Eze North Local Government Area of Enugu State, South East Nigeria. Polyoxyethylene sorbitan monooleate (Tween 80), polyethylene glycol-400 (PG-400) and Cremophor EL were products of Sigma-Aldrich, St Louise, USA. Microcrystalline cellulose (MCC) was a product of Fischer Scientific Fair Lawn, New Jersey USA. Double distilled water and n-hexane were purchased from Jeochem Chemicals Ltd. Nsukka, Enugu State, Nigeria. All other chemicals and reagents were of analytical grades and used as obtained except where otherwise specified.

Methods

Preparation of Breadfruit Seeds

The breadfruit samples were identified by Mr Patrick Obi of the Department of Pharmacognosy, Enugu State University of Science and Technology who also assigned the reference number, ESUT/DPC/TAXO/011/2021 and deposited a reference sample in the Faculty of Pharmaceutical Sciences herbarium. Initial processing of the seeds involved hand-picking from soft mashed mature breadfruit heads. The pericarp-bearing seeds were washed, parboiled for 65 min and threshed at a local threshing mill (ZKphMachines, Aba, Nigeria) to recover the clean seeds. The seeds were later sun-dried for 72 h and milled to fine powder using a domestic grinder, (GASCO Equipments, Aba, Nigeria). The resulting powder was wrapped in a black cellophane bag and stored in a refrigerator (Newclime M175 L) for further uses.

Extraction of Oil

The soxhlet solvent extraction procedure was used to extract the crude vegetable oil from the powdered seeds. In brief, for each round of extraction, 10 g of the powder was wrapped in a thimble and placed in the thimble chamber of the soxhlet extractor equipment. Two hundred and fifty millilitres (250 ml) of n-hexane was placed in the flask of the equipment and then heated at 70 °C to deliver steam to the powder until extraction was completed. The oil yield was calculated using equation 1,

$$\% \text{ oil yield} = \frac{\text{weight of oil}}{\text{weight of sample}} \times 100\% \text{ ----- eqn 1}$$

Refining of Breadfruit Oil

As part of the refining process, vegetable oil is usually degummed to remove water soluble phospholipids and other components that could cause darkening and/or produce unpleasant flavour in the oil. [13]. In brief, 250 mL of the oil was heated to 80 °C and then mixed under magnetic stirrer (100 rpm) with enough boiled water to produce a 5 % v/v mixture. The mixture was then centrifuged at 500 rpm for 20 min after which the aqueous portion was removed for bleaching. The bleaching was carried out by heating 300 g of the oil to 100 °C under vacuum for 30 min and then shaking with 9.00 g of bentonite using a mechanical shaker (DLAB SK R33-Pro). [14] The clean oil was recovered by filtration after a retention time of 30 min.

Characterization of Oil for Chemical Properties

The iodine, acid, peroxide and saponification values of the breadfruit oil were determined using various USP 31 [15] and AOCS (1995) [16] standard procedures. Acid value of oil is defined as the number in milligrams of potassium hydroxide (KOH) required to neutralize the free fatty acids present in 1 g of the oil. It was calculated using equation 2.

$$\text{Acid value} = \frac{56.1 V X N}{W}, \text{ ----- eqn 2}$$

where V = volume in mL of standard KOH used, N = Normality of the KOH solution and W = weight in gram of the oil sample.

Saponification value is the amount in mg of KOH required to neutralize the free fatty acids and saponify the esters in 1.0 g of oil. The blank titration procedure described in USP 31 [15] was used for the determination.

Peroxide value is the number that expresses in milliequivalents of active oxygen the quantity of peroxide contained in 1000 g of a substance. The

European Pharmacopoeia [17] official method was used for the determination. Briefly, 5 g of the oil was shaken with 30 mL of a mixture of glacial acetic acid and chloroform (3:2). A 0.5 mL volume of saturated potassium iodide solution was added and mixture shaken for exactly 1 min followed by addition of 30 mL of distilled water. The resulting mixture was shaken and titrated with 0.1N sodium thiosulphate solution until yellow colour was discharged. Five millilitres (5 mL) of soluble starch was then added and the titration continued until blue colour was fully discharged. A blank titration procedure without the oil was similarly carried out and the peroxide value calculated using equation 3.

$$\text{Peroxide value} = \frac{10(V_s - V_b)}{W_g} \text{----- eqn 3}$$

where V_s = volume in mL of 0.1 N sodium thiosulfate used in sample titration, V_b = volume in mL of 0.1 N sodium thiosulfate used in blank titration and W_g is the weight in gram of the oil used.

Preformulation Investigations

Drug Solubility Screening

The solubility of aceclofenac in the extracted breadfruit oil and some selected surfactants and co-surfactants was determined using the method reported in Subudhi and Mandal. [18] In brief, an excess amount of the drug (approximately 500 mg) was put in a 5 mL vial containing 2 mL each of one of the oils, surfactants or co-surfactants. The vials were securely covered and the contents mixed for 10 min on a vortex mixer, (Mixex 5000SP, Vaxas Equipments Mumbai). The mixtures were kept at $25 \pm 1.0^\circ\text{C}$ (ambient temperature) for 72 h to equilibrate. Thereafter the samples were removed and centrifuged at 3000 rpm for 15 minutes. The supernatants were separately taken and filtered through a $0.45\mu\text{m}$ whattman filter paper. The concentrations of the drugs in the various supernatants were determined at 760 nm wavelength using a uv/visible spectrophotometer (JENWAY 7305, Germany) after a 10-fold dilution with methanol.

Construction of Pseudoternary Phase Diagrams

Based on the results of the solubility screening of the aceclofenac and the component miscibility studies, Tween 80 and polyethylene glycol 400 (PEG 400) were selected as the surfactant and co-surfactant respectively for the formulation of the current SEDDS. Seven different mixtures of the Tween 80 and the PEG – 400 were prepared in 5

mL capacity test tubes in the following surfactant to co-surfactant ratios (Smix); 1 : 1, 1 : 2, 1 : 3, 2 : 3, 2 : 1, 3 : 1 and 3 : 2 and designated as Smix_{1:1}, Smix_{1:2}, Smix_{1:3}, Smix_{2:3}, Smix_{2:1}, Smix_{3:1}, and Smix_{3:2} respectively. Each of these ratio mixtures was further mixed with breadfruit oil in the following oil to Smix ratios; 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2 and 9:1 thereby generating 63 different oil–Smix mixtures. The mixtures were further blended using a vortex mixer (Mixex 5000SP, Vaxas Equipments Mumbai) at 1500 rpm for 5 min.

In a dropwise manner, double distilled water was carefully added to each mixture accompanied with gentle shaking of the tubes while being carefully observed for the formation of clear/transparent, turbid or milky emulsion. The ratio of the three components, Smix, oil and water at which the change occurred was noted and used to construct the pseudo ternary phase diagram employing Sigma Plot-14 software. Regions of maximum emulsification were delineated from the diagram from which percentage weights of the individual components that yielded optimized SEDDS were also determined.

Formulation of Aceclofenac-free SEDDS

The proportions of the breadfruit oil, Smix and double distilled water for optimized formulations were determined from the pseudoternary phase diagrams and used to formulate liquid SEDDS for Smix ratios of 1:1, 3:2 and 3:1. Carbosil (2 % w/w) was introduced into the formulations to promote droplet dispersion.[19]. Accurately weighed quantities of each ingredient except water was placed together in a 100 mL capacity glass beaker and heated over a magnetic stirrer heater set at 40°C temperature and stirring speed of 30 rpm. After 2 min, pre-determine quantity of water was added gradually with continuous slow stirring until the emulsion was formed depicted by sudden formation of transparent, translucent or milky/coudy mixtures. Weighed 30 mg of aceclofenac powder was then gradually added to each formulation with continuous stirring until the drug was completely dissolved. Table 1 shows the various materials and their percentage proportions used in the formulation of the SEDDS

Evaluation of Formulated SEDDS

Compatibility Studies

Fourier transform infrared spectroscopic analysis was used to investigate the drug–excipient compatibility in the formulated SEDDS. For each formulation, separate smears of dichloromethane solution of the two samples (pure drug and SEDD)

were made on two sodium chloride crystal sample cells. The cells were securely covered and placed in the sample holder of the FTIR machine. Sample scanning was carried out within the range of 4000 - 400 cm^{-1} .

Thermodynamic Stability Studies

One hundred millilitres (100 mL) of each optimized formulation was subjected to various extreme conditions of temperature and treatment under a set of tests referred to as; heating and cooling cycle, centrifugation test and Freeze-Thaw cycle tests to study the stability potentials of the formulations under these stress conditions.

Heating and Cooling Cycle

The samples were in a cycle of six times stored alternately at refrigerator temperatures of 4 °C and 40 °C with each storage period lasting for 48 h. The formulation(s) which remained stable after this set of treatments were taken for the next tests.

Centrifugation Test

Formulations that passed the heating and cooling cycle tests were placed in 10 mL capacity vials and centrifuged at a speed of 3500 rpm for 30 min under ambient temperature (25 °C) in a laboratory centrifuge, (CentriTemp. Shimadzu Corporation, Japan). Formulations that remained homogenous without phase separation were graded as having passed the test and were taken for the next procedure.

Freeze-Thaw Cycle Tests

The samples were stored alternately at two temperatures of -21 °C and +25 °C with each storage period of 48 h and observed for physical integrity. Samples that remained stable at the end of this test were adjudged to have passed the thermodynamic stability tests. [20]

Emulsification Efficiency Test

The emulsification efficiency of various anhydrous SEDDS mixtures and water was studied using the visual grading model reported by Khoo et al. [21] The test investigated both the rapidity of emulsion formation and the appearance of the final preparation in the process of self-emulsification. The USP XXII dissolution apparatus 2 (paddle method) was used for the current work. Briefly, 1 mL portion of each formulation was added to 500 mL of distilled water placed in the beaker of the apparatus and maintained at a temperature of 37 ± 0.5 °C. The paddle rotation speed was 50 rpm. The dispersion of the anhydrous SEEDS in water was

visually observed and interpreted using the A, B, C, D and E grading format proposed by Khoo et al. [21].

Test of Robustness to Dilution

Robustness to dilution test studies the stability profiles of SEDDS under large volume dilution and under various pH levels as may be encountered in vivo in the various sections of the gastrointestinal tract. For each optimized formulation. 3 separate 5 mL portion was removed and placed in separate 500 mL capacity flat bottomed flask. The content of the three flasks were made up to 500 mL with double distilled water, simulated gastric fluid – 0.1N HCl and simulated intestinal fluid (SIF) – phosphate buffer (pH 6.8) respectively. The content of each flask was thoroughly stirred and allowed to stand under ambient temperature for 48 h after which each formulation was visually examined. Samples that retained homogenous appearance without phase separation or drug precipitation in the three flasks were considered as robust to both pH and dilution.

Droplets Size, Polydispesity Indices and Zeta Potential Measurements

These three parameters were determined in single test runs. A zetasizer equipment, (Zetasizer Nano ZS - Malvern, UK) was used for the study. A 5 mL aliquot of each formulation was diluted to 1000 mL with double distilled water after which the mixture was thoroughly mixed with a stirring rod. The droplet sizes, zeta potential and polydispersity indices of the resulting emulsions were determined concurrently by laser diffraction analysis in which light scattering was monitored at a temperature of 25°C at angle 90°.

Determination of Cloud Point

The cloud point values of the three drug – loaded SEDDSs were determined as follows: Each formulation was diluted with water in the ratio of 1 : 100 and placed in a water bath initially set at a temperature of 25 °C. The temperature was gradually increased at a rate of 2 °C/min up to 90 °C. [22] Cloud point was regarded as the temperature at which there was a sudden appearance of cloudiness observable visually. [23]

Determination of pH and Viscosity

The pH of a 2 ml aliquot of each preparation was determined in triplicates using a digital pH meter meter (Mettler Tornado, PCE – 224HTE, China) while the viscosity was determined using a

Brookfield viscometer, (Visco - CPE40; Brookfield Engineering Inc, Middleboro).

Dissolution Rate Test

The USP apparatus II - paddle method (Model TDT-081, Electrolab, Mumbai) was used to study the release profiles of three representative SEDDS comprising ALS 01, ALS 02 and ALSS 01 as well as one commercial sample of aceclofenac tablet and the raw drug. For each round of test, a quantity of sample equivalent to 100 mg of aceclofenac was introduced into an appropriate dissolution medium maintained at a temperature of 37 ± 2 °C. The paddle speed was set at 50 rpm. At intervals of 10 min, 5 mL of the medium was withdrawn, diluted appropriately, filtered and assayed for aceclofenac using a UV spectrophotometer (JENWAY 7305, Germany) at a wavelength of 276 nm. The volume removed for assay was immediately replaced with fresh equal volume of the dissolution medium to maintain a sink condition. The concentration of aceclofenac in the dissolution medium was corrected for dilution and sampling effects using equation 4.

$$C_n = M_n \left[\frac{V_t}{(V_t - V_s)} \right] \times \left[\frac{(C_{n-1})}{(M_{n-1})} \right] \text{-----eqn 4}$$

where, C_n was the corrected concentration of the n th withdrawn sample, M_n is the measured concentration of the n th withdrawn sample, V_t is the total volume of the dissolution medium, V_s is the volume of the withdrawn sample, C_{n-1} is the corrected concentration of the previous sample. [24, 25] The cumulative percentage amount released at various time intervals were plotted against time.

Investigation of Drug Release Kinetics

The kinetics of drug release from various SEDDS formulations were investigated by fitting the release data into some mathematical kinetic models. The models studied included, zero order, first order, Higuchi [26], Korsmeyer-Peppas [27] and Hixson-Crowell [28] models. By plotting the chart of appropriate variables for each model, both the regression coefficient (r^2) and the release constants (k) were determined and used to establish the goodness of fit of each set of release data for each model.

Statistical Analysis of Data

Data analysis was done using the one-way analysis of variance (ANOVA - single factor) statistic and differences between means were interpreted using the student's t-test statistic. Statistically significant

differences were concluded at $p < 0.05$ while all values were expressed as mean \pm standard deviation (SD).

RESULTS

Breadfruit Oil Yield and Characteristics

Table 2 displays the percentage yield and some physicochemical properties of the breadfruit oil.

Aceclofenac Solubility Screening

Figure 1 shows the solubility screening chart of aceclofenac in selected solvents.

The aceclofenac was practically insoluble in water and in the crude breadfruit oil. The solubility in these two solvents were 0.184 mg/mL and 0.48 mg/mL respectively. The drug, however, showed improved solubility of 23.07 mg/mL in the hydrolysed vegetable oil. Solubility in castor oil, soya-bean oil and kulliphore were 104.39, 50.10 and 98.39 mg/mL respectively. The drug was freely soluble in Tween 80 (195.66 mg/mL) while also showing good solubility in Tween 20, the polyethylene glycols and propylene glycol.

Emulsification Efficiency Test

Two optimized formulations designated as, ALS 01 and ALS 02 rapidly formed transparent bluish-white emulsions within 17 and, 21 sec respectively. Formulations ALS 03 and 04 took longer time to emulsify and yielded whitish milky non transparent mixture. Other formulations took longer time to emulsify while some did not form stable emulsion at all even after prolonged stirring whereas some other formulations, after forming emulsions reverted back to two phases upon standing. It was observed that the two formulations that yielded clear emulsion had higher proportions of Tween 80 in the surfactant/co-surfactant mixtures (Smix).

Pseudoternary Phase Diagrams

Phase diagrams were constructed to depict visually the components and their relative concentration ranges that resulted in large regions of self-emulsification. Figure 2 shows phase diagrams for surfactant/co-surfactant mixture (Smix) ratios of 3:1, 2:1 and 2:3. Figure 3 displays the parboiled dehulled breadfruit seeds while Figure 4 shows samples of formulated SEDDS.

Thermodynamic Stability Studies

The two optimized formulations also passed the thermodynamic stability tests. They remained homogenous without phase separation or drug precipitation after storage at extreme temperatures

and vigorous centrifugation. The possible implication is that these formulations are likely to remain stable under normal storage, handling and other stressful conditions.

Component Compatibility Studies

The compatibility of the various formulation materials was investigated using the FTIR spectroscopic analysis. Figure 5 and 6 show the FTIR spectrum of the pure aceclofenac and that of the aceclofenac-loaded SEDDS respectively.

Droplets Size Distribution, Zeta Potential and Polydispersity Indices of SEDDS

Figures 7 and 8 show the charts of the droplet size distribution of formulations ALS 01(Smix3:1) and ALS 02 (Smix 2:1). The droplet sizes and other evaluated parameters for various formulations are also shown on Table 3.

The mean emulsion droplets sizes (Z average) of three optimized formulations, ALS 01, ALS 02 and ALS 03 were 84.67 ± 0.33 , 98.28 ± 0.45 and 102.11 ± 0.32 respectively. The PDI values ranged from 0.203 – 0.770 while all the SEDDS had negative zeta potentials ranging from -37.24 ± 0.04 to -29.65 ± 0.14 mV. The viscosity of the various formulations ranged from 19.34 ± 2.2 to 38.35 ± 4.5 cP whereas the cloud point range was 77 ± 0.33 - 87 ± 7.05 . Formulations ALS 01, 02 and 03 exhibited transmittance values above 99 % while formulations ALS 04, 05 and 06 showed poor transmittance values below 80 %. The pH of the various formulations was around the neutral range and all were robust to media pH and volume adjustments.

In Vitro Dissolution Studies

The dissolution profiles of various samples are shown in Figures 9 and 10.

The figures suggested that the three SEDDS formulations experienced initial rapid/burst releases of their drugs thereby releasing more than 75 % of their content in less than 30 min and with $t_{1/2} < 20$ min in the phosphate buffer. Formulations ALS 01, ALS 02 and ALSS 01 attained maximum drug releases of 84.14 ± 0.65 , 78.89 ± 0.55 , and 81.33 ± 0.51 % at 30, 30 and 40 min respectively. Release of aceclofenac into the phosphate buffer solution (pH 6.8) were significantly higher ($p < 0.05$) than releases into the simulated gastric fluid 0.1N HCl (pH 0.2). No formulation attained 50 % release in the 0.1N HCl (pH 0.2) medium. This suggests that though the SEDDS formulations were robust to pH changes, actual drug release was pH dependent.

Fitting of Release Data into Selected Kinetic Models

Table 4 presents the summary of the regression coefficients and rate constants for the various models and the “n” values of the Korsmeyer-Peppas plots.

DISCUSSION

The percentage yield of the oil was comparable to the values of 15.58 – 19.30% reported by Nwabueze and Nwafor, [29] and those of cottonseed (15.00 – 24.00%) and soybean (17.00 – 21.00%), obtained by Pritchard [30].

The mean peroxide value of 6.33 ± 0.73 was considered good for edible oil. A peroxide value below 10 meq/kg is an evidence of fresh oil free from oxidative degradation and rancidity. [31] Similarly the acid value of the current oil was comparable to 22.45 % obtained by Ajiwe et al. [32] Low acid value is a desirable characteristic of edible oils. The degree of unsaturation and susceptibility to oxidative degradation of a vegetable oil may be deduced from the iodine value. High values are indicative of the presence of large proportion of unsaturated fatty acids. [33, 34] The 24.16 ± 0.54 value obtained for our sample is relatively good. On the other hand, high saponification value may be an evidence of oil deterioration during storage and/or the presence of long carbon chains and large molecular weight fatty acids.[35] Breadfruit oil has long chain, high molecular weight fatty acids hence the relatively high value of the saponification number. [36]

Aceclofenac exhibited poor solubility in both distilled water (0.184 mg/mL) and crude breadfruit oil (0.48 mg/mL). Poor aqueous solubility was expected since aceclofenac is a hydrophobic compound and belongs to group II of the biopharmaceutics classification system (BCS).[37] Similarly, presence of long chain unsaturated fatty acids in the crude oil limits its solubilising capacity. Soybean and castor oils showed superior solubilizing effects of 50.10 and 104.39 mg/mL respectively over the breadfruit oil. Among the tested surfactants, aceclofenac exhibited the highest solubility of 195.66 mg/mL in Tween 80 and 106.13 mg/mL in polyethylene glycol (PEG-400). The two solvents were therefore selected as the surfactant and co-surfactant respectively for the current formulations.

Spontaneous formation of emulsion upon introduction of anhydrous SEDDS into an aqueous medium is a major attribute of self-emulsifying drug delivery systems. Rapid emulsification is important

for fast gastrointestinal spreading of SEDDS, quick release, fast absorption and adequate systemic uptake of drugs. The self-emulsification efficiency

study was carried out to observe the rapidity at which various formulations go into in vitro self-emulsification upon mild stirring in water.

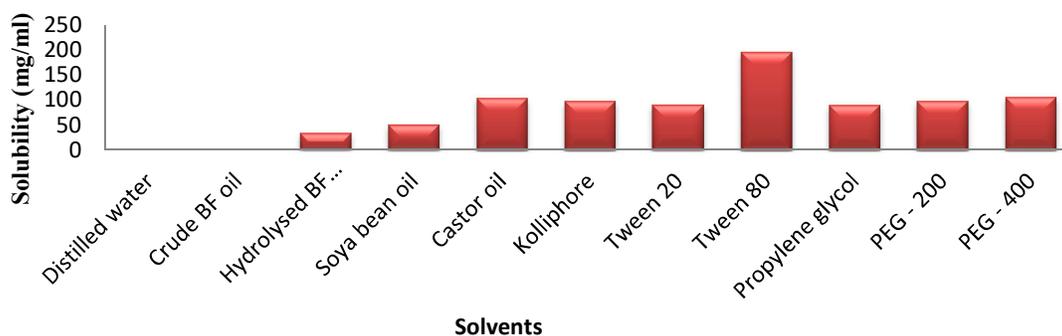


Figure 1: Solubility chart of aceclofenac in selected solvents

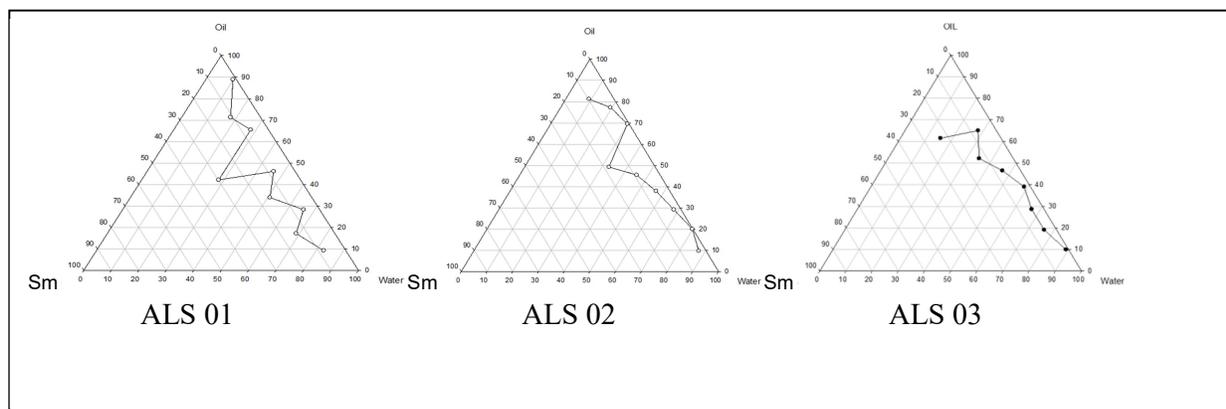


Figure 2: Pseudoternary phase diagrams for ALS 01 (Sm 3:1), ALS 02 (Sm 2:1) and ALS 03 (Sm 3:2)



Figure 3: Dehulled breadfruit seeds

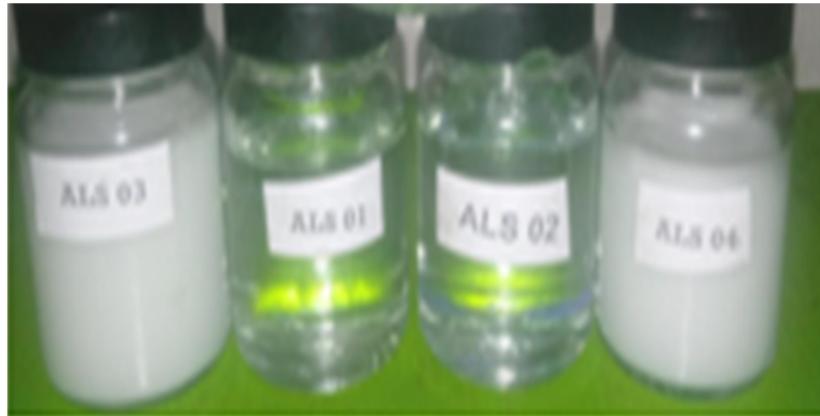


Figure 4: Formulated SEDDS

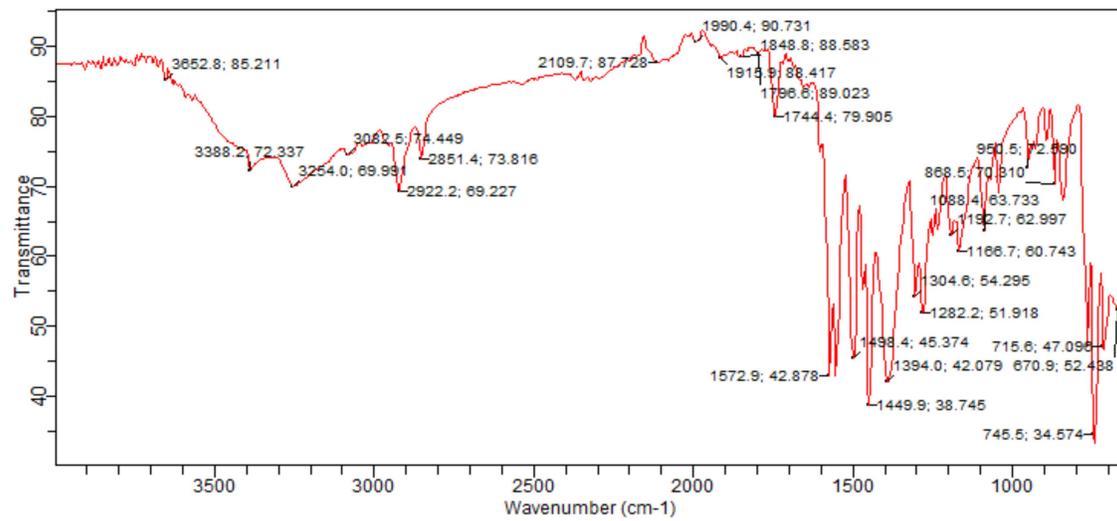


Figure 5: FTIR spectra of pure aceclofenac

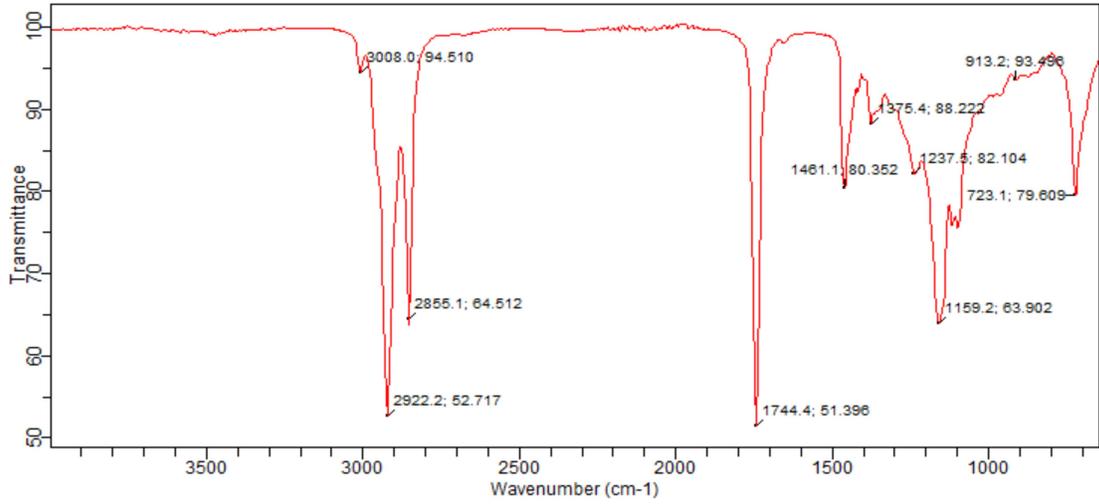


Figure 6: FTIR spectra of aceclofenac-loaded SEDDS

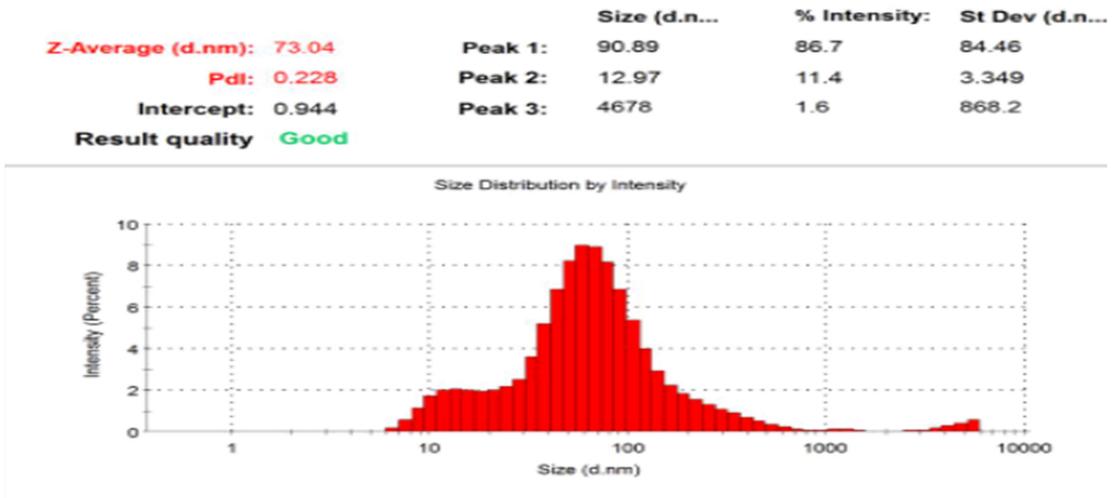


Figure 7: Size distribution of ALS 01

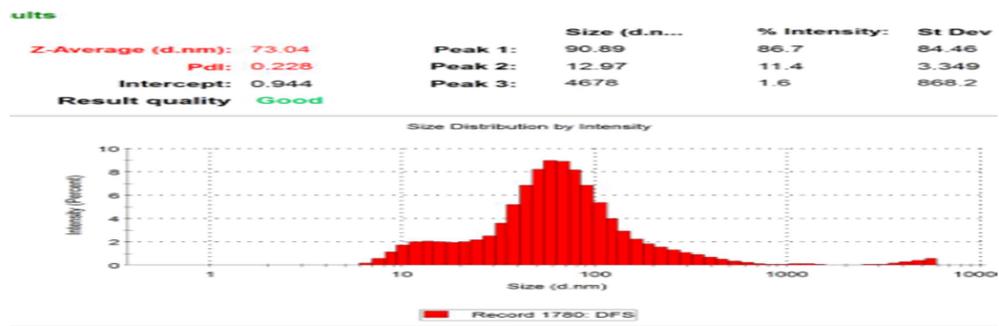


Figure 8: Size distribution of ALS 02

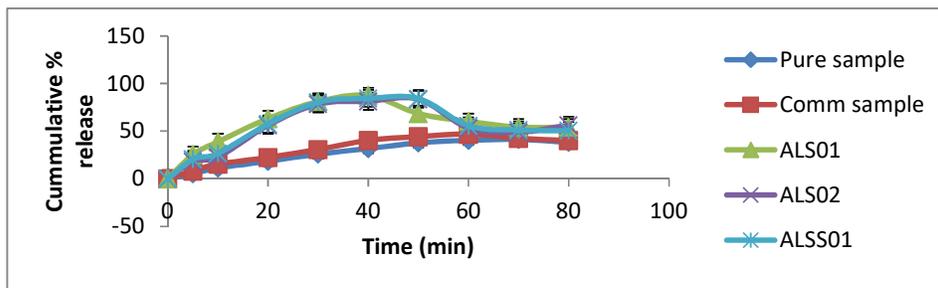


Figure 9: Aceclofenac release profiles of various formulations and pure sample in phosphate buffer (pH 6.8)

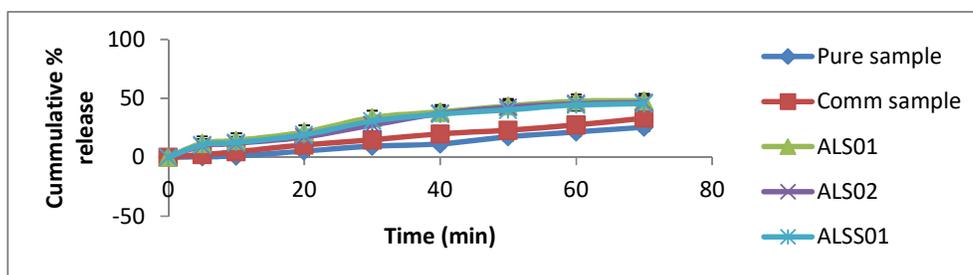


Figure 10: Aceclofenac release profiles of formulations and pure sample in 0.1N HCl (pH 0.2).

Table 1: Formulation of aceclofenac-loaded SEDDS

Components (w/w %)	Formulations/Smix ratios		
	ALS 01(Smix 1:1)	ALS 02 (Smix 3:1)	ALS 03 (Smix 3:2)
Aceclofenac	30.00	30.00	30.00
Bread fruit oil	17.65	17.18	18.32
Tween-80/PEG400	37.90	41.34	46.30
Carbosil	2.00	2.00	2.00
Water	12.52	9.48	3.38
Total	100.00	100.00	100.00

Table 2: Percentage yield and physicochemical properties of breadfruit oil

Parameter	Result
% yield	22.32
Color	yellow
Odour	characteristic
Viscosity (cP) at 25 °C	29.43 ± 0.46
Acid value (%)	5.86 ± 0.54
Iodine value	24.16 ± 0.54
Peroxide value	6.33 ± 0.73
Saponification value	317.31 ± 0.97

Table 3: Physicochemical characteristics of the formulated SEDDS

Code	pH	Viscosity (cPs)	Transmittance (%)	Cloud point (°C)	ZP (mV)	Droplet size (nm)	PDI
ALS 01	7.8 ± .67	31.19 ± 0.7	99.73 ± 2.4	87 ± 7.05	-37.24 ± 0.04	84.67 ± 0.33	0.203
ALS 02	7.2 ± 7.8	19.34 ± 2.2	99.96 ± 5.4	77 ± 0.33	-31.66 ± 0.45	98.28 ± 0.45	0.229
ALS 03	7.7 ± 2.5	21.34 ± 0.6	99.32 ± 0.4	70 ± 7.54	-34.03 ± 0.74	102.11 ± 0.32	0.341
ALS 04	7.4 ± 7.8	28.74 ± 1.1	62.96 ± 5.2	N/A	-29.65 ± 0.14	698.56 ± 1.34	0.613
ALS 06	6.7 ± 2.3	33.08 ± 1.2	60.87 ± 2.1	N/A	-30.98 ± 0.47	997.43 ± 53	0.712
ALS 08	8.2 ± 0.3	38.35 ± 4.5	70.76 ± 0.2	N/A	-36.72 ± 0.15	1612.14 ± .05	0.770

Values = mean ± SD; n = 3, N/A – Not applicable, ZP = zeta potential

Table 4: Summary of release kinetic data of various kinetic models

Batch	Kinetic models										
	Zero order		First order		Huguchi		Korsmeyer – Peppas			Hixson- Crowell	
	r ²	k	r ²	k	r ²	k	r ²	k	n	r ²	k
ALS 01	0.806	12.38	0.61	-0.09	0.783	35.9	0.881	0.006	0.014	0.981	-0.029
ALS 02	0.805	10.35	0.61	-0.09	0.783	35.9	0.889	0.006	0.014	0.901	-0.204
ALSS 01	0.896	1.261	0.8	-0.01	0.869	11.42	0.813	0.003	2.322	0.91	-0.034
ALSS 02	0.937	8.127	0.8	-0.05	0.879	27.37	0.923	0.008	0.01	0.976	-0.295

Formulations ALS 01 and ALS 02 which exhibited rapid formation of transparent bluish-white emulsions within 17 and 21 sec respectively fall into grade A of the Khoo et al [21] grading system while formulations ALS 03 and ALSS 01 that formed whitish-milky emulsion are of group C which are characterised by formation of fine milky emulsion within 2 min. Formulations ALS 01 and ALS 02 had higher concentrations of the surfactant mixture than ALS 03 and ALSS 01. The possible explanation for the direct proportionality between speed of self-emulsification and the concentration of Smix is that the concentration of surfactants influenced the quality and rapidity of self-emulsification by reducing the interfacial tension between the oil and water phases and by the ability of the co-surfactant to enhance interfacial film curvature.[38] Co-surfactants equally enhance the hydrophilicity of the SEDDS system thereby promoting faster dispersion of the oil phase (SEDDS) in the aqueous medium. [39]

It was observed that the formulation prepared with Smix ratio of 3:1 yielded largest self-emulsification region. It also exhibited finest droplet sizes and lower polydispersity index than the rest of the formulations. The observation again confirms the influence of surfactant in the formation, quality and stabilization of emulsions. successfully produced.

Generally emulsions with PDI value below 1 is considered as good in terms of droplet size uniformity. [42] This value suggests close droplet size distribution within each formulation. This is advantageous for reproducible membrane permeation, drug loading and release.

The zeta potentials of the various formulations are also shown in Table 3. Zeta potential is an important surface parameter of the emulsion droplets. It is indicative of the predominating surface charge of the dispersed droplets and is critical for the emulsion stability. Similar charges on the droplet surfaces ensure inter droplet repulsion and adequate and continued dispersion of the droplets thereby reducing the chances of coalescence and phase separation. High zeta potential is associated with greater inter-droplet repulsion. The optimized SEDDS had negative surface charges and zeta potentials ranging from -37.24 ± 0.04 mV to -29.65 ± 0.14 which is considered as good for the stability of the various formulations. A zeta potential value of ± 30 mV is sufficient to ensure sustained dispersion of emulsion droplets.[43]

Most SEDDS formulations are prepared as liquid dosage forms to be directly administered as such or

The FTIR spectral peaks at 3254.0 and 3651.8 followed by a band at 1304.6 are indicative of the presence of OH/COOH groups while the peak at 3082 is suggestive of the presence of unsaturated aromatic bonds. The peak at 1744.4 is characteristic of carbonyl group (alkyl carbonate) while the peak at 2922.2 is descriptive of methylene C-H asym/sym stretch, Exact peaks at 2922.2, 1744.4 and 28551.1 cm^{-1} were prominently observed in both spectra for pure aceclofenac and the aceclofenac-loaded SEDDS suggesting that no molecular alteration from chemical interaction between the drug and the excipients occurred.[40, 41] Observed shifts in peaks at the lower range of the spectra may be attributed to the fact that the aceclofenac in the SEEDS formulation is in a solubilized state.

Droplet size is a critical parameter in the stability, loading capacity and membrane permeation of emulsion-loaded active ingredients. Droplet size reduction to micro or nano scale can also confer on some drugs novel physicochemical, pharmacokinetic and even therapeutic characteristics that are lacking in the original source drugs. The droplet size distributions obtained for the various formulations show that emulsions in the nano, micro and macro size scales were

poured into gelatin capsules. In both cases, the viscosity of the preparation plays important roles. Very light preparations may be difficult to handle due to easy spillage or sorption through gelatin capsule shells. On the contrary, highly viscous preparations may face the problem of poor pourability, difficulty in filling into narrow-necked bottles and poor patient acceptability for oral administration.

A well formulated oral SEDDS must remain stable while in transit or resident within the gastrointestinal tract. The preparation must be robust to varying physiologic pHs and fluid volumes to avoid loss of emulsion characteristics and drug precipitation at any point in the GIT. Results in Table 3 show that the formulations maintained their stability under simulated gastric and intestinal fluid pHs and their varying volumes

Similarly, transparency has inverse relationship with the droplet sizes of the SEDDS formulations and can be used to monitor the stability of the preparation. The transmittance values of the optimized formulation are shown on Table 3 and indicate that formulations with higher surfactant/co-surfactant concentration exhibited higher transmittances possibly due to more efficient emulsification. Rani et al. [44] proposed that

transmittance value $\geq 99\%$ signifies good emulsification.

Cloud point determination was done to ascertain the minimum temperature at which the product transparency is lost signalling the onset of temperature related deterioration. The cloud points of all the formulations were well above normal room temperature. This suggested that the formulation will remain stable and transparent under normal environmental and storage temperatures. [45]

The release profiles of the various formulations indicated that aceclofenac showed preferential releases into the phosphate buffer as against the 0.1N HCl.

The possible reason was that aceclofenac, being a weak acid molecule ($pK_a = 4.7$) ionized more in low pH environment resulting in lower solubility. [10] It was also generally observed that, for both media, release of drug from SEDDS were higher and faster than releases from the commercial aceclofenac tablets and the raw aceclofenac powder. It may therefore be averred that the SEDDS system actually improved the solubility of aceclofenac in both dissolution media.

The release data obtained from the dissolution profile studies were subjected to statistical analysis using the Excel Ad-in single factor analysis of variance (ANOVA) software with $\alpha = 0.05$. The obtained p value was $0.00428 < 0.05$ and f value $5.21868 > 0.05$ hence the null hypothesis ($H_0: \mu_1 = \mu_2 = \mu_3 = \mu_4 = \mu_5$) was rejected. It was then concluded that there were significant differences among the dissolution profiles of the various formulations including the commercial sample and the raw aceclofenac. A similar statistical analysis for the two SEDDS only, gave a $p > 0.05$ denoting no significant difference between the release profiles of the SEEDS formulations.

Drug release data yielded mixed release kinetics models with Hixson-Crowell model predominating. Hixson-Crowell r^2 values of the four tested formulations were above 0.90. Release data for other formulations, showed relative good fit into the Korsmeyer–Peppas model with r^2 values above 0.81. The preferential fitting of the release data into Hixson–Crowell model may be attributed to the nature of SEDDS. The drug is presented to the media in solubilised form and as such influence of diffusion path length as is applicable in the Higuchi model and impact of undissolved portion of the drugs as in the case in zero and first order models are minimal or even non-existent. Ramteke [46] noted that the Hixson–Crowell model “is used to describe a system in which, it is considered that the release rate of drug particles is limited by the

dissolution rate and not by the diffusion that take place within the polymeric matrix.”

CONCLUSION

Poor aqueous solubility of many clinically important drugs like aceclofenac is a major challenge to their oral administration. Formulation of such drugs in form of self-emulsifying drug delivery system has been reported to enhance their solubility. This work has successfully demonstrated that the solubility of aceclofenac can be enhanced by loading it into a self-emulsifying drug delivery system prepared using breadfruit oil as the major lipid phase. This has been further demonstrated by the higher in vitro release of the drug from SEDDS formulation vis-a-vis some equivalent conventional aceclofenac formulations and the raw powder samples. Increase in aqueous solubility will likely result in enhanced drug absorption and higher systemic bioavailability. The implication of the results is improved drug delivery and better treatment outcome as well as economic benefits arising from the utilization of a local raw material. It may then be concluded that breadfruit oil, apart from its food value, possess good potential for pharmaceutical application.

REFERENCES

1. Midha K, Nagpal M, Singh G, Aggarwal G. Prospectives of Solid Self-microemulsifying Systems in Novel Drug Delivery, *Current Drug Delivery*, 14(8), 2017, 1078-1096. doi: 10.2174/1567201813666160824123504
2. Singh S, Bajpai M, Mishra P. Self-Emulsifying drug delivery system (SEDDS): an emerging dosage form to improve the bioavailability of poorly absorbed drugs. *Critical Reviews Therapeutic Drug Carrier Systems*. 37(4), 2020, 305-329 doi: 10.1615/CritRevTherDrugCarrierSyst.2020.033111.
3. Wadhwa J, Nair A, Kumria R. Emulsion forming drug delivery systems for lipophilic drugs. *Acta Polonica Pharmaceutica – Drug Research*. 69(2), 2012, 179-191
4. Halim A, Jindal K, Tarique M. Solubility enhancement of poorly soluble drug by self emulsifying drug delivery system: comprehensive review. *World Journal of*

- Pharmaceutical Research, 10. 2021. 840–52
5. Amara S, Bourlieu C, Humbert L, Rainteau D, Carrière F Variations in gastrointestinal lipases, pH and bile acid levels with food intake, age and diseases: possible impact on oral lipid-based drug delivery systems. *Advanced Drug Delivery Reviews*, 142. 2001, 3–15.
 6. Akula S, Gurram AK, Devireddy SR. Self-microemulsifying drug delivery systems: an attractive strategy for enhanced therapeutic profile, *International Scholarly Research Notices*, 20(14), 2014, 1811-1823.
 7. Rani ER, Radha GV. Insights into Novel Excipients of Self-Emulsifying Drug Delivery Systems and Their Significance: An Updated Review. *Critical Reviews on Therapeutic Drug Carrier Systems*, 38(2), 2021, 27-74. doi: 0.1615/CritRevTherDrugCarrierSyst.2020034975
 8. de Oliveira MC, Bruschi ML. Self-emulsifying systems for delivery of bioactive compounds from natural origin. *AAPS PharmSciTech*. 23, 2022. 1–19.
 9. Ruiz PSL, Serafini MR, Alves IA, Novoa DMA. Recent progress in self-emulsifying drug delivery systems: A systematic patent review (2011-2020). *Critical Reviews on Therapeutic Drug Carrier Systems*, 2022, 39(2), 1-77 DOI:10.1615/CritRevTherDrugCarrierSyst.2021038490
 10. US National Library of Medicine, National Center for Biotechnology, PubChem CID: 71771
 11. Tropical Plants Database, Ken Fern. *Tropical-the-ferns.info*. Available at, <tropical.theferns.info/viewtropical.php?id =Treculia+Africana> Assessed, June 19, 2021
 12. Amos-Tautua B. M. W., Onigbinde A. O. Physicochemical properties and fatty acid profiles of crude oil extracts from three vegetable seeds. *Pakistan Journal of Nutrition*, 12(7). 2013, 647 – 650.
 13. Zufarov O., Schmidt S., Sekretar S. Degumming of rapeseed and sunflower oils, *Acta Chimica Slovaca*. 1(1), 2008, 321–328
 14. Usman, MA Ekwueme VI., Alaje TO., Mohammed AO. Characterization, acid activation, and bleaching performance of ibeshe clay, Lagos, Nigeria, *ISRN Ceramics*, 2012, 2012, 97 - 102 Article ID 658508
 15. United States Pharmacopoeia/National Formulary 31. *The United States Pharmacopeia Convention*. Rockville MD USA Volume III.
 16. AOCS Official methods, Sampling and analysis of commercial fats and oils. *American Oil Chemists Society*, Chicago. (1995).
 17. AOCS Official methods, Sampling and analysis of commercial fats and oils. *American Oil Chemists Society*, Chicago (1997).
 18. Subudhi¹ BB., Mandal S. Self-microemulsifying drug delivery system: formulation and study of intestinal permeability of ibuprofen in rats. *Journal of Pharmaceutics*, 33(8), 2013, Article ID 328769, <http://dx.doi.org/10.1155/2013/3287>
 19. Obite N, Ofokansi KC, Nzekwe IT, Esimone CO, Okoye I. Self-nanoemulsifying drug delivery systems based on melon oil and its admixture with a homolipid from *Bos indicus* for the delivery of indomethacin, *Tropical Journal of Pharmaceutical Research*, 10(3). 2011, 299 – 307.
 20. Potharaju S, Mutyam SK, Liu M, Green C, Frueh L, Nilsen A, Pou S, Winter R, Riscoe MK, Shankar G. Improving solubility and oral bioavailability of a novel antimalarial prodrug: comparing spray-dried dispersions with self-emulsifying drug delivery systems. *Pharmaceutical Development and Technology*, 25(5), 2020, 625-639 doi: 10.1080/10837450.2020.1725893. Epub 2020 Feb 12.
 21. Khoo SM, Humberstone AJ, Porter CJ, Edwards GA, Charman WN. Formulation design and bioavailability assessment of lipidic self-emulsifying formulations of Halofantrine.. *International Journal of Pharmacy*, 167. 1998. 155-164
 22. Kovvasu SP, Kunamaneni P, Joshi R, Betageri GV. Self-emulsifying drug delivery systems and their marketed products: a review. *Asian Journal of Pharmaceutics*. 13 (2). 2019. 73 – 94
 23. Penjuri SCB, Damineni S, Ravouru N, Poreddy SR. Self-emulsifying drug delivery system (SEDDS) of Ibuprofen:

- formulation, in vitro and in vivo evaluation. *Ceska a Slovenska Farmacie*, 66, 2017, 23–34
24. Mazumder B., Dey S., Bhattacharya S., Sarkar S., Mohanta B. Studies on formulation and characterization of cellulose-based microspheres of chlorpheniramine maleate. *Archives of Pharmaceutical Sciences Research*, 1, 2009. 66-74.
 25. Sachan NK., Bhattacharya A. Modelling and characterization of drug release from glutinous rice starch based hydrogel beads for control drug delivery. *International Journal of Health Research*, 2(1), 2009. 93-99.
 26. Huguichi T. Mechanism of Sustained Action medication: theoretical analysis of rate of release of solid drugs dispersed in solid material. *Journal of Pharmaceutical Sciences* 52, 1963. 1145 -1149.
 27. Korsmeyer, RW., Gurny R., Doelker E., Ruri P., Peppas N.A. Mechanisms of solute release from porous hydrophilic polymers. *International Journal of Pharmacy*, 15, 1993, 25-35.
 28. Hixson AW., Crowell J.H. Dependence of reaction velocity upon surface agitation: theoretical consideration, *Journal of Industrial and Engineering Chemistry Research*, 23, 1931. 923931. (DOI; 10.1021/ie502609018).
 29. Nwabueze O., Nwofor C. Extractive performance of solvents on the indices of African breadfruit (*Treculia africana*) seed oil. *African Journal of Food Science Research*, 2(6). 2014. 99-104
 30. Pritchard JIR. Analysis and properties of oil seeds, In: Rossell JB, Pritchard JLR. Eds, *Analysis of oil seeds, fats and fatty foods*, Elsevier Applied Science Publishing, New York, 2021, 80–98.
 31. JAOC, Standard methods of oil analysis in Japan, *Japanese Association of Oil Chemists*, 2(4), 1972, 12-71.
 32. Ajiwe, VIE., Okeke CO., Agbo HU. Extraction and utilization of breadfruit seed oil (*Treculia Africana*). *Bioresource Technology*, 53. 1995. 183-184
 33. Aremu MO., Olonisakin A., Bako DA., Madu PC. Compositional studies and physicochemical characteristics of cashew nut (*Anacardium occidentale*) flour. *Pakistan Journal of Nutrition*, 5(4), 2006, 328-333
 34. Xie J, Huang S, Huang H, Deng X, Yue P, Lin J, Yang M, Han L., Zhang D. Advances in the application of natural products and the novel drug delivery systems for psoriasis. *Frontiers in Pharmacology*, 12. 2021. 2164 -2174 doi: 10.3389/fphar.2021.644952
 35. Ardabili GA., Farhoosh R., Haddad-Khodaparast MH. Chemical composition and physicochemical properties of pumpkin seeds (*Cucurbita pepo* Subsp. *pepo* Var. *Styriaka*) grown in Iran. *Journal of Agricultural Science and Technology*, 13, 2011. 1053-1063.
 36. Amos-Tautua, BMW., Onigbinde AO. Physicochemical properties and fatty acid profiles of crude oil extracts from three vegetable seeds. *Pakistan Journal of Nutrition*, 12(7), 2013. 647 -655
 37. Legrand E. Aceclofenac in the management of inflammatory pain. *Expert Opinion on. Pharmacotherapy*, 5, 2020. 1347–1357.
 38. Halder S, Islam A, Muhit MA, Shill MC, Haider SS. Self-emulsifying drug delivery system of black seed oil with improved hypotriglyceridemic effect and enhanced hepatoprotective function, *Journal of Functional Foods*, 78, 2021, 104391. <https://doi.org/10.1016/j.jff.2021.104391>
<https://doi.org/10.1016/j.jff.2021.104391>
[et rights and content](https://doi.org/10.1016/j.jff.2021.104391)
 39. Chowdary KAV., Babu VP, Rao BS. Formulation and in-vitro characterisation of self emulsifying drug delivery system of diclofenac for the enhancement of dissolution and solubility. *International Journal for Resaerch in Applied Science and Engineering*. 5(11). 2017, 663– 672
 40. Nandiyanto BAD, Ragadhita ROR. How to read and interpret FTIR spectroscopy of organic material. *Indonesian journal of science and technology*. 4(1). 2019. 97-118. <http://dx.doi.org/10.17509/ijost.v4i1.15806>
 41. Mathias J. A beginner's guide to interpreting & analyzing FTIR results: Innovative solutions for materials analysis. Available at: <https://www.innovatechlabs.com/newsroom/1882/interpreting-analyzing-ftir-results/> Assessed on June 8, 2021.

42. Cavalcanti IMF, Mendona EAM, Lira MCB, Honrato SB, Camara CA, Amorim RVS, Filho JM, Rabello MM, Hernandez MZ, Ayala AP, Santos-Magalhães NS. The encapsulation of β -lapachone in 2-hydroxypropyl- β - cyclodextrin inclusion complex into liposomes: A physicochemical evaluation and molecular modeling approach. *European Journal of Pharmaceutical Sciences*, 44. 2011. 332–340
43. Muller RH, Jacobs C, Kayser O. Nanosuspensions as particulate drug formulations in therapy: rationale for development and what we can expect for the future. *Advanced Drug Delivery Reviews*, 47. 2001. 3–19.
44. Rani DT, Pankaj S., Sawati S., Parul S. Novel self-nanoemulsifying drug delivery systems (SNEDDS) for oral delivery of lipophilic drugs. *Indo American Journal of Pharmaceutical Research*:7(01). 2017. 7361 -7368.
45. Laldusanga P. Recent developments in novel drug delivery systems for wound healing, *Expert Opinion on Drug Delivery*, 12(12), 2015. 1895-1909, DOI: [10.1517/17425247.2015.1070143](https://doi.org/10.1517/17425247.2015.1070143)
46. Ramteke K. Mathematical models of drug dissolution: a review. *Scholarly Academic Journal of Pharmacy*, 3(5). 2014. 388-396.