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DESIGN AND EVALUATION OF ENCAPSULATED COW FAT-BASED SOLID LIPID NANOCARRIER FOR IMPROVED BIOAVAILABILITY OF A POORLY WATER SOLUBLE NON STEROIDAL ANTI-INFLAMMATORY DRUG: EFFECT OF SURFACTANT IONIC STATE ON PRODUCT PERFORMANCE

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ABSTRACT

Oral route delivery of many non-steroidal anti-inflammatory drugs possessing poor gastrointestinal fluid solubility is associated with low gastrointestinal absorption and poor bioavailability. Nanoparticle technologies are being employed as effective techniques to overcome the challenge of poor aqueous solubility of many drugs. The study aimed at utilizing cow fat as solid-lipid nanocarrier system to enhance the aqueous solubility of diclofenac. Preformulation studies including melting points, gas chromatography-mass spectrophotometery, dynamic scanning calorimetry and Fourier transform infrared spectroscopy were carried out on the cow fat prior to product preparations Six (6) diclofenac-loaded solid lipid nanoparticle formulations containing varied proportions of stearic acid, cow fat and selected surfactants were prepared by the hot homogenization and ultra-sonication processes. The resulting preparations were characterized for surface morphology, particle size distribution, drug loading efficiency and drug release profiles. Thereafter, the SLNs were lyophilized and filled into number 4 empty gelatin capsule shells for further studies. The melting point of the fat was 43.13 ± 0.64 ^{o}C. Spectral analyses of the optimized SLN formulation revealed the existence of smooth surface with amorphous texture. The mean particle size of the SLNs formulated with stearic acid as solid lipid was in the range of 56.28 ± 0.56 - 100.23 ± 0.37 nm while formulations prepared with cow fat had their mean particles sizes ranging from 97.24 ± 04 to 171.29 ± 0.08 nm. The drug loading efficiencies of various formulations ranged from 72.32 ± 0.97 – 94.43 ± 0.11 % with the rate of releases being formulation component dependent. Quantitative data obtained suggested that design of diclofenac dosage form as solid-lipid nanosystem improved its gastrointestinal fluid solubility.

Keywords: GC-MS, FTIR, SLN, Friability, Gastrointestinal, Absorption

INTRODUCTION

Low solubilization of organic compounds in the gastrointestinal fluid remains a serious challenge to optimal absorption and systemic distribution of many pharmacologically effective drugs. Factors like complexity of chemical structures, large molecular weight and dominance of non-polar compounds have been suggested to be responsible for poor water solubility of many new drug entities (Merisko-Liversidge and Liversidge 2008, Szymański *et al*.2012). It is also thought that high throughput screening and other advanced drug development technologies have high tendency to result in complex, multidimensional, lipophilic and high molecular weight compounds and this, not only limit their solubility in polar solvents but also inhibit their passage through various gastrointestinal membrane transport routes Szymański *et al*.2012. Many novel formulation approaches and technologies are currently being used and/or investigated for improving systemic delivery of poorly water soluble drugs (Park et al., 2020; Sirvi et al., 2022).

Most widely investigated and commercialized nano technologies include, liposomes, niosomes, self-emulsifying drug delivery systems (SEDDS), hydrogels, microemulsions, solid lipid nanocarriers, among others. Among these multiplicity of approaches, SLN systems have been credited with superior attributes like, process simplicity, widely available and cheap inputs, good stability and high drug loading and delivery efficiency as well as robust funtionalization potential Gugleva, 2021). They are also reported to enjoy high mucous and epithelial membrane penetrability due to their lipophylicity and untra fine sizes (Abo El-Enin et al., 2022, Mirchandani et al., 2021, Pedro et al., 2016).

SLN is a homogenous mixture of sold state lipid(s), surfactant(s) and aqueos phase (water) Structurally the components of the mixture are stearically oriented to produce an inner solid lipid core and an outer shell made up of single layer of hydrophilic surfactant or surfactant mixtures By virture of the bipolar configuration, the SLN system is able to host both lipophlic molecules (drugs and other compounds) in the lipidic core and hydrophilic compounds within the hydrophilic surfactant in the outer shell (Almawash et al., 2023, Eskandani et al., 2022). Ideal lipids for SLN formulation naturally exist in solid state at room temperature and exhibit melting points that are well above the physiologic body temperature (Gabriella et al., 2023). The surfactant may be cationic, anionic non-ionic or amphoteric depending on desired functionality required of the inal product (Talegaonkar and Bhattacharyya, 2019). Apart from reducing the interfacial and surface tension between the lipid and water interphase, surfactants also modulate the nanoparticle suface charge and zeta potential of the system. During preparation, particle size reduction is achieved by many techniques

including use of ultrasonic wave (probe), ultrasonic bath, high pressure/high speed homogenization and microwave systems (Haque et al., 2018, Wang et al., 2018, Sathya et al., 2018).
Animal fats have been successfully used in the design of pharmaceutical delivery systems. Obite et al., (2011) used cow (*Bos indicus*) fat for the formulation of self emulsifying drug delivery system (SEDDS) for improved aqueous solubility of metronidazole Intestinal cow fat is solid at room temperature and has a melting point range of 40 – 45 ^{o}C which is well above the human physiologic body temperature. This property is specifically required for good SLN formulation (Gabriella et al., 2023) thereby making cow intestinal fat a good lipid candidate for the formulation of SLN. Despite these advantages, no research work was found in the literatures to have employed this edible widely available and nutritious material as an alternative to the mostly synthetic and semi synthetic lipids being utilized for SLN preparation. The current work is therefore an attempt to investigate the potentials of cow intestinal fat to function effectively as the solid lipid component of SLN systems designed to enhance the aqueous solubility and invitro release of the drug. Diclofenac, a poorly water soluble drug belongs to group II of the biopharmaceutics classification systems (BCS) with characteristic poor aqueous solubility and high membrane permeability (Amidon 1995). Solubility is their absorption rate limiting step. Improving the aqueous solubility using cow fat based SLN will not only enhance the invivo absorption of the drug but subsequently boost its bioavailability and therapeutic effectiveness. This will also create additional economic value for the cow fat and will likely stimulate more investigations on the role of animal fats in pharmaceutical processing and drug delivery.

MATERIALS AND METHODS

Sample fat was extracted from the intestine of a commercially sacrificed female cow (Bos indicus (Zebus) at an abattoir in Ogige market of Nsukka Local Government Area, Enugu State, and South East Nigeria on 10th September 2023. Stearic acid, Poloxamer 188, Sodium lauryl sulphate (SLS) and 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP) were products of Sigma-Aldrich (Vienna, Austria). Diclofenac powder was obtained from Medrel Pharmaceuticals, Pvt Ltd, India. All other chemicals and reagents were of analytical grades and were used without modifications except where otherwise specified.

Extraction of cow fat

Cow fat was manually rendered from the excised intestine of the female cow, *Bos indicus (*white Northern Nigeria Fulani specie*)*. The method used by Obite et al (2011) was employed with slight modification. In brief, the rendered fat was immersed in hot water (80 – 90 ^{o}C) for 1 h and thereafter cooled to room temperature. Extraneous matters were strained off using a piece of clean nylon cloth. Finally, the fat was recovered by decanting the lower aqueous layer.

Formulation Preparation and optimization of Diclofenac-loaded SLN

Previous works that utilized design of experiment method reported that the optimum percentage composition of the various SLN components included; solid lipid, 0 – 30 % w/w, aqueous medium, 0.99 – 99 %w/w and stabilizer/surfactant (dissolved in aqueos component), 0.5 – 5 %w/w (Dobreva et al., 2020, Thulasi, et al., 2022, Wei et al., 2016). On the bases of this our current SLN formulation was optimized using the procedure reported in Zang et al., (2007).Three different formulations containing equal quantity of , diclofenac, water and any of the non-ionic, anionic or cationic surfactants were prepared and subjected to standard characterization tests upon which the optimized formula was selected. The same procedure was adopted to optimize the cow fat-loaded SLN. Material components used for the current studies are shown in table 1.

Table 1: Formulation components of various batches of experimental SLN

Sample codes	Formulation components (mg)							
	Diclofenac powder	Stearic acid (lipid)	Cow fat (lipid)	DW	Poloxamer (ns)	SLS (as)	DOTAP (cs)	Total
SLN 1	100	30		65	5			200
SLN 2	100	30		65		5		200
SLN 3	100	30		65			5	200
SLN 4	100		30	65	5			200
SLN 5	100		30	65		5		200
SLN 6	100		30	65			5	200

Key: DW = distilled water, ns = non-ionic surfactant; as = ionic surfactant; cs = cationic surfactant; DOTAP = 1,2-dioleoyl-3-trimethylammonium-propane

Preparation of SLN

The drug-loaded SLN was prepared using the hot homogenization technique followed by ultrasonication as described in previous works (Florina et al., 2022, Katri, et al, Luigi et al, 2023]). Briefly, for each formulation batch, the solid lipid component (stearic acid or cow fat) was melted on a water bath under magnetic stirring. The Diclofenac was then introduced into the melted lipid under continued stirring and heating until the temperature of the mixture was brought to and retained at a temperature 10 ^{o}C above the lipid's natural melting point. In a separate set up, the surfactant was dissolved in deionized water (10 %v/v) and the resulting solution heated to the same temperature as the lipid phase (70 ^{o}C) and then gradually added to the lipid phase under gentle magnetic stirring (350 rpm) until a stable pre-emulsion was formed. Final formation of SLN was achieved by hot ultrasonocation of the pre emulsion using an ultra sonicator, (UP 200H, Hielscher Ultrasonics GmbH, Germany) with an ultrasonic frequency of 25 kHz and an amplitude of 100 % for 8 min. The resulting SLN was stored in a refrigerator at 4 ^{o}C for further studies.

Freeze drying/ lyophilization of prepared diclofenac SLN

The soft solid mass of the SLN was cut into several small pieces using a clean stainless steel kitchen knife and then placed in a deep freezer with the refrigerator temperature maintained at – 20 ^{o}C for 24 h. Thereafter, the chipped SLN were freeze dried (lyophilized) in a deep freezer-dryer equipment (Scientz No10 Series, China). The freeze drying conditions were, -40 ^{o}C and 0.200 mbar temperature and

vacuum respectively for 72 h. (Safvan et al., 2022). The dry SLN was gently pulverized to fine powder and screened through a No. 80 sieve and thereafter stored in a desicator for further uses.

Pharmaceutical encapsulation of SLN powder

Appropriately weighed quantities of the freeze dried SLN powder equivalent to 100 mg of diclofenac were separately weighed out and manually filled into No. 4 empty gelatin capsule shells.

Preformulation evaluation of components and characterization of diclofenac-loaded SLN

Determination of the melting point of cow fat

The melting point of the cow fat was studied using the USP/NF 36 (USP. 2018) melting point apparatus (ElecteroThermal®). About 10 mg quantity of the fat was forced into a glass capillary tube by pushing one open end of the tube into a soft mass of the fat, The tube, with the fat was then placed in the melting point apparatus and the temperature gradually adjusted upwards from ambient point while observing the fat for commencement and completion of melting. The temperature at which the fat melted completely was recorded as its melting point.

Gas chromatography-mass spectroscopic analysis of cow fat

The technique reported in Muruganandam et al., (2017) was used to conduct the GC-MS study of the cow fat using a hyphenated gas chromatography/mass spectrometer (Perkin Elmer GC 680/Clarus 600). For the GCMS component, an ionization energy of 70 eV was utilized while Helium gas (99.999 %) flowing at a rate of 1 ml/min with an injection volume of 1 µl and injector temperature of 250 ºC served as the carrier gas The base oven temperature which was set at 2 ºC was increased gradually at a rate of 10 ºC/min up to 300 ºC for a total of 6 min. The mass spectral readings were also taken at 70 eV at a scanning interval of 0.5 sec with total GC running time of 32 mins. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas.

Fourier transform infrared spectroscopic analysis pure diclofenac and the formulated SLN

FTIR spectroscopy of the pure diclofenac and the formulated SLN were conducted using standard procedures on a FTIR equipment, (Agilent Technologies USA) to determine if any chemical interaction occurred between the drug and the excipients. Briefly, the test samples were carefully blended with dry Potassium Bromide (KBr) and then compressed into discs which were placed on the equipment sample holder and thereafter scanned in the wavelength range of 4000–500cm^{-1}.

Scanning electron microscopy

A scanning electron microscope (JSM-6360LV. Hitachi, Tokyo, Japan) was used to study the surface morphology of the diclofenac-loaded SLN. Prior to the testing 100 µL of the SLN sample was dried for 24 h under vacuum on aluminium stub. An ultra thin film of powdered SLN was smeared on the aluminium plate and sputter-coated with thin gold platinium layer under Argon atmosphere. Thereafter, the sample was scanned and micrographed at selected magnification and at acceleration voltage of 15 KV (Kheradmandnia et al. 2010).

Particle size analysis, zeta potential and polydispersity index determination

The above parameters were determined concurrently in single test runs on a nanosizer equipment (Malvern Zetasizer Nano ZS90, UK) which employed the dynamic light scattering technology. Product samples for examination were suspended in triple distilled water with the standard operating temperature and light scattering angle set at 25 ºC and 90º respectively for the readings. The zeta potentials were measured at electric field strength of 23 V/cm (Manjunath and Venkateswarlu, 2005),

Determination of drug loading efficiency of various formulated SLN

The drug loading efficiencies (DLEs) of the various formulations were determined by subtracting the quantity of drug retained in the aqueous phase of the SLN from the total quantity of drug initially incorporated into each formulation. In a short detail, 4 mL of melted drug-loaded SLN was diluted to 8 mL with sodium lauryl sulphate solution and the resulting mixture centrifuged (Remi Instruments Ltd.Mumbai, India) at 6000 rpm for 1 h (Dilip et al., 2013). Upon standing, two separate phases were observed (aqueous and lipid phases). A ten-fold methanol dilution of the aqueous supernatant was assayed for diclofenac on a UV spectrophotometer (JENWAY 7305, Germany) at a λmax of 282 nm. The drug loading efficiency (DLE) was calculated using equation 1.

$$\text{DLE} = \frac{CW-AW}{CW} \text{ x } 100 \text{ \% where, --} \quad (1)$$

LE = loading efficiency

CW = calculated weight of diclofenac in the SLN portion taken for assay

AW = assay weight = weight of diclofenac in the SLN portion taken determined from assay procedure

Pharmaceutical quality analysis of the SLN capsules

Three quality assessments of the SLN capsules were conducted according to the methods of the British Pharmacopoeia [BP, 2020]. The tests included:

Capsule friability studies

Erweka model friabilator (Erweka 50254 Germany) was used to study the friability of the diclofenac-loaded SLN capsule.using the BP standard procedure. The percentage friability values were calculated as per equation 2.

$$\text{Percentage friability} = \frac{\text{W0}-\text{Wt}}{\text{W0}} \text{ x } 100 \quad (2)$$

Where, Wo is the initial total weight of six capsules before the test, and Wt is the total weight of the same six capsules after the test

Capsule disintegration studies

Capsule disintegration studies were conducted using a DT apparatus, (DCR Instruments, Jogeshwari East,Mumbai, India). For each of the formulations being tested, one capsule was dropped into each of the 6 tubes of the tester and the temperature of both the disintegration medium and water in the equipment tank maintained at 37 ± 2 ºC. The testing process was allowed to proceed until complete disintegration as described in the BP was attained.

Drug release studies

In-vitro dissolution rate tests of randomly selected capsules of each formulation in simulated gastric solution of 0.1 N HCl (pH 1.2) and in phosphate buffer solution (pH 6.8) were determined using the USP apparatus II equipment, (Metrolab H44 - 6). The volume and temperature of the dissolution media were 900 mL and 37 ± 0.5 ºC respectively. For each run, one capsule was placed inside the dissolution medium with the paddle rotating at 50 rpm. At intervals of 1 h, a 5 mL portion of the test media was withdrawn with a pipette, filtered and diluted (3-fold) and then assayed for quantity of

diclofenac on a UV/VIS spectrophotometer (JENWAY 7305, Germany).

Statistical analysis

Data obtained from the various tests were expressed as mean ± standard deviation and statistically significant differences were determined at $p < 0.05$ using one-way analysis of variance (ANOVA) in GraphPad Prism version 7.0 software.

RESULTS AND DISCUSSIONS

Melting point of cow fat sample

The melting point of the cow fat used in the study was determined at 43.13 ± 0.64 ºC. This value is optimum for the design of SLN which requires that the lipid component remain in solid state at ambient temperature and exhibit a melting point well above (not less than 40 ºC) the physiologic human body temperature (Patel et al, 2013). The solid state of the final products both on storage and upon oral ingestion is guaranteed by the relatively high melting point of the lipid component. This solid state enhances drug loading capacity, increases formulation stability as well as guarantees slow digestion of the nanoparticle ensuring controlled and prolonged release of the active ingredients (Cavendish et al,. 2019). Other effects of lipid melting point on the performance of SLN have been proposed (Mona and Ali 2020). Lipids that have high melting points form large particle-sized SLN and produce more viscous SLN. The 43.13 ± 0.64 ºC melting point determined for cow fat is moderate to ensure fine nanoparticles with good drug loading capacity.

Observed SEM micrograph (figure 1) show that the SLN were predominantly ammorphours in shapes with smooth topography. This observation was, however, in contrast to some previous work which reported near spherical diclofenac nanoparticles (Liparulo et al., 2020). The amorphous morphology may be a consequence of the presence of cow fats which contained a lot of long chain unsaturated fatty acids.

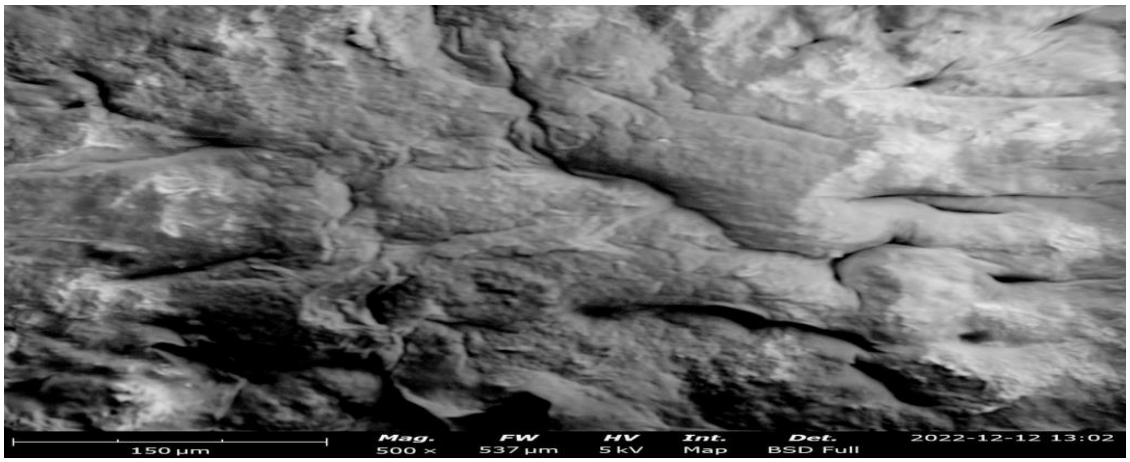

Figure 1: SEM nicrograph of SLN 5

GCMS analysis of cow fat

Lipds are generally composed of fatty acids, fatty esters, fatty alcohols, triglycerides and partial glycerides all with varied structures, carbon chain lengths, hydrophobicity,and different levels of saturation (Omeh et al., 2022, Yongtao et al., 2020).These parameters influence the properties of the formulated SLN (Mukherjee et al., 2009). GCMS analysis was conducted to investigate the biochemical/fatty acid composition of the cow fat used in the study. Twenty eight compounds made up of mainly fatty acids, estes, ethers, aldehyde and ketones were identified with oleic acid and fatty esters predominating. Table 2 shows the generated GCMS data. A lot of the components were long chain polyunsaturated fatty acids with implication of poor drug solubilising and low loading capacity (Omeh et al., 2024).

Table 2: Data and information generated from GCMS analysis

Peak no.	RT (minute)	Peak area (%)	Compounds	Molecular formula	Molar mass (g/mol	Fragment ion (m/z)
6	20.443	0.1122	Hexadecenoic acid,Z-11	C_{16} H_{30} O_2	254	55,83,111,147,206,254
8	21.366	0.2782	-methyl-Z,Z-3,13-octadecadienol	C_{19} H_{36} O	280	55,81,109,165,207,254,280
	23.168	0.23	11-dodecen-1-ol trifluoroacetate	C_{14} H_{25} F_3 O_2	280	55,73,97,129,146,165,192,221
10	25.503	18.669	Oleic acid	C_{18} H_{34} O_2	282	55,95,147,185,221,282
11	25.626	2.099	9-0xabicyclo[6.1.0] nonane	C_8 H_{14} O	126	55,97,126
13	25.898	5.4809	9-octadecenoic acid Z-2-hydroxy-1-ydroxymethyl)ethyl ester	C_{21} H_{38} O	354	55,97,147,221,280,354
17	27.344	12.059	Oleic acid	C_{18} H_{34} O_2	282	55,95,133,207,253,282
18	27.732	8.1739	Z-10-tetradecen-1-ol acetate	C_{16} H_{30} O_2	254	55,95,129,166,207,254
19	27.992	10.669	Z-13-methyl-11-pentadece-1-ol acetate	C_{18} H_{34} O_2	282	55,81,117,149,185,227,264,282
20	29.709	1.025	9-0xabicyclo[6.1.0] nonane cis	C_8 H_{14} O	126	55, 81, 98, 117,126
21	30.127	2..283	Hexadecanoic acid ethyl ester	C_{18} H_{36} O_2	284	55,88,115,135,157, 1185,213
23	32.717	0.254	Hexadecylpentyl ether	C_{21} H_{44} O	312	55,81,97,116,135,174,207,
24	33.622	4.333	9,12-octadecadienal	C_{18} H_{32} O	264	55,81,97,109,137,180,207,235
25	34.100	0.957	Succinic acid tridec-2-yl pent-4-en-1-yl ester	C_{22} H_{36} O_4	364	55,88,117,137,185,207,269
27	36.099	0.956	9,12-octadecadienal	C_{18} H_{32} O	264	55,88,98,117,135,155,177
28	38.526	-3.648	2-ethyl-3-trimethylsilyloxy(trimethylsilyl) butyrate	C_{12} H_{28} O_3 Si_2	276	73,109,147,185,221,200

FTIR spectroscopy

Figure 2 and figure 3 show the FTIR spectra of the pure diclofenac and the SLN (containing the diclofenac, fat and surfactant) respectively. The pure diclofenac which has a chemical formular of $C_{14}H_{10}C1_2NNaO_2$ exhibited characteristic peaks with tentative functional group assignments as shown in table 3 while the peaks and associated functional groups for the formulated SLN are shown in table 4.. The SLN clearly showed more peaks possibly due to the presence of additional functional groups from the excipients (fat and surfactant and their complexes. Some peaks that are present in the diclofenac spectrum were absent in that of the SLN. This may suggest the occurrence of some chemical interactions among the functional groups of the drug and those of the other components. Dilip et al. (2013) proposed that the disappearance of some peaks of the diclofenac as seen in this case and predominance of spectral peaks of the fat suggested that there were some interactions among the various molecules of the two compounds.

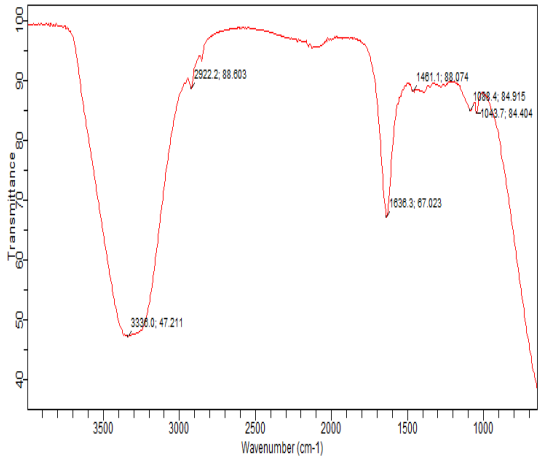

Figure 2: FTIR spectrum of pure diclofenac

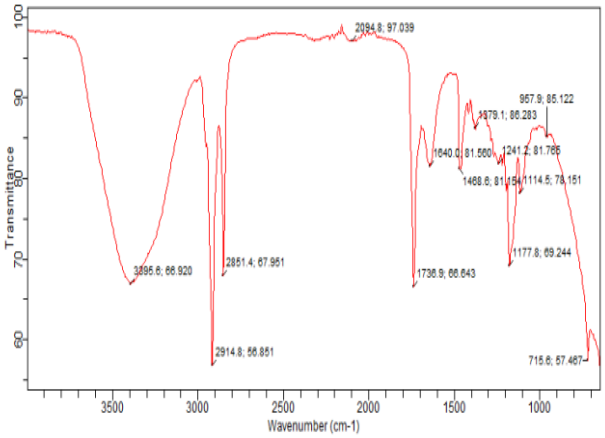

Figure 3: FTIR spectrum of formulated SLN

Table 3: FTIR spectral peaks of pure diclofenac and their corresponding tentative functional group assignments

Spectral peak	Spectral assignment
1043.7	Cyclohexane ring
1086.4	aliphatic chloro compounds,
1481.1	secondary amine
1608.3	>N-H bend
2822.2	C-H stretch (CH3-O-)
3338.08	aliphatic secondary amine (>N-H} stretch

Table 4: FTIR spectral peaks of SLN and their corresponding tentative functional group assignments

Spctral peak	Peak assignment
1177.67	secondary Amine
957.85	C-N stretch
1579.1	sromatic ethers
1020.67	aryl -O stretch
1730.6	secondary amine
2094.8	>N-H bend
3198.66	Carboxylate (carboxylic acid salt)

Particle size analysis zeta potential and polydispersity index

Figure 4 (A and B) are the graphical output of the DLS machine for the analysis of formulation SLN 5 which was selected as the optimized cow fat-based formulation. Both figures show that SLN 5 formulated with the cow fat and sodium lauryl sulphate had mean particle size of 97.24 ± 04 nm confirming that the current process yielded nanoscale particles which has been described as particles having average size range from 1 to 100 nm, The mean particle size of formulations SLN 4 and SLN 6 were 171.29 ± 0.08 and 165.78 ± 0.55 respectively. The particle sizes of formulations SLN 1, SLN 2 and SLN 3 all of which were prepared with stearic acid as the solid lipid were in the range of 56.28 ± 0.56 - 100.23 ± 0.37 nm. Comparatively SLNs prepared with stearic acid showed relatively smaller particles sizes than the ones prepared with the cow fat. This was independent of the surfactant type used. Formulation SLN 5 exhibited the finest particle size among the cow fat-based formulations. Fine particle sizes enhance the stability of SLN formulations and increases their membrane permeation and systemic circulatory half life. (Omeh et al., 2022). Particle size range of 50 -100 nm has been recommended for stable SLN.drug delivery systems. (Gabriella et al., 2023]). Nanosization also increases the absorption surface area and facilitates longer cellular membrane attachment; these factors increase drug bioavailability and subsequent therapeutic effectiveness.

The zeta potential of the optimized cow fat based formulation (SLN 5) was determined at -2.003 mV while the polydispersity index (PDI) was 0.47. The ZP and the PDI of the formulations seem to be influenced by the class of surfactant used with SLS (anionic surfactant) producing SLNs with higher ZP and PI. This observation may be attributed to the inherent negative charges possessed by the fatty acid

molecules which may have been augmented by the negative charge of the surfactant. On the contrary, the positive charge of the cationic surfactant (DOTAP) may have neutralized the opposite charge of the fatty acid dominated system. Similarly, higher concentration of similarly charged ionic species would have translate to higher inter particle repulsion and greater dispersion with minimal particle agglomeration. Negative ZP offers the advantage of more intimate and prolonged surface attachment of the carrier to the cell membrane since the later is reported to exhibit positive surface environment. The PI recorded in the current work showed that the nanoparticles had close particle size distribution which favours product stability. PI values less than 1 is indicative of a mono dispersed system. The higher loading efficiency of SLN 5 may be due to the fine particle sizes which provided larger surface areas for drug adsorption.

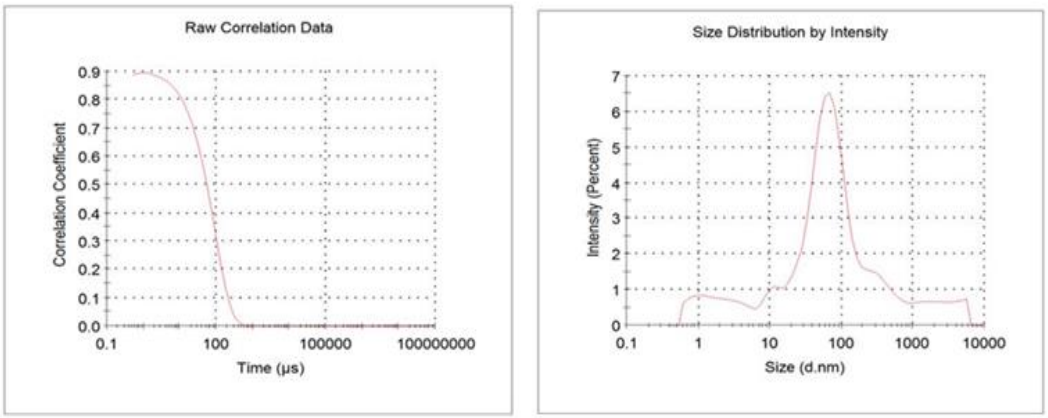

Figure 4: (A and B): DLS generated particle size distribution curves

Formulation of SLN capsules

Encapsulation of the SLN powder was necessary to: provide easy handling of the preparation, ensure consistent unit dosing, protect preparation and enhance stability and mask unpleasant taste of product.

Physicopharmaceutical evaluation of diclofenac SLN capsules

Table 5 and table 6 display some physical parameters of the formulated SLN. All the products had good appearance with no defects such as locking, telescoping, dust or dents. All the formulations equally complied with official specification for the individual parameters and as such passed the tests. The BP limit for friability is 0 - 1 % while the DT for conventional capsule should not be more than 15 min. Uniformity of weight studies requires that for tablets/capsules of weight 80 to 200 mg, not more than 2 tablets/capsule should deviate from the mean weight of 20 tablets/capsules with more than ± 7.5 % (BP 2020).

The drug loading efficiency of the SLN refers to the percentage of the total quantity of drug incorporated into the formulation that is taken up into the hydrophobic core (portion) of the SLN. The loading efficiency of the SLN prepared with stearic acid ranged from 80 to 88 % while the ones formulated with cow fate had LE values ranging from 60 to 76 %.

Table 5: Physicopharmaceutical parameters of formulated SLN

Parameter	Formulation batche codes					
	SLN 1	**SLN 2**	**SLN 3**	**SLN 4**	**SLN5**	**SLN6**
MPS (nm)	100.23 ± 0.37	56.28 ± 0.56	74.88 ± 0.66	171.29 ± 0.08	97.45 ± 0.71	165.78 ± 0.55
ZP (Mv)	-2.331	-2.976	1.883	-1.832	-2.003	1.432
PI	0.76	0.57	0.65	0.68	0.47	0.73
LE (%)	78	88	80	60	76	70

Table 6: Physicopharmaceutical parameters of encapsulated SLNs

Parameter	Formulation batch codes					
	SLN 1	**SLN 2**	**SLN 3**	**SLN 4**	**SLN5**	**SLN6**
Appearance	Good	Good	Good	Good	Good	Good
% friability	0.05	0.3	0.3	0.45	0.13	0.51
DT (min)	4	5	3	6	3	4
Uniformity of wgt	Pass	Pass	Pass	Pass	Pass	Pass

Drug release studies

The drug release profiles of various formulations are shown as figure 5. All the batches exhibited similar drug release pattern irrespective of the type of lipid and/or surfactant class used. There were initial rapid (burst) releases which were followed by relatively slower drug releases over time. The three stearic acid-based formulations released 25 - 35 % of their cargo drug within the first hour of the test whereas about 19 - 21 % of the drug load was released from the cow fat based formulations within the same period. Drug release rate within these periods increased increasingly. Beyond the third hour, rate of drug release decreased increasingly. However, drug releases from all the formulations were sustained up to the 7th hours of the test. Different formulations exhibited varied release rates with the stearic acid-based formulations showing faster and higher releases at all test points. Table 7 shows the release half life of each formulation. Dilip et al., (2013) suggested that burst releases followed by gradually

decreasing release rates was due to the existence of un-trapped drugs held at the surface of the SLN matrix adjacent to the dissolution medium. This portion of the drug freely went into solution. Thereafter, drug release rates were influenced by the path length of media penetration into the matrix and subsequent diffusion path length of absorbed drug out of the matrix into the medium. As these travel distances increased, the rate of release conversely decreased. This observation was in tandem with the Higuchi (1963) drug release kinetic model which proposed the planer concept of liquid front model for release of drugs from matrix systems.
The differences in the drug release rates observed for the stearic acid formulation as against the cow fat formulations were reflective of the differences in the formulations' loading efficiencies as observed earlier. The former group of formulations (stearic acid) exhibited higher loading capacities and consequent higher drug releases.. Release rate from the cow fat-based formulations may have also been slowed down due to the natural tendency of the lipophilic diclofenc to preferentially partition into the lipid core in preference to the surrounding hydrophilic dissolution media. Among the cow fat based formulations SLN 5 which contained a cationic surfactant (sodium lauryl sulphate) exhibited fastest drug release rate possibly due to the higher particle dispersion, and greater drug-loading surface areas. Unfortunately ionic surfactants have been associated with gastro side effects which make non ionic types better choice for oral formulations.

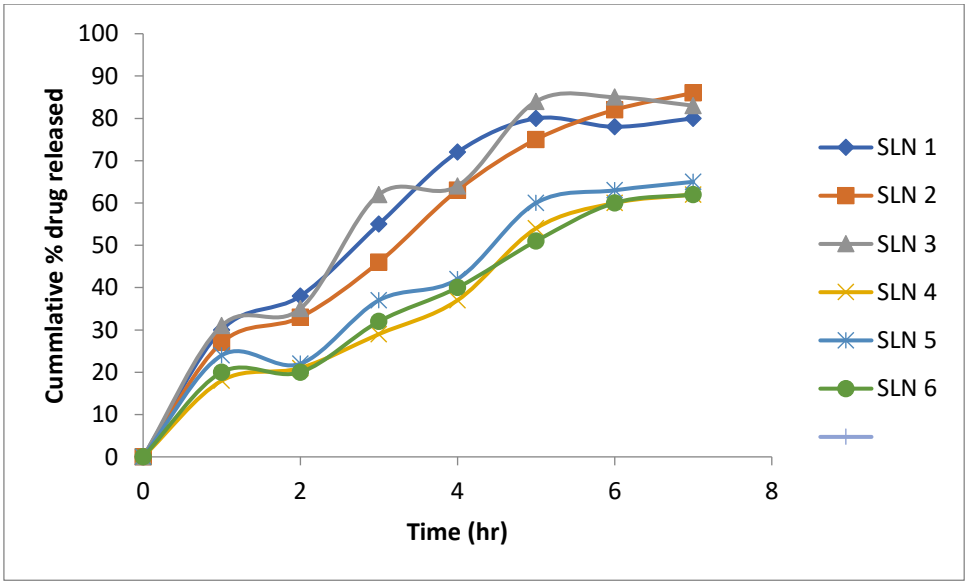

Figure 5: Drug release profiles of various SLN formulations in phosphate buffer (pH 6.8)

Table 7: Time for 50 % drug release ($t_{1/2}$) of various SLN formulations

Formulation	SLN 1	SLN 2	SLN 3	SLN 4	SLN 5	SLN 6
$t_{1/2}$ (hr)	2.5	3.7	3.7	4.3	4.5	4.4

CONCLUSION

Encapsulated diclofenac loaded solid lipid nanoparticles based on stearic acid and cow fat as the solid lipids were successfully formulated. Selection of surfactants based on ionic statues did not play significant role on the physic-pharmaceutical properties of the various formulations. However, stearic acid based SLN displayed superior relevant characteristics such as finer particle sizes, higher zeta potentials, greater loading efficiency and faster drug release rate profiles with formulation containing sodium lauryl sulphate as surfactants displaying optimum performance. FTIR analysis revealed occurrence of some interactions among the diclofenac active ingredient and cow fat component molecules. It may, therefore be concluded that cow fat has the potential to serve as the solid lipid component of solid lipid nanoparticle drug carrier. Future studies may undertake the modification (hydrolysis) of cow fat to improve its loading efficiency and drug release profile.

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