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Research article

PEGylated aceclofenac solid lipid microparticles homolipid-based solidified reverse micellar solutions for drug delivery

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ABSTRACT

Aceclofenac is a non-steroidal anti-inflammatory drug with poor aqueous solubility and a short half-life resulting in low bioavailability. Aceclofenac-loaded solid lipid microparticles based solidified reverse micellar solution (SLMs-SRMS) for oral drug delivery was investigated to improve the bioavailability and control drug release. Hot homogenization method was adopted to prepare the SLMs using a homolipid irvingia fat and Phospholipon® 90H with or without propylene glycol 6000 (PEGylation) in different ratios and characterized *in vitro*. The *in vivo* anti-inflammatory activity of the drug was determined on mice inflamed with carrageenan as phlogistic agent. Results showed that the morphology and particle sizes of the SLMs were spherical and smooth and ranged between 5.24 ± 0.01–97.44 ± 0.18 μm. EE % ranged between 67 - 81 %. A significant ($p < 0.05$) viscosity of 490 $mPasec^{-1}$ was obtained. FTIR spectra indicated compatibility amongst the constituents. DSC showed a broad peak which depicted an imperfect matrix resulting in a deformation of crystal arrangement creating many spaces for drug entrapment. Delayed drug release was observed in almost all the formulations in SIF (pH, 6.8). Anti-inflammatory activity showed a significant inhibitory effect ($p < 0.05$, up to 90 %). Hence, the aceclofenac-loaded SLMs-SRMS showed desirable characteristics and could be used for controlled delivery of aceclofenac and thus alternative to conventional aceclofenac oral formulation.

1. Introduction

Low aqueous solubility and erratic drug release of some poorly soluble drugs have been recorded as major challenges in pharmaceutical research. These challenges result in poor bioavailability after oral administration and hence, poor therapeutic efficacy. To address this challenge, Research and Development has developed some strategies that could improve the aqueous solubility of poorly soluble drugs such as solid lipid microparticles, solid dispersions, liquid-solid compacts, and so forth. Among these, solid lipid microparticles (SLMs) have been deemed a robust approach in the improvement of the solubility of a poor aqueous drug. The SLMs are lipid-based microscale drug carriers possessing a matrix made from fatty acid, glyceride, fatty alcohol, solid wax, and so on. SLMs are fine spherical particles usually <100 μm in diameter. An incorporated drug can be distributed homogeneously throughout the polymer matrix (microparticles), or it can be encapsulated into a polymer surrounding to form a drug reservoir (microcapsules). SLMs blend numerous advantages of the drug delivery system. SLMs contain physiologically related materials and hence show high drug encapsulation with tolerance in living systems (Umeyor et al., 2012). The solid carrier shields incorporated drug substances from destruction and aid in potential drug targeting. This matrix produces a better biocompatibility system, that reduces the hazards and toxicity during the periods of treatment when compared to polymer microparticles (Nnamani et al., 2010). The matrices have solid cores that decrease the mobility and leakage of the entrapped drug from the system. This drug delivery has the potential to mask the objectionable taste of some bitter drugs (Umeyor et al., 2012; Chime et al., 2013). Moreover, Solid lipid microparticles equally have topical applications as reported that occlusion characteristics due to film formation on the skin improved drug penetration through the stratum corneum. When compared to many colloidal carriers, it is more economical in production, high in both drug stability and encapsulation efficiency, allow sustained drug release, and enhance drug dissolution profile (Momoh and Esimone, 2012).

In this context, drug substance may be modified by linkage of polyethylene glycol (PEG) chains through a process of PEGylation.

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Polyethylene glycols (PEGs) are synthetic polymers with biocompatibility. The U.S Food and Drug Administration (FDA) approved the PEGylation of drugs as safe for human consumption (Xia et al., 2019). PEGylation enhances the solubility of poorly aqueous soluble drugs. It increases drug stability, prolongs drug release, and retention time of drug conjugated *in vivo*. PEGylation has been investigated to improve the stability of some novel drug delivery such as microemulsion, dendrimers, liposomes, micelles, nanoparticles, and so on (Momoh et al., 2020a; Thai et al., 2020). PEGylated mucin-based microparticles were reported to give competing merit for insulin shelter in an acidic medium (Momoh et al., 2020a). More so, research reported that PEGylation of miconazole nitrate enhanced drug stability, prolonged drug release, and increased anti-fungal activity (Kenechukwu et al., 2018). Thus, PEGylation improves drug efficacy, stability, decreases the dosing frequency of drug products, and so forth.

Irvingia wombolu (Var excelsa) is a tropical economic tree belonging to the Irvingiaceae family. Irvingia species are commonly known as African mango, bush mango or wild mango. The tree bears green oval shaped fruits with fibrous bitter and unpalatable pulps containing endocarps that encapsulate the nuts. The nut is edible with characteristics of gummy properties and fat (irvingia fat). These properties may lead to the application in the food, pharmaceutical, and cosmetic industries. The irvingia fat (IF) is also known as Dika fat. The dika nut has been reported to produce about 58% fat (Okoronkwo et al., 2014). The IF is soluble in several organic solvents. The fat composition may vary based on the topography and storage conditions. Research showed that the fat is not good for deep-fat frying applications (Okoronkwo et al., 2014). It has a low melting point which may be useful in the manufacture of margarine and cream. It has low acid content, relative low iodine number, high saponification value, and free fatty acids with few unsaturated bonds which indicates that the oil will have low susceptibility to oxidative rancidity after extraction when stored in a conducive environment (Etong et al., 2014). Dika fat has been found useful as a plasticizer and reported to be mainly saturated (lauric and myristic acid) which is the characteristic of vegetable oils (Etong et al., 2014). Anti-malarial activity of homolipid-based artemether microparticles was investigated to find that IF improved solubility and bioavailability of the drug (Agubata et al., 2015). Some reports have shown physicochemical properties of irvingia species (Ogaji et al., 2012; Etong et al., 2014; Okoronkwo et al., 2014).

The employment of blends of homolipid (irvingia fat) as solid reverse micellar solution (SRMS) in SLMs formulations makes the formulation more useful and efficient for delivering lipophilic drugs (Uduma et al., 2017). This kind of formulation has been reported to generate controlled drug release with improved drug absorption (Umeyor et al., 2012; Uduma et al., 2017). Hence, this qualitative change enhanced controlled drug delivery, precludes drug expulsion, and improved the encapsulation efficacy of lipophilic drugs (Umeyor et al., 2012). Importantly, SRMS matrices were considered as the sustained release for both lipophilic and lipophobic drug substances (Chime et al., 2013).

Currently, there is a dearth of data reported on the employment of PEGylation of aceclofenac homolipid-based SLMs-SRMS. Hence, concerning the above benefits this work was to formulate, characterize, and investigate the *in vivo* and *in vitro* activity of aceclofenac-loaded-SRMS based SLMs blends of irvingia fat and Phospholipon 90H for controlling drug release, enhancing stability, dissolution and oral bioavailability of aceclofenac.

2. Materials and methods

2.1. Materials

The following are the study materials employed in the preparation: Pure samples of aceclofenac (Evans Pharmaceutical limited, England), activated charcoal (Bio lab, UK), Phospholipon 90H (P90H) (Phospholipid GmbH, Köln, Germany), Cremophor® RH 40 (BASF, Germany), PEG 6000 (Qualikems, India), Poloxamer (Merck, Darmstadt Germany), Soluplus® (BASF, Ludwigshafen, Germany). Other chemicals and solvents are of analytical grades. *Irvingia wombolu* fat *was* processed in our Drug Delivery Research Unit, Department of Pharmaceutical and Industrial Pharmacy.

2.2. Extraction and purification of irvingia fat

The pure kernels of *Irvingia wombolu* were procured from Ogige market in Nsukka, Enugu, Nigeria, in August, 2019 and were documented by a consultant taxonomist at the herbarium section of the Department of Pharmacognosy and Environmental Medicine, Faculty of Pharmaceutical Sciences, University of Nigeria Nsukka, Enugu State. The kernels seeds were cracked and properly dried. The seeds were ground using a milling machine (Moulinex Pikalica) and extracted in a Soxhlet extractor using n-hexane at a sample: solvent ratio 1:2 for 10 h according to Onyishi et al. (2014) with little modification. The extract was transferred into a flat plate to facilitate complete evaporation at room temperature. The extracted irvingia fat was successively depolymerized using warm water at 70 °C and ethyl acetate at a ratio of 1:1. The depolarized extract was allowed to pass via a column of 2 % w/v activated charcoal and bentonite (1:9) at 70 °C at 10:1 w/w ratio of column material to enable further purification. Irvingia The depolarized extract was allowed to pass via a column of 2 % w/v activated charcoal and bentonite (1:9) at 70 °C at 10:1 w/w ratio of column material to enable further purification. Irvingia fat (dika wax/fat) is kept in the refrigerator until used.

2.3. Formulation of the lipid matrices

The lipid matrices consisting of phospholipid 90H (P90H), irvingia fat (IF), and polyethylene glycol 6000 (PEG 6000) were prepared by fusion method with little modification (Kenechukwu et al., 2018). In brief, IF (500 mg) and P90H (4.5 g) were measured and melted together at P90H: IF ratio of 1:9 w/w using a thermo-regulated water bath (HH. W21.Cr42II, China) at 70 °C with continuous stirring until a homogeneous admixture and then allowed to solidify to obtain IFP90H matrix. Moreover, the PEGylations of the lipid matrix (IFP90H) were done by melting the lipid matrix and PEG 6000 at IFP90H: PEG ratios of 1:8, 1:4, and 1:2, respectively to get 5 g w/w lipid matrices at 70 °C. These were agitated continuously, then left to solidify and called PEGylated lipid matrices. Hence, a total of six lipid matrices consisting of four PEGylated matrices PGU (containing plain 1:2 w/w IFP90H: PEG), PGL1 (containing 1:8 w/w IFP90H: PEG), PGL2 (1:4 w/w IFP90H: PEG), and PGL3 (1:2 w/w IFP90H: PEG), and two unPEGylated matrices UPGL (containing 1:9 P90H: IF) and irvingia fat matrix (IFM 5 g w/w) were obtained. All were kept in air-tight and moisture resistant amber bottles.

2.4. Formulation of drug-loaded solid lipid microparticles (SLMs) based SRMS

The SRMS-SLMs containing 5 %w/w PEGylated lipid matrices [PGL1 (1:8), PGL2 (1:4), PGL3 (1:2)], and unPEGylated lipid matrices of irvingia fat matrix (IFM) and UPGL with 1.5% w/v Cremophor® RH 40 (Surfactant), 0.5% w/w Poloxamer (solubilizer) and sufficient distilled water to produce 100% w/w were formulated using hot homogenization method with little modification of Kenechukwu et al. (2018) as presented in Table 1. In brief, each lipid matrix was melted at 60 °C and the surfactant aqueous phase containing poloxamer and Cremophor® RH 40 at the same temperature were incorporated into each lipid matrix with little agitation using magnetic stirrer (SR1UM 52188, Remi Equip., India) and then, further homogenize at 10,000 rpm for about 5 min with an Ultra-Turrax mixer (T 25 digital Ultra-Turrax, IKA, Staufen, Germany) while submerged in a thermo-regulated water bath to obtain hot O/W emulsion. Then, appropriate quantities of aceclofenac powder (500 mg) were dispersed in each 5 % w/w molten lipid matrix (PGL 1:8, PGL 1:4, PGL 1:2, UPGL and IFM) previously melted at 60 °C. A 5 % w/w lipid

Table 1. Quantities of materials for the unloaded and drug loaded SLMs based SRMS.

Batch	LM: PEG	Crem (% w/w)	Polo (% w/w)	DW (% w/w)	Drug (mg)
PGL1	1:8	1.5	0.5	100.0	500.0
PGL2	1:4	1.5	0.5	100.0	500.0
PGL3	1:2	1.5	0.5	100.0	500.0
UPGL	10:0	1.5	0.5	100.0	500.0
IFM	10:0	1.5	0.5	100.0	500.0
PGU	1:2	1.5	0.5	100.0	-

Key: PGL1 (containing IFP90H: PEG in ratio 1:8); PGL2 (containing IFP90H: PEG in ratio 1:4) PGL3 (containing IFP90H: PEG in ratio 1:2); UPGL (loaded unPEGylated lipid matrix, containing P90H: IF in ratio 1:9), PGU (containing 1:2 of IFP90H: PEG without drug load), IFM (loaded irvingia fat matrix 5% w/w); LM (lipid matrix); Crem (cremophor RH 40); Polo. (poloxamer); DW (distilled water).

matrix containing 1:2 IFP90H: PEG (PGU) produced was left without drug loading and served as a control. Then, the formulated SLMs based SRMS were transferred to clean containers, closed tightly and stored in a refrigerator until further use.

2.5. Determination of encapsulation efficiency (EE %) and drug loading capacity (DLC)

The content of the aceclofenac in the SLMs was determined using the UV spectrophotometric method. A 5 mL of each SLMs was incorporated into microconcentrator (Vivaspin® 6, Vivascience, Hanover, Germany) consisting of filter membrane with molecular weight cutoff (MWCO) of 10, 000 Da, then centrifuged (laboratory centrifuge model SM800B uniscope surgifriend medicals, England) at a speed of 4000 rpm for 2 h. Then, 5 mL syringe, 1 mL each of the supernatant was withdrawn and made up to 5 mL with phosphate buffer (pH, 6.8), then centrifuged again at 4000 rpm for 1 h to obtain clearer supernatant. Then, a 0.5 mL of the supernatant was withdrawn and assayed using a UV/Vis Spectrophotometer (Spectrum lab, 752s, Netherlands) at 290 nm wavelength. The drug encapsulated in the SLM was determined to obtain the EE % using Eq. (1) by Momoh et al. (2020a), while the drug loading capacity (DLC) expresses the quotient between the entrapped drug and the entire quantity of the lipids (Momoh et al., 2020b). Drug loading capacity (DLC) was calculated using the relationship in Eq. (2):

$$\textit{Encapsulation efficiency}\ (\%) = \frac{\textit{Real drug loading}}{\textit{Theoritical drug content}} \times\ 100 \qquad (1)$$

$$\textit{Drug loading capacity}\ (\%) = \frac{\textit{Entrapped drug}}{\textit{Total weight of lipid}} \times\ 100 \qquad (2)$$

2.6. Rheological study of formulations

The viscosity of the samples was obtained using an electronic viscometer (NDJ-5S labscience, England) having spindle no. 2 at a speed of 60 rpm. The spindle connected to the coupling nut was inserted into the SLMs contained in a 50 mL beaker maintained at 32 °C to cover the groove. Then, the viscosity ($mPasec^{-1}$) of each batch was noted and recorded at each speed of the rotation.

2.7. Time dependent pH stability study and physical examination

2.7.1. Time-dependent pH stability study and physical examination

The pH of the loaded and unloaded SLMs was ascertained in a time-dependent pattern: day 1, day 8, day 15, day 30 and day 60 with a pH meter. This was done by immersing the electrode of the pH meter into the SLM formulations up to three times and the average value calculated, while the pH meter was calibrated using standard buffer solution (pH, 7.0) before each test. More so, the SLMs was physically examined for colour, homogeneity, and consistency.

2.8. Morphology and particle size determination

Particle size analysis of the SLMs was determined using Hund binocular microscope (Wetzlar, Germany). Briefly, a small dispersion of the SLMs (1 drop) was dropped on a microscope slide, a coverslip used to cover it and then the picture was observed using a Hund binocular microscope (X400 magnification) connected with a Motic image analyzer (Moticam, Xiamen, China). Then, mean particle sizes and morphology were determined.

2.9. Fourier transformed infra-red spectroscopy (FT-IR)

The FT-IR was used to examine any chemical reaction between aceclofenac and other excipients of the SLMs using Shimadzu FT-IR 8300 Spectrophotometer (Shimadzu, Tokyo, Japan). A little quantity of the drug, irvingia fat, PEG 6000, P90H and the SLMs were scanned over a wavelength of 4000–400 cm^{-1} at a resolution of 4 cm^{-1} and the spectrum was recorded in transmittance mode.

2.10. Differential scanning calorimetry

Thermal characteristics of aceclofenac, irvingia fat, P90H, PEG, and SLMs based SRMS formulation were ascertained with a DSC 204 F1 instrument (Netzsch, Germany). The instrument was calibrated with indium and sapphire before analyzing samples under a nitrogen atmosphere (20 ml/min). Briefly, approximately 5 mg of the formulations packed in an aluminum pan was hermetically sealed. Then heated at a rate of 10 °C/min between 30 – 250 °C, and kept at this temperature for 3 min to allow complete melting and the thermographs recorded.

2.11. In vitro release study

The freshly prepared drug release medium consisted of 500 mL of simulated gastric fluid (SGF, pH 1:2) and simulated intestinal fluid (SIF, pH 6.8) were maintained at 37 ± 1 °C. A volume (5 mL) of SLMs-SRMS equivalent to 50 mg of the drug was collected and enclosed with a dialysis bag MWCO 5000–8000) with hermetically sealed ends. The enclosed bag containing the dispersion was freely hung in 500 mL of SGF (pH, 1.2) in a beaker assembled on a magnetic stirrer at 50 rpm. Then, at 5, 10, 15, 20, 30, 40, 50, 60, 80, 100, 120 min, 5 mL was collected from the medium and simultaneously, 5 mL of fresh SGF of pH 1.2 was added as a replacement. The absorbance of each of the withdrawn samples was assayed using a UV spectrophotometer at 219 nm wavelength (a predetermined wavelength). This procedure was repeated for SIF (pH, 6.8) and all were determined in triplicate. The amount of drug released and the percentage of drug released was calculated and plotted against time.

2.12. In vivo anti-inflammatory study

Anti-inflammatory property of the aceclofenac-loaded SLMs based SRMS was performed using the rat paw oedema model (Anosike et al., 2009). Earlier before the animal experiment, the Animal Ethics Committee of at the Faculty of Pharmaceutical Science, University of Nigeria, Nsukka approved the research protocol for the use of experimental animals. Hence, the animal experimental protocols were observed following the guidelines of the Animal Ethics Committee. Carrageenan was the employed phlogistic agent. Wistar rats (100–128 g) were divided into six experimental groups (n = 6). The animals fasted for 12 h before the commencement of the experiment to enable consistent hydration and reduce variability in oedematous response. An approximately 5 mg/kg per body weight of PEGylated (PGL1, PGL2, PGL3 and PGU) and unPEGylated (UPGL) aceclofenac-loaded SLMs based SRMS were orally given to the animals. Placebo group took 0.9% normal saline. And the standard

Table 2. Physicochemical properties of SLMs.

Sample	EE (% ± SD)	DLC (% ± SD)	V ($mPasec^{-1}$ ± SD)	pH				
				Day 1	Day 8	Day 15	Day 30	Day 60
PGL1	67.00 ± 0.10	19.00 ± 0.10	12.50 ± 0.15	4.01 ± 0.20	3.90 ± 0.40	3.90 ± 0.11	3.90 ± 0.11	3.90 ± 0.30
PGL2	71.80 ± 0.12	25.00 ± 0.10	20.50 ± 0.11	4.11 ± 0.10	4.11 ± 0.30	4.10 ± 0.20	4.01 ± 0.30	4.01 ± 0.20
PGL3	81.10 ± 0.11	27.00 ± 0.11	34.00 ± 0.00	4.40 ± 0.30	4.30 ± 0.20	4.10 ± 0.11	4.01 ± 0.20	4.01 ± 0.10
UPGL	85.00 ± 0.11	27.00 ± 0.13	490.00 ± 0.11	4.40 ± 0.11	4.30 ± 0.10	4.20 ± 0.20	4.20 ± 0.20	4.01 ± 0.10
IFM	81.40 ± 0.21	27.00 ± 0.11	490.00 ± 0.01	4.20 ± 0.50	4.20 ± 0.11	4.20 ± 0.60	4.20 ± 0.70	4.01 ± 0.11
PGU	_	_	11.50 ± 0.11	4.70 ± 0.20	4.60 ± 0.11	4.60 ± 0.30	4.50 ± 0.20	4.20 ± 0.10

Key: PGL1 (containing IFP90H: PEG in ratio 1:8); PGL2 (containing IFP90H: PEG in ratio 1:4) PGL3 (containing IFP90H: PEG in ratio 1:2); UPGL (loaded unPEGylated lipid matrix); IFM (loaded irvingia fat matrix); PGU (containing 1:2 of IFP90H: PEG without drug load).
EE (encapsulation efficiency); LC (drug loading capacity); V (viscosity).

(reference) group took pure drug of 5 mg/kg. At half an hour, post-treatment, 0.1 mL fresh concentrated carrageenan was injected into the sub-plantar region of the right hind paw of the Wistar rats to induce oedema. Using a plethysmometer, the volume of water displaced by the treated right hind paw of the animals were noted and recorded pre and post 1, 2, 3, 4, 5, 7, and 8 h after the carrageenan administration. The mean oedema inhibition at the time interval was determined as the difference in volume displaced by the injected paw (DV_t - DV_o) (Anosike et al., 2009). The % oedema inhibition was calculated using Eq. (3) (Chime et al., 2013).

$$\%\ Inhibition = \frac{DV_{o-}\ DV_t}{DV_o} \times 100 \quad (3)$$

DVt = volume of edema at a given time; DVo = volume of edema in control rats at zero time.

2.13. Statistical analysis

Data were analyzed using SPSS Version 16.0 (SPSS Inc. Chicago, IL, US) and one-way analysis of variance (ANOVA). Values were presented as mean ± SD (standard deviation). Differences between means were determined by a two-tailed student's T-test and $p < 0.05$ was considered statistically significant.

3. Results and discussion

3.1. Encapsulation efficiency and drug loading capacity

The power of a formulation to entrap an incorporated drug is an essential characteristic that can be conveyed by encapsulation efficiency and drug loading capacity (DLC). The EE % determines the total amount of drugs encapsulated by microparticles, while the DLC measures the quotient of the weight of the encapsulated drug and the entire quantity of the lipids (Momoh et al., 2019). These properties of the SLMs as shown in Table 2 include 81, 85, and 67, 72, 81 % and 27, 27, 26, 26, and 27 % for IFM (irvingia fat matrix), UPGL (unPEGylated lipid matrix) and, PEGylated lipid matrix [PGL1, PGL2, PGL3 (containing IFP90H: PEG 1:8, 1:4, and 1:2)], respectively. The formulation UPGL (containing loaded unPEGylated lipid matrix) exhibited the highest encapsulation efficiency (85 %) followed by the SLM PGL3 (1:2 of IF90H: PEG) (81 %) and IFM (81 %), whereas the least EE % was observed in PEGylated lipid matrix (67 %) with the highest PEG 6000. Hence, the higher the PEGylation of the SLMs the lower the encapsulation. This depicted that more drugs were entrapped within the PEG 6000. The DLC followed a similar trend. Some factors that may have influenced the EE % and DLC include the lipophilicity of the drug and its excipients (Kenechukwu et al., 2015). The high lipophilic aceclofenac may have contributed to the improved EE % in the SLMs based SRMS.

3.2. Rheology of SLMs

Viscosity may be referred to as a flow resistance of fluid in the structured state, developing as viscous or elastic flow resistance to oscillating movement. The results of dynamic viscosity on shear stress as represented in Table 2 deduced that IFM (irvingia fat matrix) and UPGL (unPEGylated matrix) exhibited the highest viscosities (490 mPa/s). This may be implicated by the absence of PEGylation or degree of inter-particle dispersibility. The high viscosity depicted more resistance to flow in the structured nature (Kenechukwu et al., 2018). Hence, formulations IFM and UPGL (SLMs without PEG) are denoted to have consistent gel-like structures and will likely cause a delay in the drug release. The SLMs PGU (containing 1:2 of IFP90H: PEG without drug load), PGL1 (containing 1:8 of IFP90H: PEG), PGL2 (containing 1:4 IFP90H: PEG), PGL3 (1:2 of containing IFP90H: PEG) were depicted with the lowest values of viscosity which may be due to the presence of PEG incorporated. It can be observed that the presence of the aceclofenac (API) did not affect the rheological property of the SLMs.

3.3. Physical examination and pH stability of SLM

The result of the pH test of the aceclofenac based SRMS presented in Table 2 indicated more stable acidic pH with a slight decrease as time went on. This depicted that the formulations require a buffer system. This slight change in the pH of the SLMs may not be assigned to degradation of the API since the unloaded SLM (PGU) equally had a significant ($p < 0.05$) pH reduction from 4.70 ± 0.20 to 4.20 ± 0.10. Previous reports had similar results (Attama et al., 2009; Chime et al., 2012). Though, it may be associated with the hydrolysis of ester linkage of the drug to give diclofenac and glycolic acid. Hence, the diclofenac may have a complex with phospholipid within the periphery leaving the glycolic acid in the aqueous medium thereby increasing the acidity.

3.4. Particle sizes and morphology

The particle size range of the formulations include 8.27 ± 0.22–36.89 ± 0.08 μm, 8.11 ± 0.13–33.80 ± 0.05 μm, 7.23 ± 0.01–31.13 ± 0.10 μm, 18.73 ± 0.11–97.44 ± 0.18 μm, 5.24 ± 0.01–12.39 ± 0.03 μm, and 12.14 ± 0.11–44.05 ± 0.05 μm for batches PGL1, PGL2, PGL3, UPGL, IFM, PGU as shown in Figure 1, respectively. Particle size may occasionally be as a result of excipient employed, the crystalline habit of the particle size, method of size reduction, the level, pressure, and forces involved during size reduction, and so on (Attama et al., 2009). Drug load was insignificant on the particle size. Formulation with the lowest particle size was IFM (5.24 ± 0.01–12.39 ± 0.03 μm), while unPEGylated SLM had the highest particle sizes

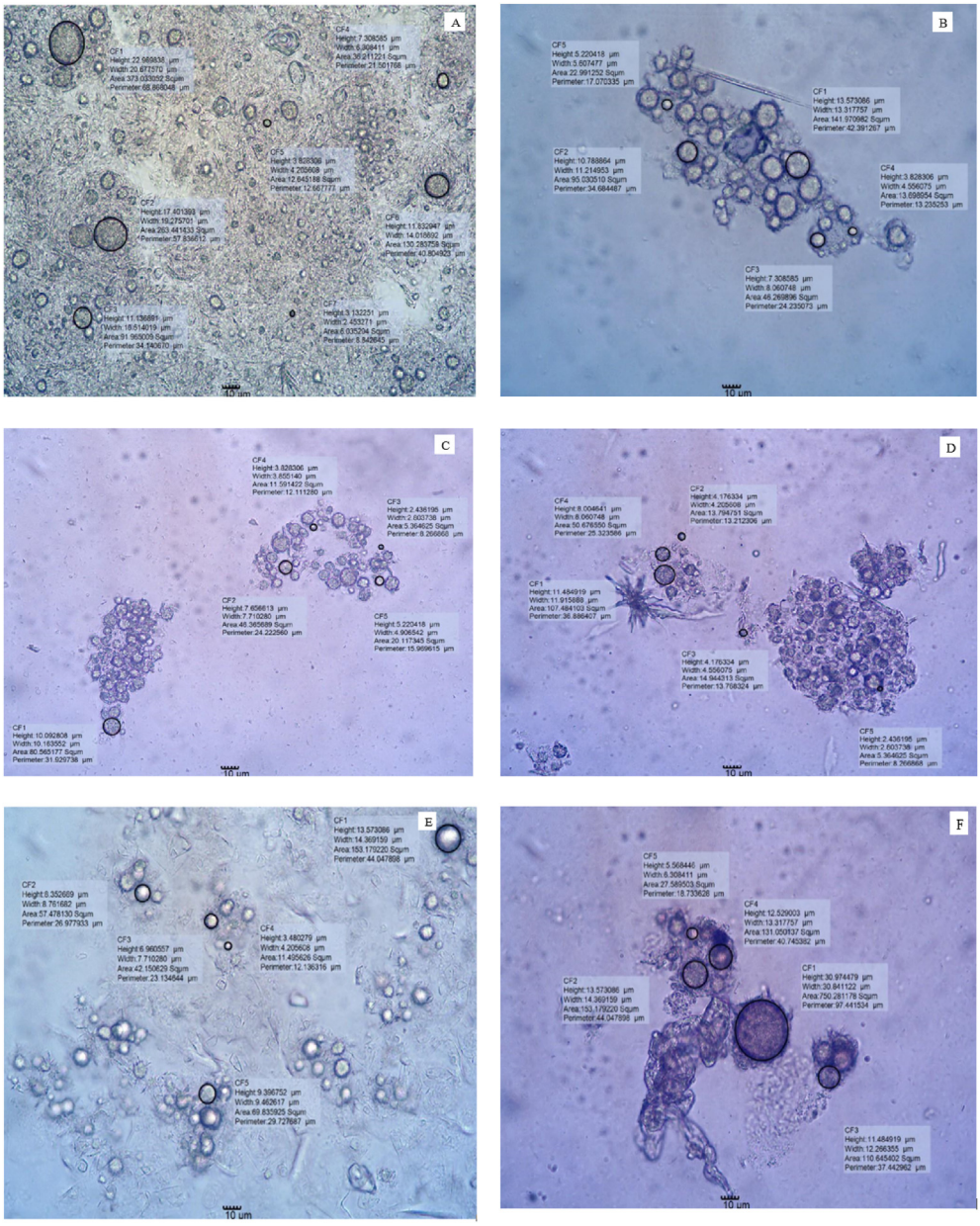

Figure 1. Photomicrographs of IFM (A), PGL3 (B), PGL2 (C), PGL1 (D), PGU (E) and UPGL (F). Key: A (IFM, loaded irvingia fat matrix); B (PGL3, containing IFP90H: PEG in ratio 1:2); C (PGL2, containing IFP90H: PEG in ratio 1:4); D ((PGU, containing 1:2 of IFP90H: PEG without drug load); E (PGL1, containing IFP90H: PEG in ratio 1:8); F (UPGL, loaded unPEGylated lipid matrix).

(18.73–97.44 µm). More so, the presence and concentration of PEGylation (PEG 6000) exhibited a significant decrease in particle size as observed in SLMs based SMRS formulations PGL1, PGL2, and PGL3. This result supported a previous report on the effect of PEGylation on particles sizes (Kenechukwu et al., 2018). The mean particle diameter of formulations affects the bioavailability of the prepared drug and could ascertain the route of drug administration. The little particle size of SLMs (<20 µm) is allowed by single-cell contact such as 8.27 ± 0.22–36.89 ± 0.08 µm and 8.27 ± 0.13–36.89 ± 0.05 µm exhibited by batches PGL1 and PGL2, respectively, while bigger sizes (>50 µm) used to have a higher reactive effect due to attractive force (Chime et al., 2012). This implied that none except some of UPGL SLMs may be reactive due to the absence of attractive forces.

The particle morphology as shown in the photomicrographs (Figure 1) depicted smooth and spherical particles which were within the micrometer limits of SLMs based SRMS formulations.

3.5. Characterization of SLM based SMRS

3.5.1. FTIR

The FTIR was used to study the possible interaction between the drugs and carriers in the drug formulation. The peaks in the FTIR charts represents different functional groups. Alteration in the peaks detects interaction between the drug and the carrier. The FT-IR spectra of irvingia fat, PEG 6000, Phospholipon 90 H, aceclofenac and SLMs based SRMS were recorded in the range of 4000-650cm^{-1} (Figure 2). The spectrum identifies the surface functional groups and also illustrates the changes in biosorbent after thermal and modified treatment. The spectrum for irvingia fat exhibited major characteristic absorption bands to form a broad and strong band in the functional group region of 2918.5–3200.0, 1740.7–1750.0, and 1095.8–1350.0 cm^{-1} which is associated with C–H (vinyl or aldehyde) or OH (carboxylic); C=O (aldehyde or ketones); and C–C stretch (aromatic amines), respectively.

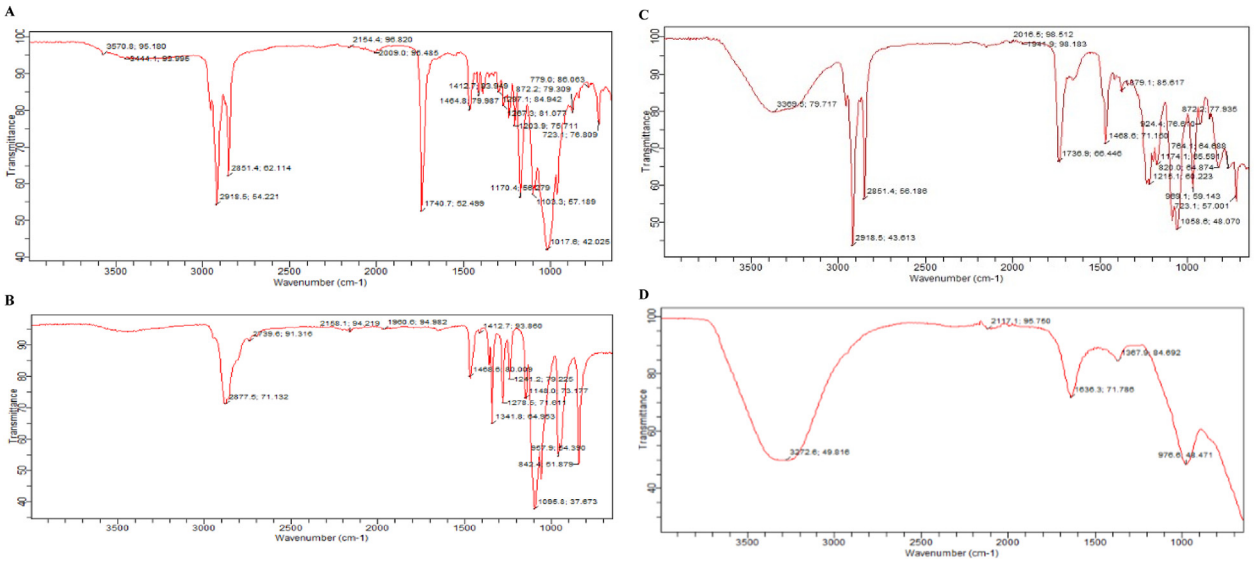

Figure 2. FTIR of irvingia fat (A), PEG 6000 (B), phospholipon 90H (C), and PGL2 (D, containing IFP90H: PEG in ratio 1:2).

This correlated with earlier reports (Santoni and Pizzo, 2013; Hamarneh et al., 2010). PEG 6000 exhibited frequencies and vibrations of major bands of multiple intensities at the region of 2877.5–3200 and 1095.8–1350.0 cm^{-1}, associated with C–H (vinyl or aldehyde) or OH (carboxylic) and C–C stretch (aromatic amines), respectively. This is consistent with previous reports (Bley et al., 2010; Maghraby and Elsergany, 2014). The FTIR of Phospholipon 90H showed vibrations of principal characteristic band intensities with their functional groups at the following ranged regions; 3600–3309.5, 2918.5–2800, 1736.9–1720, and 1216.1–1058.6 cm^{-1} which are associated with at OH (alcohol), N–H (amine), or ≡CH (alkyne); C–H (vinyl or aldehyde) or OH (carboxylic); C=O (aldehyde or ketones); and C–C stretch (aromatic amines), respectively. The spectrum is consent with some earlier reports (Yue et al., 2010: Singh et al., 2013). The FTIR spectrum of pure aceclofenac showed wavelengths of 3319.10–3700 cm-1 for OH (alcohol), N–H (amine), or C≡H (alkyne); 2800–2936.8 cm-1 for C–H (vinyl or aldehyde) or OH (carboxylic); 1650–1771.5 cm-1 for C=O (aldehyde or ketones); and 1300–821.94 cm^{-1} for C–C vibration. This is similar to earlier

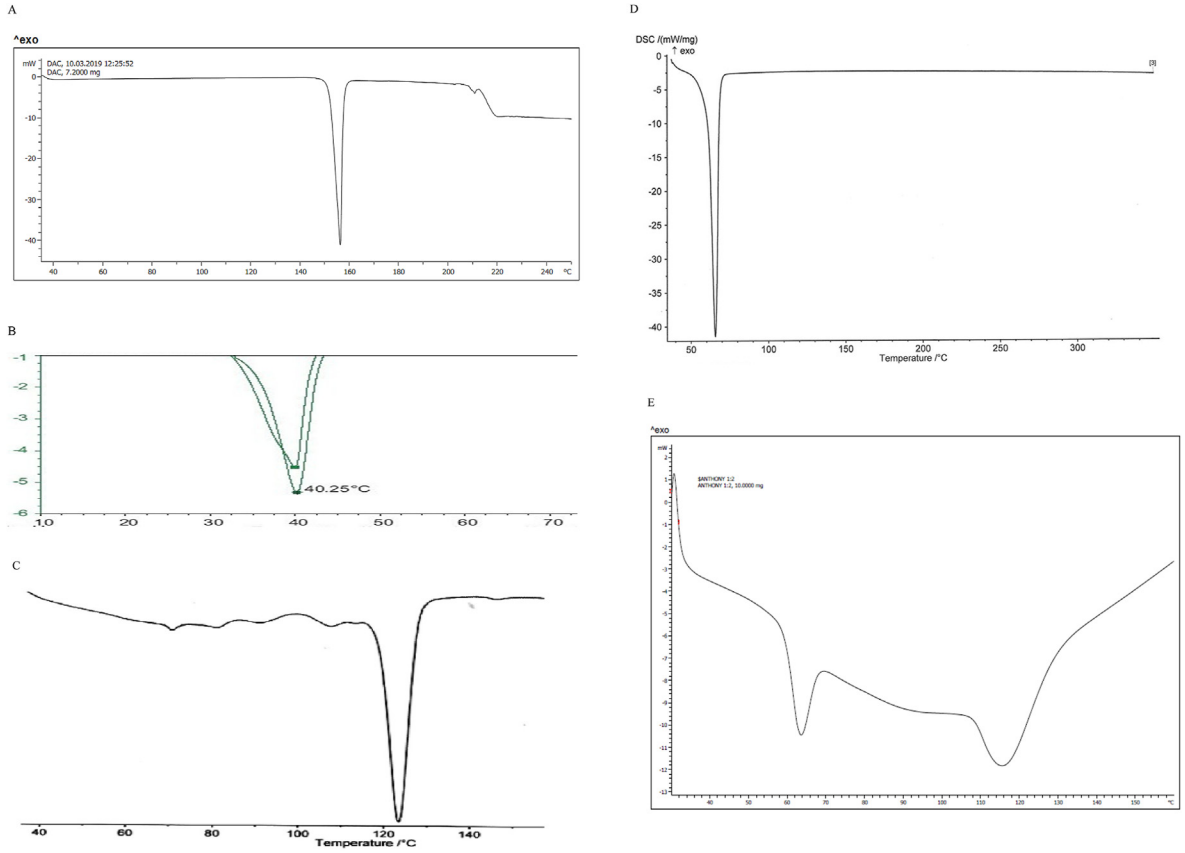

Figure 3. DSC thermographs of aceclofenac (A), irvingia fat (B), P90H (C), PEG 6000 (D), and SLMs PGL2 (E).

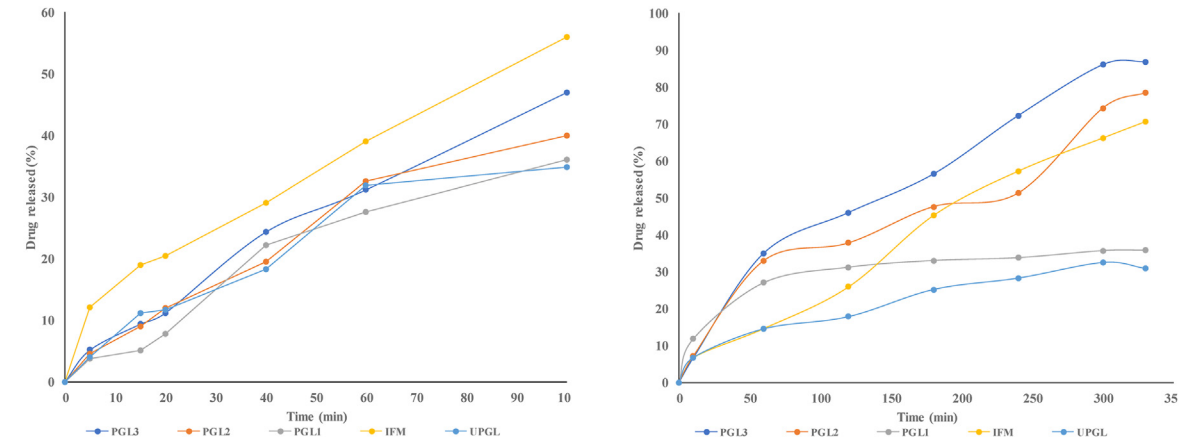

Figure 4. Drug release of SLMs in (a) simulated gastric fluid (SGF, pH 1.2 and (b) simulated intestinal fluid (SIF, pH 6.8). Key: IFM (loaded irvingia fat matrix), PGL1 (containing IFP90H: PEG in ratio 1:8); PGL2 (containing IFP90H: PEG in ratio 1:4) PGL3 (containing IFP90H: PEG in ratio 1:2); UPGL (loaded unPEGylated lipid matrix).

findings (Maulvi et al., 2011; Bley et al., 2010). The FTIR of the selected SLM based SRMS exhibited major principal peaks at 3272.49–3700.00, 2117.1–2200, and 1030.71–1000.00 which corresponds to the following functional groups of OH (alcohol), N–H (amine), or C≡H (alkyne); C=C (alkyne); and C–C stretch (aromatic amine), respectively. There was no disappearance or appearance of a new functional group in the SLM based SRMS formulation. Hence, no chemical drug-carrier interaction was detected.

3.5.2. DSC

Figure 3 shows the DSC thermograms of irvingia fat (IF), Phospholipon 90 H (P90H), polyethylene 6000 (PEG) and selected SLM based SRMS (PLG2). The DSC thermograms of IF, P90H and PEG showed sharp endothermic peaks at 44.3, 125.2, and 65.7 °C, respectively. The melting transition of aceclofenac (AF) depicted a sharp exothermic peak representing melting at 154.56 °C with an enthalpy of −41.20 mW/mg. These sharp peaks depicted crystalline nature of the materials. The melting transition of SLM PLG2 (containing IFP90H: PEG in ratio 1:4) showed two broad exothermic peaks corresponding to melting at 63.33 and 115.37 °C with an enthalpy of -10.31 and −11.83 mW/mg, respectively. The first peak was close to the melting peak of PEG 6000 (65.7 °C), while the second peak was close to the peak of the Phospholipon 90 H (125.2 °C) where the drug and solubilized and entrapped. The broad peak of the SLM based SRMS is an indication that the matrix produced an imperfect structure resulting in a deformation of crystal arrangement which might have generated many spaces for drug entrapment. The exothermic peak showed polymorphic transitioning (crystallization) of the formulation to more amorphous state. More so, a higher enthalpy value indicates better-arranged crystal structures (Kenechukwu et al., 2018; Kenechukwu et al., 2015).

3.6. In-vitro drug release studies

The *in vitro* drug release study of the formulations as presented in Figure 4 depicted a gradual release of the drug in the different pH media without a burst release effect. This implied that the drug was well uniformly dispersed and encapsulated within the matrices. The time to release 40 % (T_{40}) of the drug in SGF (pH, 1.2) is 100 min for PGL2, PGL3, and IFM. Formulations PGL1 and UPGL could not release up to 40 % within 2 h experimental time. The delay may be related to the high PEG concentration of the formulation (PGL1, containing IFP90H: PEG 1:8), and large particle sizes (UPGL). Formulation IFM (containing irvingia fat matrix) exhibited a significant ($p < 0.05$) higher drug release of 56 % in SGF than others. This high drug release may be attributed to its low particle sizes (5.24 ± 0.01–12.39 ± 0.03 μm) and the presence of surfactant. This is in consent with some previous reports (Agubata et al., 2015; Ugwu et al., 2020). In SIF (pH, 6.8), the time to release 75 % (T_{75}) of the drug was 300 min for PGL3 and 330 min for PGL2, whereas PGL1, IFM, and UPGL showed a delayed drug release. Drug release may be affected by some factors such as particle sizes, excipient type and concentration, formulation technique, medium and pH, and so forth.

The formulation UPGL (unPEGylated SLM) released <40 % in both release media which may be attributed to its larger particle sizes (≤97.44 ± 0.18 μm) and the highest viscosity (490 $mPasec^{-1}$). The delayed drug release observed in formulations PGL1 (containing 1:8 of IF/P90H: PEG) may be associated with the higher concentration of PEG 6000 though the pH of the dissolution medium may also have played part. The delayed drug release found in all the SLM formulations including IFM may also be due to gradual hydration and swelling of the hydrogels within the matrix which prolong the residence time of the drug. SLM equally played a role in the delay release pattern since none of the formulations released up to 100 % of the drug within the experimental time. It was earlier reported that SLM formulations prolong drug release owing to the incorporated solid lipid matrix (Kenechukwu et al., 2015). More so, the 3-dimensional network structure of hydrogel (excipient) supplies more diffusion barriers to prolong drug release (Kenechukwu et al., 2018). Hence, an increase in the concentration of PEG and the use of hydrogel may enhance drug release control and sustain the drug release and could lead to once daily drug administration. This delayed drug release of aceclofenac is of therapeutic benefit since the drug has a short half-life (1.8–3.5 h) making the dosing frequency to be two to three times daily within the dose range of 100–200 mg.

3.7. Anti-inflammatory study

The anti-inflammatory activities of the SLMs as observed in Figure 5 depicted 43–46 %, 38–51 %, 55–72 %, 66–86 %, and 60–88 % oedema inhibition at 0.5, 1, 2, 3, and 4 h post-drug administration, respectively. The inhibition was observed to be continually increased. The highest oedema inhibitions of 90 and 88 % were observed with IFM and PGL3 at 2 and 4 h, respectively. The highest inhibition of IFM may be attributed to the presence of fatty acids of *Irvingia wombolu* which have been reported as absorption enhancer (Agubata et al., 2015). Irvingia fat has been shown to have melting point within the range of about 40–44 °C

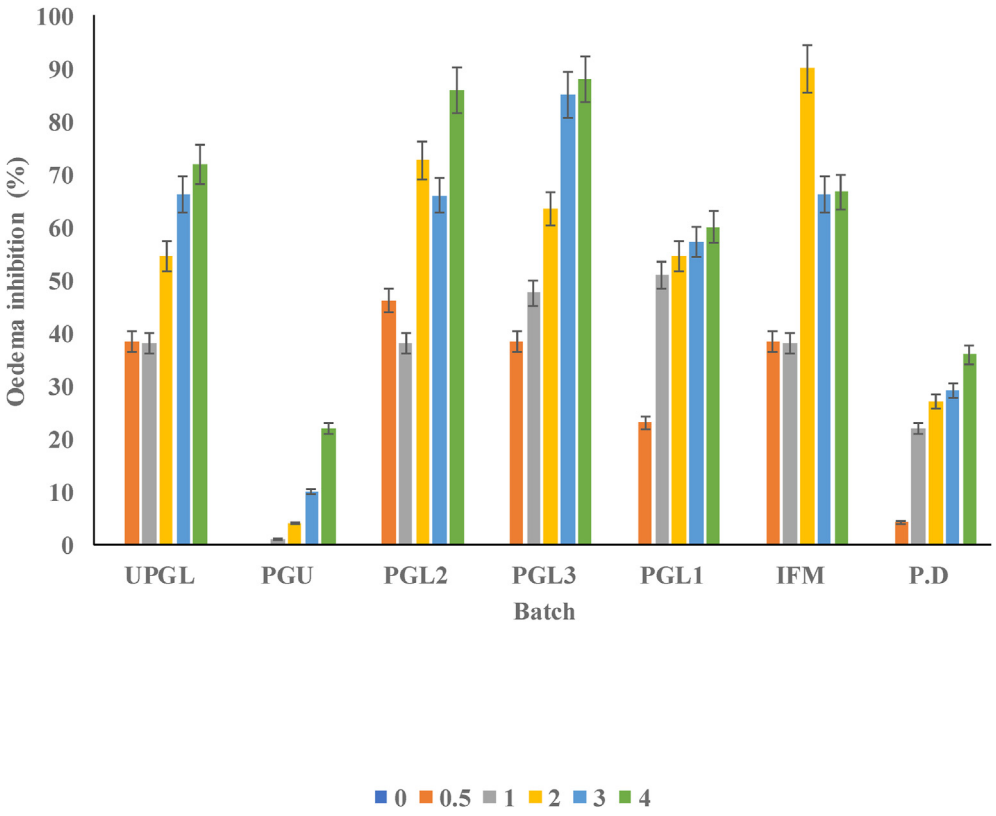

Figure 5. Anti-inflammatory activity of the SLMs. Key: UPGL (loaded unPEGylated lipid matrix), PGU (containing 1:2 of IFP90H: PEG without drug load), IFM (loaded irvingia fat matrix), PGL1 (containing IFP90H: PEG in ratio 1:8); PGL2 (containing IFP90H: PEG in ratio 1:4) PGL3 (containing IFP90H: PEG in ratio 1:2).

which is nearer to the body's temperature. The high inhibition observed in PGL3 may be due to faster hydration and swelling of the entrapped aceclofenac in the SLM matrix due to the lower ratio concentration of PEG 6000. Myristic acid found in both phospholipid and irvingia fat with low PEG 6000 might also contribute to high oedema inhibition in SLM PGL3. All the SLMs showed significant ($p < 0.05$) oedema inhibition when compared to the pure drug and unloaded SLM (PGU). SLM has been shown to enhance bioavailability and protect entrapped drugs against decay. Aceclofenac has been reported to be sensitive and degradative in many media and light environments (Bhinge et al., 2008). More so, SLM might have improved the trans-mucosal permeation of aceclofenac or the drug's lymphatic transport.

4. Conclusion

Solid lipid microparticles homolipid-based solidified reverse micellar solutions was formulated for the delivery of aceclofenac which offered a novel carrier for the poorly aqueous soluble substances. Owing to the lipid-based nature of SLMs, a higher bioavailability of aceclofenac with prolonged drug release with decreased dosing frequency was achieved. The content of irvingia fat whose melting peak was close to body temperature synergistically improve aceclofenac penetration *in vivo*. SLMs offer some advantages of good physical stability and decrease drug degradation with enhanced bioavailability of the lipophilic drug. The formulation also offers flexibility in terms of production and routes of administration with an improved anti-inflammatory property. More so, the SRMS property enhanced the prolonged control characteristics of the formulation. Thus, aceclofenac solid lipid microparticles homolipid-based solidified reverse micellar solutions offers a promising alternative to conventional oral drug delivery of aceclofenac.

Declaration

Author contribution statement

Calister E. Ugwu: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Jude N. Oraeluno: Analyzed and interpreted the data.

Kingsley C. Eze: Contributed reagents, materials, analysis tools or data.

Caleb O. Ezenma, Anthony O. Nwankwo: Performed the experiments.

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Data availability statement

Data included in article/supplementary material/referenced in article.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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