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NEW APPROACH TO SOLID LIPID MICROPARTICLES USING BIOCOMPATIBLE HOMOLIPIDS-TEMPLATED HETEROLIPID MICROCARRIERS FOR CIMETIDINE DELIVERY

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Abstract
Tallowation refers to the modification of lipid molecules using tallow fat while P90Gylation is the modification of lipid molecules by one or more phospholipid chains. Phospholipon® 90G (P90G) contains about 94.0 % of phosphatidylcholine stabilized with 0.1 % ascorbyl palmitate and is parenterally safe (GRAS) FDA approved excipient with wide application in drug delivery including surface modification of lipid particles (templation). P90Gylated-goat fat conjugate has numerous advantages: wetting, solubilisation, drug stabilization, emulsification and modified release. Here we report an evaluation of solid lipid microparticles (SLMs) formulated from this system and compared the effect of tallowation of the P90Gylated-goat fat matrices as an alternative microcarrier system for oral cimetidine administration in ulcerated rats. The results of this study showed that the templated homolipid mixtures had more spaces in them that entrapped cimetidine than P90Gylated-goat fat conjugate alone. Outstanding amongst them were the tallowated P90Gylated-goat fat matrices generated from 1:1 admixture of goat and tallow fats which showed 45.37 % encapsulation efficiency and the 2:1 templated matrix which exhibited the highest ulcer inhibition of 81.20 % superseding that of CEMTAB® which maximally inhibited ulcer at 72.50 %.

Keywords: Solid lipid microparticles, homolipids, heterolipid, P90Gylation, tallowation, goat fat; cimetidine.

INTRODUCTION

Novel pharmaceutical materials are achieved when one or two excipients are mixed at certain proportions. This novelty has been shown to be justified and recently received increased attention as to create new entities that are recognized to perform better or different functions compared to the native materials (Radtke and Muller, 2001), and to generally achieve wide applications (Muller *et al.*, 2002).

P90Gylation represents a conjugate system of lipid matrix with good drug delivery potentials (Nnamani, 2010, Nnamani *et al.*, 2010). However, mixtures of goat fat and P90G (P90Gylated-goat fat) as well as tallow fat-goat fat admixtures and P90G (tallowated P90Gylated-goat fat) as solidified reverse micellar solutions (SRMS) were employed to prepare SLMs used to deliver oral cimetidine to ulcerated rats.

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According to the biopharmaceutical classification system (BCS), cimetidine belongs to the class III drugs which are characterized by high solubility and low permeability. The aim of the present study was to enhance the permeability of cimetidine in intestinal Peyer's patches by increasing its surface area in the form of SLMs to enable the M-cells internalize and trancytose these microparticles to underlying lymphocytes.

MATERIALS AND METHODS

Materials

Phospholipon® 90G (P90G) (Phospholipid GmbH Köhn, Germany), is a purified, deoiled and granulated soy lecithin with phosphatidylcholine content of at least 90 %. Cimetidine CEMTAB® (Fidson Drugs, Nigeria), sorbic acid, sorbitol (BDH, England), and polysorbate 80 (Tween® 80, Uniqema, Belgium) were used as procured from their manufacturers without further purification. Homolipids (tallow fat and goat fat) were from batches prepared in the Pharmaceutics Laboratory of the University of Nigeria, Nsukka. Distilled water was obtained from the University of Nigeria, Nsukka (Lion water).

Extraction and purification of homolipids

Goat fat was extracted from the adipose tissue of *Capra hircus* according to an earlier method (Attama *et al*, 2003; Nnamani *et al*., 2010). Briefly, the adipose tissue was collected from freshly slaughtered goat, manually freed of extraneous materials, crushed and boiled in distilled water for 45 min, filtered through a muslin cloth and allowed to solidify at room temperature. The solid fat was manually removed and bleached/deodorized by passing it through a mixture of activated charcoal and bentonite (2:1) at 100 ºC at a ratio of 10 g of the fat to 1 g of the column material.

The above procedure was repeated using tallow fat from *Bos indicus* to obtain tallow fat.

Formulation of P90Gylated-goat fat matrices

The lipid matrices used in the formulations were a 4:1 mixture of goat fat and P90G according to an earlier described method (Nnamani *et al*., 2010; Nnamani, 2010). In brief, the lipids were weighed with an electronic balance (Mettler H8, Switzerland), melted together at 60 ^{o}C on a thermo-regulated water bath shaker (Heto, Denmark) and stirred until solidification.

Preparation of binary lipid matrices

Binary mixtures of goat fat and tallow fat in the ratios of 1:1, 1:2 and 2:1 were prepared by fusion as described elsewhere (Nnamani, 2010).

Incorporation of Phospholipon® 90G into the binary lipid matrices

The various lipid matrices of 1:1; 1:2, and 2:1 were further mixed with Phospholipon® 90G in a 4:1 ratio such that they separately contained 25 % (w/w) of P90G in each 1:1; 1:2 and 2:1, binary mixtures of all three binary solid lipid solutions. The lipids were prepared by fusion prior to microparticle preparation.

Differential scanning calorimetry (DSC) of lipid matrices

Melting transitions and changes in heat capacity of the pure goat fat and tallow fat as bulk materials, and as physically structured (P90Gylated) lipid matrices were determined by DSC (NETZSCH DSC 204 F1, Germany). Approximately, 3 – 5 mg of each lipid matrix was weighed (Mettler M3 Microbalance, Switzerland) into an aluminium pan, hermetically sealed, and the thermal behaviour determined

in the range of 35-190 ^{o}C under a 20 ml/min nitrogen flux at a heating rate of 10 ^{o}C/min. The baselines were determined using an empty pan and all the thermograms were baseline-corrected.

Formulation of unloaded SLMs

SLMs were formulated to contain 5 % w/w of the lipid matrix previously molten (4:1 mixture of goat fat and P90G), graded concentrations of polysorbate 80 (0.0, 0.75, 1.5, and 2 % w/w), 4 % w/w of sorbitol, 0.1 % w/w of sorbic acid and enough distilled water to make 100 % w/w (Nnamani *et al*., 2010). The hot homogenization method was adopted.

Previously molten P90Gylated binary lipid matrices were employed to prepare SLMs using 1.5 % of polysorbate 80.

Formulation of cimetidine-loaded SLMs using P90Gylated-goat fat matrices

By adding cimetidine (graded concentrations of 50, 100 and 200 g %) to the lipidic phase (goat fat structured with P90G) and following the previously described procedure, cimetidine-loaded SLMs were obtained.

Formulation of cimetidine-loaded SLMs using the binary structured lipid matrices

By adding cimetidine (10 % w/w) to the binary structured lipidic matrices of goat fat-tallow fat, and following the previously described procedure using 1.5 % polysorbate 80, cimetidine-loaded SLMs were obtained. In each case, three determinations were undertaken for each ratio combination of the matrices and mean values noted.

Evaluation of SLMs

DSC of drug and drug-loaded SLMs

Melting transitions and changes in heat capacity of cimetidine and its SLM-containing formulations were ascertained by DSC as previously described, at different scan ranges of 35 – 190 ^{o}C.

Particle size analysis and morphology of SLMs

Particle size analysis was carried out on the SLMs after production using a digital light microscope (Leica Diestar, Germany) and images captured with Moticam 1000 camera. The morphology (shape and surface) of the particles was also noted. The SLM were also subjected to time-resolved particle size analyses for 12 months at 6 months intervals to check the effect of storage on the particle size.

Drug encapsulation efficiency

Approximately 6 ml of the cimetidine-loaded SLMs was added into a microconcentrator (5, 000 MWCO Vivascience, Germany). This was centrifuged (TDL-4 B. Bran Scientific and Instru. Co., England) at 3,000 rpm for 120 min. The supernatants were adequately analyzed by UV/Vis Spectrophotometer (Unico 2102, England) at 254 nm. The amount of drug encapsulated in the microparticles was calculated with reference to a standard Beer's plot to obtain the % encapsulation efficiency (EE) using the formula below:

$$EE\ (\%) = \frac{\text{Real drug loading}}{\text{Theoretical drug loading}} \times 100 \quad \ldots\ldots (1)$$

***In vitro* diffusion studies**

Franz diffusion cells with a receiver compartment volume of 20 mL and effective diffusion area of 2.84 cm^2 were used to evaluate drug delivery characteristics from the selected compositions. A Millipore membrane filter (0.22 µm), (Millipore Corporation, Billerica, MA) was used. The receptor phase (phosphate buffer solution, PBS, pH 7.4) was

continuously stirred and kept at a temperature of 37 ± 0.5 °C during the experiments. A 1 ml volume of the drug-loaded SLM formulations was placed in the donor compartment. At appropriate time, 1 ml of the sample was withdrawn from the receiver compartment and the same amount of fresh solution was added to keep the volume constant. Each experiment was run in three independent cells. The samples were analyzed spectrophotometrically at a wavelength of 254 nm and the concentration of cimetidine in each sample was determined from a standard curve. Each data point represented the average of three determinations. The release study was carried out for 24 h period. Sink conditions were maintained throughout the experiment.

In vivo investigation of ulcer

The aspirin model was employed to induce ulcer in the experimental rats. Male Wistar albino rats weighing 220-250 g obtained from the animal house of the Department of Pharmacology and Toxicology, University of Nigeria, Nsukka were used. The rats were placed on standard feed and housing conditions for some time to acclimatize and fasted over night before the experiment. Thirty-six fasted rats were divided into 9 groups of 4 rats each.

Firstly, only 4 rat groups were employed and the first three groups received (1 ml p.o.) cimetidine-loaded SLMs containing 5, 10 and 20 g % while the 4th group received blank SLM without cimetidine (1 ml p.o.).

Secondly, the structured-binary lipid matrices loaded with cimetidine (10 g %) were investigated for anti-ulcer activity in another set of ulcerated rats. Groups 1-6 were given 2 ml of the six different batches of the SLM preparations (i.e. three drug-loaded and three zero-drug loaded SLMs corresponding to the 1:1; 1:2 and 2:1 structured matrices of goat fat and tallow fat) formulated with or without cimetidine (10 g %). Groups 7 and 8 received I ml p.o. of 200 mg of CEMTAB® dispersed in distilled water and 2 ml of distilled water p.o. respectively, while Group 9 received 200 mg of pure cimetidine powder in distilled water orally. One hour post administration, all rats were given 200 mg/kg of Aspirin p.o. and two hours later, they were sacrificed using ether, their stomachs isolated and cut along the greater curvature. The stomachs were washed and viewed with an X_{10} magnifying lens.

Ulcer scores were calculated as thus: ≤1 mm = 1; >1 mm but ≤ 2 mm = 2; > 2 mm = 3

The scores were summed, divided by X_{10} magnification and averaged by number of animals to get the mean ulcer indices from where the percentage ulcer inhibition was calculated as:

$$UI = \frac{ulcer\ index\ (control) - ulcer\ index\ (test)}{ulcer\ index\ (control)} X\ 100$$

…… (2)

Stability studies of the formulations

The method of Nnamani *et al*., 2010 was employed here. Briefly, some 6 ml volumes of each microparticle were stored in closed glass bottles and placed at 4-6 ^{o}C; 25 ^{o}C, and 40 ^{o}C away from direct light. Aliquots were withdrawn every 6 months to determine particle size and morphology as earlier described.

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Determination of injectability

Injectability, defined as the smallest needle gauge that a microparticulate sample could pass through, was determined according to the method of Nnamani e*t al.* (2010).

Statistical analysis

All experiments were performed in replicates for validity of statistical analysis. Results were expressed as mean ± S.D. ANOVA and student's t-test were performed on the data sets generated using Predictive Analytics SoftWare (PASW Statistics 18.0, 2009) formerly called SPSS. Differences were considered significant for p-values < 0.05– 0.001.

RESULTS AND DISCUSSION

Characterization of lipid matrices

The melting endotherm for tallow fat was 54.5 ^{o}C with an enthalpy of -5.067 mW/mg (Nnamani *et al.*, 2010). The DSC thermogram of goat fat showed an endothermic peak at 53.7 ^{o}C with an enthalpy of -6.42 mW/mg. This melting point value was slightly different from that in the literature reported by Attama and Müller-Goymann, 2006 and the possible reason may be a question of sensitivity of the DSC machines used. The lower melting peak would belong to unstable modification, while the higher peak belongs to stable modification (Nnamani *et al.*, 2010).

The higher the enthalpy of the transitions, the more crystalline the matrix and consequently, the more difficult it may be for any drug to be encapsulated (Attama *et al.*, 2007). This is because highly crystalline matrices have perfect crystals without much space to entrap any drug. Comparatively, it could be said that goat fat is more crystalline (higher enthalpy) than tallow fat.

Structuring of bulk crystalline matrices with P90G generally produce matrices with lower melting endotherms as well as enthalpies (Nnamani et al., 2010; Attama et al., 2007). For instance, when tallow fat was structured with P90G, the melting peak and enthalpy changed from 54.5 ^{o}C and -5.067 mW/mg to 52.2 ^{o}C and -5.501 mW/mg; goat fat changed from 53.7 ^{o}C and -6.42 mW/mg to 50.8 ^{o}C and -2.813 mW/mg. This is because it has recently been shown that P90G is a good surface modifier for solid lipid particles (Schubert *et al.*, 2005; Schubert and Muller-Goymann, 2005) with resultant improvement in targeting and pharmacokinetics (Huang *et al.*, 1992; Heiati *et al.*, 1998). The phospholipids bilayer structure formed around the lipid core may increase the drug loading capacity, as biologically important molecules can be anchored on the colloidal particle surface, and surface-modification also enables stabilization of colloidal particles especially when generation of the microparticles is carried out in an aqueous medium (Sastry, 2000).

The thermotropic phase behaviour of a lipid matrix changes on encountering guest molecules such that the thermodynamic variables of melting temperature and changes in enthalpy depend on the nature of interaction between the constituents (Attama *et al.*, 2007). Indeed, considering that the degree of lipid crystallinity and the possible modifications in the lipid's solid state are correlated with drug incorporation and release rates, and considering that the drug's solid-state form (amorphous or crystalline) in solid dispersions influences dissolution rates, it is important to pay special attention to these parameters (Mehnert and Mader, 2001; Savolainen *et al.*, 2002).

The bulk materials of goat fat and tallow fat were subjected to DSC analysis as starting materials. This characterization step was necessary in

order to detect possible modifications in the physicochemical properties of the drug intended to be incorporated into SLMs and of the lipophilic excipients. It has been proved that although particles were produced from crystalline materials, the presence of emulsifiers, the preparation method and the high-shear dispersion may account for changes in the crystallinity of matrix constituents compared with the bulk materials. This may lead to liquid, amorphous or only partially crystallized metastable systems (Westesen *et al.,* 1997).

Moreover, the Phospholipon® 90G used in this study mainly contains linoleic, oleic, stearic and palmitic acids, which are fatty acids of different chain lengths and degrees of saturation (Stuchlik, 2001). The interaction of these fatty acids with the diverse fatty acids present in goat and tallow fats may have resulted in the partly amorphous nature of the lipid matrix containing the phospholipids (Kameda, 2004). The fatty acid present in goat fat is C16:0, C18:0, and C18:1, somewhat similar to that of theobroma oil and tallow fat alike (Attama and Muller-Goymann, 2006) which remain as liquid crystals (solid/liquid) over a wide temperature range indicated by their very broad endotherms (Nnamani *et al.,* 2010).

Fig. 1 shows the collective thermograms obtained when goat fat was combined with tallow fat in different ratio combinations of 1:1; 1:2, and 2:1. Mixtures of lipids have been shown to possess varied and mixed transition peaks and have been suggested as alternatives to lipid modification by chemical techniques as the latter often leads to products of decreased *in vivo* tolerability (Kim *et al.,* 2005).

A 1:1 (i.e. 50:50) mix of both lipids yielded a hybrid matrix with melting endotherm of 54.0 ^{o}C and an enthalpy of -5.803 mW/mg. This, more or less, tended towards that of tallow fat (54.5 ^{o}C and -5.067 mW/mg) rather than goat fat (53.7 ^{o}C and -6.42 mW/mg), signifying a somewhat amorphous system. The 1:2 mix of goat and tallow fats yielded a matrix which melted at 54.1 ^{o}C but had an enthalpy of -8.298 mW/mg suggesting a high crystalline matrix which is probably going to expel any drug it entraps upon storage. The 2:1 mix of goat and tallow fats also gave a hybrid which melted at 53.6 ^{o}C with an enthalpy of -7.811 mW/mg, suggesting less crystalline system than the former.

Crystallinity of lipid matrices affects the functional properties of the SLMs derived from them. Lipid mixtures can result in increased or decreased crystallinity. Directly, after preparation, lipids crystallize partially in higher energy modifications (α, β') with more imperfections in the crystal lattice (Freitas and Muller, 1999; Radtke and Muller, 2001). If however, a polymorphic transition to β modification takes place during storage, any incorporated drug could be expelled from the lipid matrix and it can then neither be protected from degradation nor released in a controlled manner. To overcome such problem, use of mixtures of lipids which do not form highly ordered crystalline arrangement is required. Such lipid matrix could be achieved by using solid lipid and liquid lipid

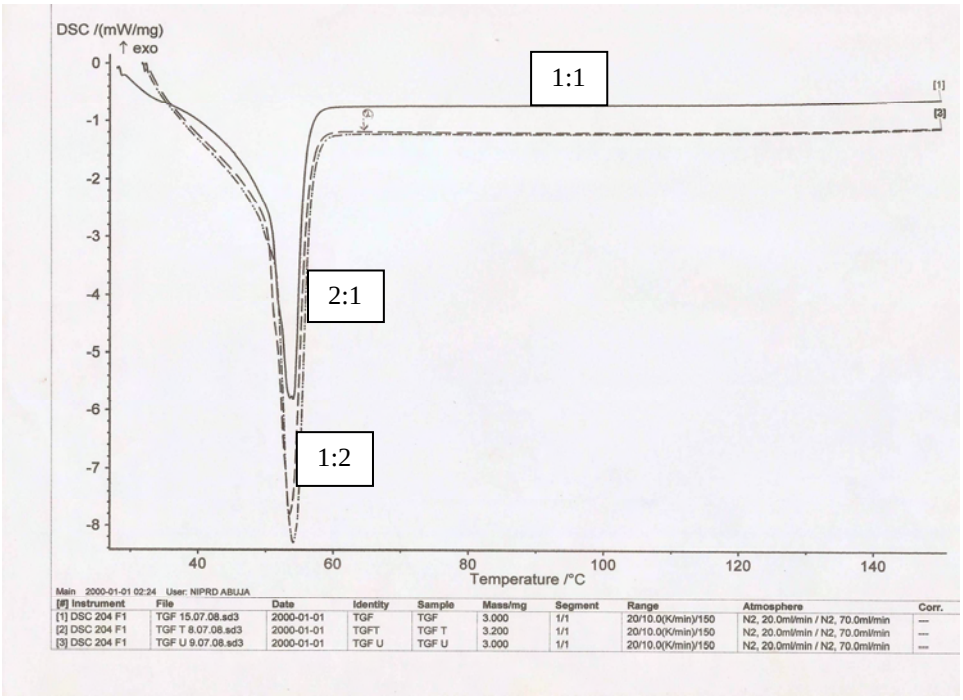


Fig. 1: DSC thermograms of binary mixtures of goat fat - tallow fat matrices

(Jenning *et al.*, 2000) or solid lipid mixtures of complex nature such as mono-, di- or triglycerides of different chain lengths (Wissing *et al.*, 2004). Mixture of lipids also modifies the polymorphic properties of the individual lipids, and has been shown to generate lipid matrices of low crystallinity (Attama *et al.*, 2006).

In contrast, when these binary-lipid matrices were structured with P90G, their properties were further modified. The results of the physical structuring of goat fat and tallow fat combinations as presented in Fig. 2 were also compared. The matrix obtained when 1:1 (i.e. 50:50) mix of goat fat and tallow fat were structured with P90G melted at a peak temperature of 51.4 ^{o}C with an enthalpy of -2.52 mW/mg. When the matrix containing 1:2 mix of goat and tallow fats was structured with P90G, the resultant matrix showed an endothermic peak of 51.7 ^{o}C and enthalpy of -2.766 mW/mg. On further variation of 2:1 mix of both fats, the resultant structured matrix melted at 52.0 ^{o}C with an enthalpy of -4.433 mW/mg. Comparatively, all tallowated P90Gylated-goat fat matrices showed low crystallinity in all proportions compared to the earlier values of the binary mixtures of goat and tallow fats prior to physical structuring with P90G. However, this suggests that tallowation of P90Gylated-goat fat matrices created enormous spaces for possible drug localization in the order of 1:1>1:2>2:1.

Morphology and particle size analysis of SLMs containing different concentrations of cimetidine

The result of the particle size analysis of the SLMs is presented in Table 1 while the morphology is shown in Fig. 3. The increase in particle size with increasing drug loading has been observed by other authors (Chorny *et al.*, 2002, Nnamani, 2010).

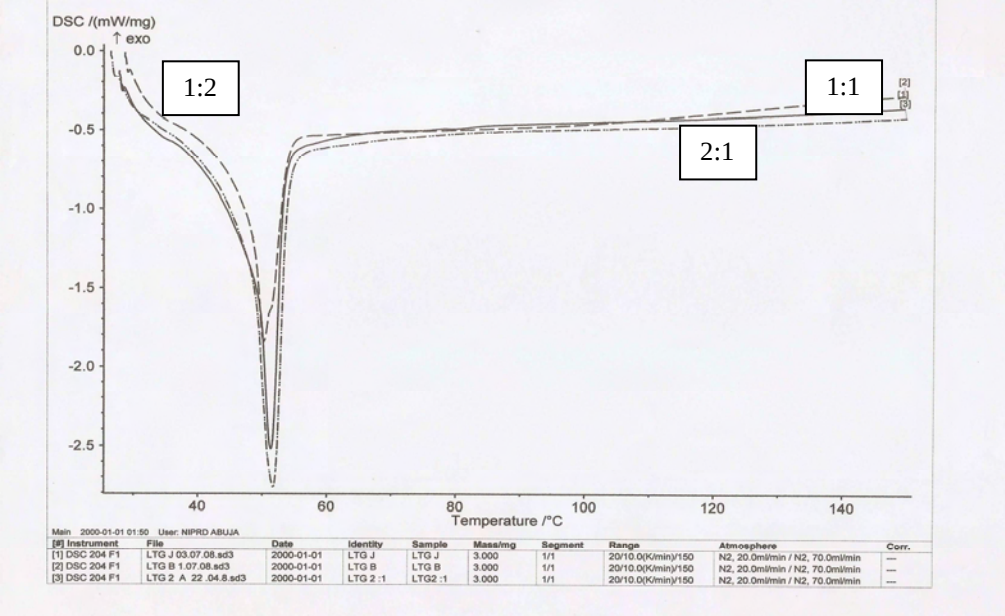


Fig. 2: DSC thermograms of P90G-structured goat fat – tallow fat matrices

Table 1: Properties of the SLMs loaded with graded concentrations of cimetidine

Formu-lations (GF/P90G)	Drug composition (g)	Average particle size (µm)		Drug encapsulation efficiency (%)	Injectability (Gauge) at 25 °C		
		After preparation	After 6 months storage		1 week of preparation	After 6 months	After 12 months
SLM-0	0.00	3.50±0.9	5.34±0.3	-	27	23	18
SLM-1	0.05	4.23±1.2	5.97±1.6	22.54 ± 3.40	27	23	18
SLM-2	0.10	10.71±1.5	56.68±10.8	25.00 ± 7.50	27	18	NS
SLM 3	0.20	21.36±2.0	110.76±35.9	17.21 ± 4.90	27	23	NS

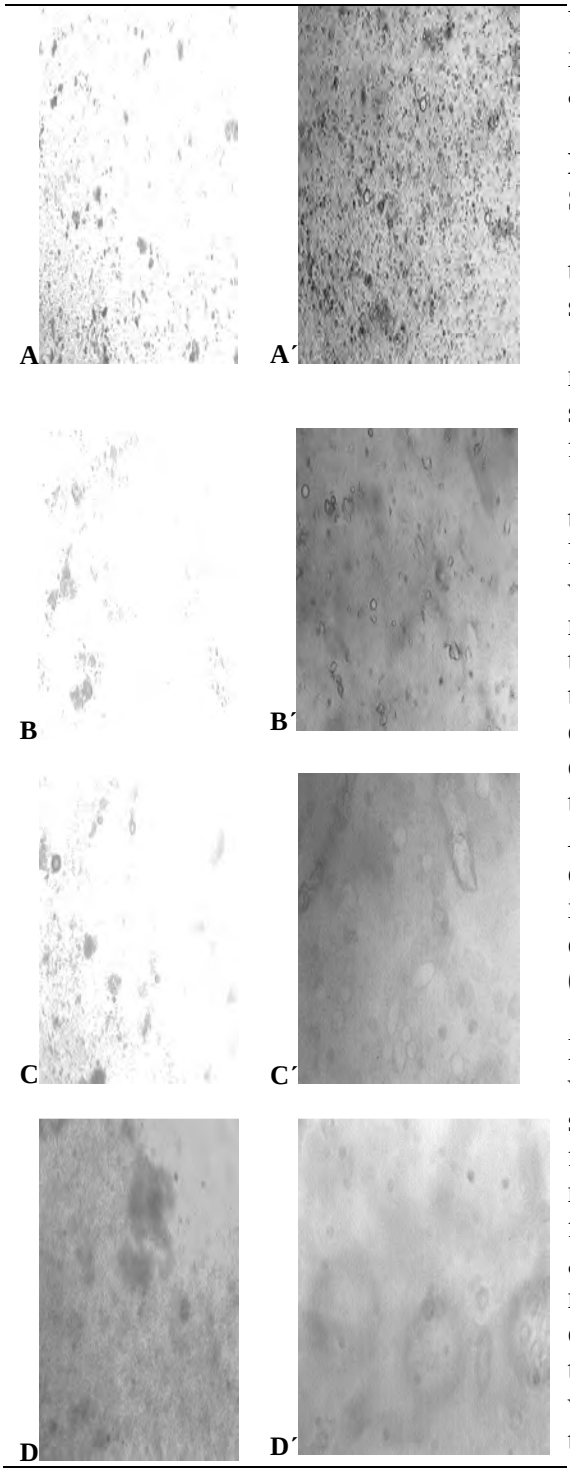


Fig. 3: Photomicrographs of the SLM dispersions; (A) Blank SLM; (B) 0.05 %, (C) 0.10 % and (D) 0.2 % w/w cimetidine-loaded SLMs within one week of preparation and (A´- D´) after six months of preparation.

Upon storage, the microparticles grew in size in line with the findings of other authors (Muller *et al*, 2000).

DSC properties of cimetidine and SLM formulations

Fig. 4 shows the DSC trace of the pure cimetidine sample which showed a melting peak temperature of 145.3 °C with an enthalpy of -2.759 mW/mg. This however falls within the specified melting temperature range for a pure cimetidine sample.

Table 2 shows the properties of the formulated SLMs produced using P90Gylated-goat fat (^{a}Formulations) as well as tallowated P90Gylated-goat fat matrices (^{b}Formulations). Summarily, the blank SLMs (^{a}formulations) was the most crystalline (-18.32 mW/mg) of all formulations whereas the SLMs containing 0.1 g w/w of cimetidine was the least crystalline (-9.56 mW/mg). As a result, this concentration of cimetidine was further employed to investigate the structured-binary combinations of goat fat and tallow fat (^{b}formulations).

Fig. 5 shows the result of the DSC analysis of the SLMs (with or without cimetidine) produced using the structured matrices of goat and tallow fats. A plain structured 1:1 (i.e. 50:50) mix of this matrix yielded an SLM formulation without drug which melted at 118.1 ^{o}C with an enthalpy of -12.1 mW/mg, while its drug-containing counterpart traced two endothermic transitions which occurred at 120 ^{o}C with an enthalpy of -15.67 mW/mg for the lower peak and at 124 ^{o}C with an enthalpy of -16.43 mW/mg for the higher peak. This high temperature of melting of the SLMs was quite closer to the melting point of the incorporated drug rather than the lipid matrices.

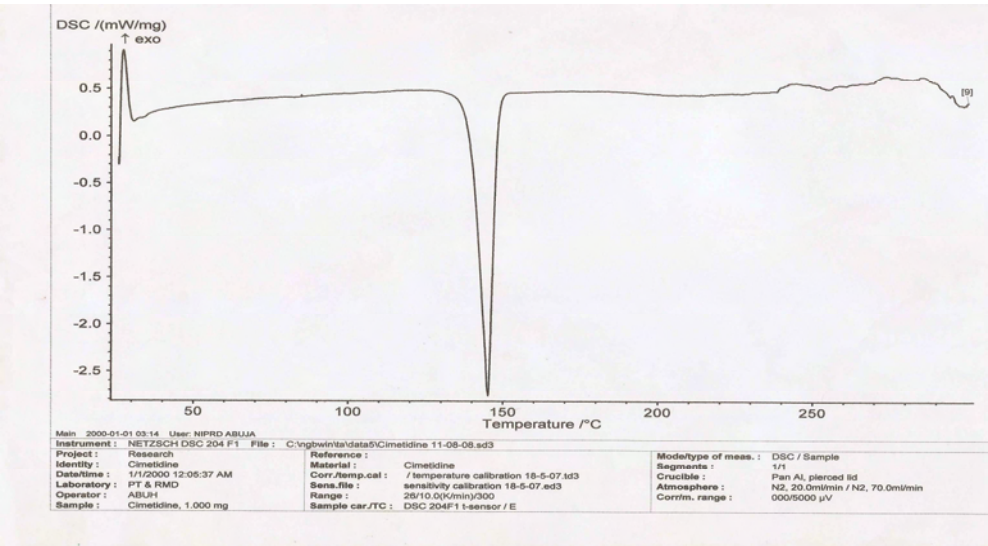
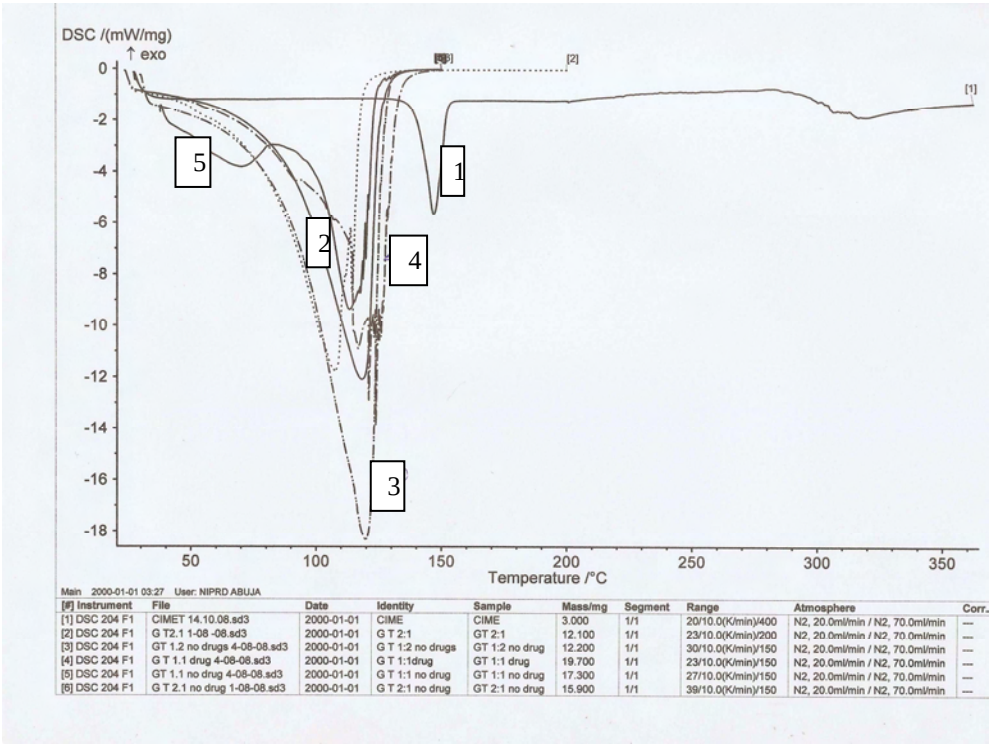


Fig. 4: DSC thermogram of pure cimetidine



[#] Instrument	File	Date	Identity	Sample	Mass/mg	Segment	Range	Atmosphere	Corr.
[1] DSC 204 F1	CIMET 14.10.08.sd3	2000-01-01	CIME	CIME	3.000	1/1	20/10.0(K/min)/400	N2, 20.0ml/min / N2, 70.0ml/min	---
[2] DSC 204 F1	G T2.1 1-08 -08.sd3	2000-01-01	G T 2:1	GT 2:1	12.100	1/1	23/10.0(K/min)/200	N2, 20.0ml/min / N2, 70.0ml/min	---
[3] DSC 204 F1	GT 1.2 no drugs 4-08-08.sd3	2000-01-01	G T 1:2 no drugs	GT 1:2 no drug	12.200	1/1	30/10.0(K/min)/150	N2, 20.0ml/min / N2, 70.0ml/min	---
[4] DSC 204 F1	G T 1.1 drug 4-08-08.sd3	2000-01-01	G T 1:1drug	GT 1:1 drug	19.700	1/1	23/10.0(K/min)/150	N2, 20.0ml/min / N2, 70.0ml/min	---
[5] DSC 204 F1	GT 1.1 no drug 4-08-08.sd3	2000-01-01	G T 1:1 no drug	GT 1:1 no drug	17.300	1/1	27/10.0(K/min)/150	N2, 20.0ml/min / N2, 70.0ml/min	---
[6] DSC 204 F1	G T 2.1 no drug 1-08-08.sd3	2000-01-01	G T 2:1 no drug	GT 2:1 no drug	15.900	1/1	39/10.0(K/min)/150	N2, 20.0ml/min / N2, 70.0ml/min	---

Fig. 5: DSC thermograms of SLM formulations with P90G-structured Goat fat and tallow fat matrices containing cimetidine

However, when the 1:2 mix of the same matrix was used, the resultant blank SLM melted at 119.8 ^{o}C with an enthalpy of -18.32 mW/mg, while its drug-containing counterpart melted at 120.82 ^{o}C and had an enthalpy of -17.52 mW/mg. On further varying the composition to 2:1, the resultant SLM recorded a melting peak of 113.2 ^{o}C and -9.336 mW/mg value of enthalpy, while its drug-loaded counterpart melted at 107.5 ^{o}C with an enthalpy of -11.77 mW/mg.

It follows that with lipid drug delivery systems, polymorphic transformations may occur during dosage form preparation and subsequent storage. During the melt solidification, triglycerides and fatty acids in particular can crystallize into different polymorphic forms (i.e., the thermodynamically unstable α-form, the β′- form, the stable β-form) depending on lipid composition and cooling rates. Polymorphic transformations may cause changes in active and auxiliary substances solubilities and melting points. In particular, the conversion of one polymorph into another may change the physical properties of the substance (Reithmeier *et al.,* 2001).

Drug encapsulation efficiency

Table 1 shows the result of the properties of the cimetidine-loaded SLMs formulated with P90Gylated-goat fat matrices. A maximum drug loading efficiency of 25.0 ± 7.5 % was obtained which corresponded to the SLMs containing 0.1 g w/w of cimetidine. This was not surprising because the DSC analysis of the SLMs suggested high crystallinity (Table 2, [a]formulations) except for the SLMs containing 0.1 g w/w of cimetidine which was the least crystalline. The low crystallinity meant more spaces for cimetidine entrapment even though the encapsulation efficiency was generally low. This is probably due to the fact that cimetidine is a high dose drug with usual dosage of 200 or 400 mg. As a matter of fact, it could not have been easy to entrap the 10 g w/w of this heavy drug in the 5 g w/w of the lipid matrix. Besides, cimetidine is slightly water soluble. As a matter of fact, this concentration of cimetidine was selected to load the various tallowated P90Gylated-goat fat matrices of 1:1, 1:2 and 2:1. The drug encapsulation efficiency therefore increased due to tallowation (Table 3). In any case, the 1:1 matrix gave the highest loading efficiency of 45.37 ± 9.26 % whereas the 1:2 gave 40.30 ± 10.20 % while the 2:1 gave 38.87 ± 3.90 % (Table 3).

***In vitro* drug diffusion studies**

The *in vitro* drug release studies of the SLMs containing cimetidine in tallowated P90Gylated-goat fat matrices behaved in a different way entirely as shown in Fig. 6. The graph suggests low *in vitro* release profiles. This may probably be due to the fact that cimetidine is slightly soluble in water. The highest release profile of 15.72 mg % was seen at 9 h in the batch corresponding to 1:2 structured combinations of goat fat and tallow fat. The release profile traced an erratic nature.

The batch corresponding to the 2:1 matrix had a more controlled release of cimetidine over a period of 10 h achieving a maximum of 13.60 mg %. The 1:1 batch had the least release profile achieving a maximum of 7.07 mg % at 6 h. The percentage amount of drug released was generally lower than the theoretical drug loading.

The release was generally erratic with plateau and troughs here and there until it continuously decreased in quantity of drug released even though sink conditions were maintained throughout the release study. Yet the quantities released were very small. This may probably be due to the fact that cimetidine is a high-

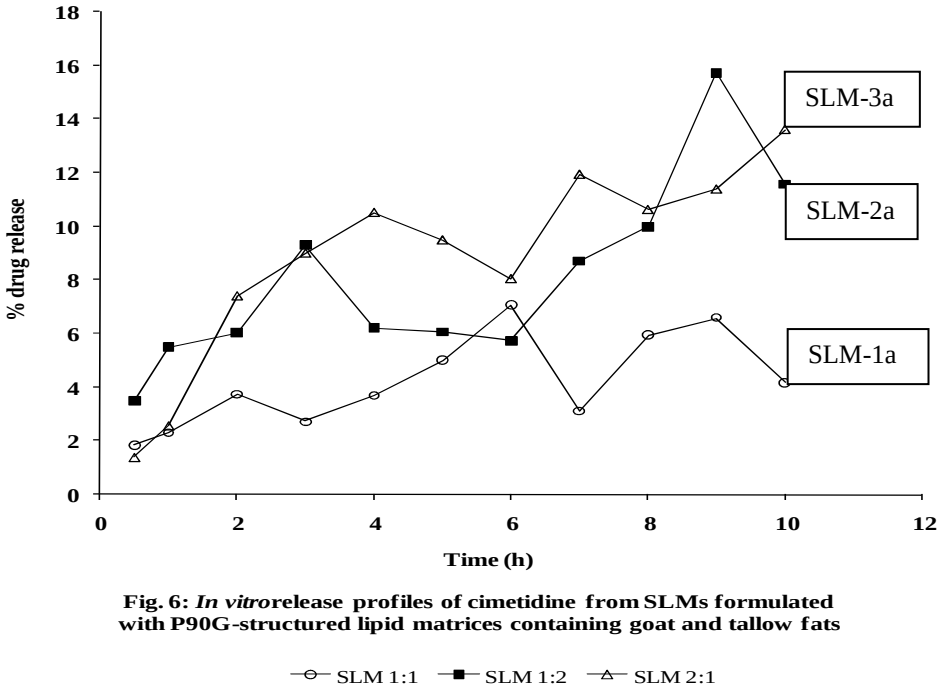


Fig. 6: *In vitro* release profiles of cimetidine from SLMs formulated with P90G-structured lipid matrices containing goat and tallow fats

Table 2: DSC properties of the SLMs produced using [a]P90Gylated-goat fat and [b]tallowated P90Gylated-goat fat matrices.

[a]Formulations	Lipid matrix (mg)			Drug Composition (g) w/w	Melting point (^oC)	Enthalpy (mW/mg)
	Goat fat	Tallow fat	P90G			
Blank SLM	G	-	P	0	119.8	-18.32
Cimetidine SLM	G	-	P	0.05	104.7	-16.70
Cimetidine SLM	G	-	P	0.10	80.75	-9.56
Cimetidine SLM	G	-	P	0.20	114.7	-13.11
[b]Formulations	Lipid matrix (mg)			Lipid composition (g) w/w	Melting point (^oC)	Enthalpy (mW/mg)
	Goat fat	Tallow fat	P90G			
Blank SLM	G	T	P	1:1	118.1	-12.10
0.1 Cimetidine SLM	G	T	P	1:1	120	-15.67
,,	,,	,,	,,	,,	124	-16.43
Blank SLM	G	T	P	1:2	119.8	-18.32
0.1 Cimetidine SLM	G	T	P	1:2	120.82	-17.52
Blank SLM	G	T	P	2:1	113.2	-9.36
0.1 Cimetidine SLM	G	T	P	2:1	107.5	-11.77

G means goat fat; T means tallow fat; P means P90G. [a]Formulation means SLMs formulated with P90Gylated goat fat while [b]Formulations mean the SLMs formulated with tallowated P90Gylated goat fat matrices.

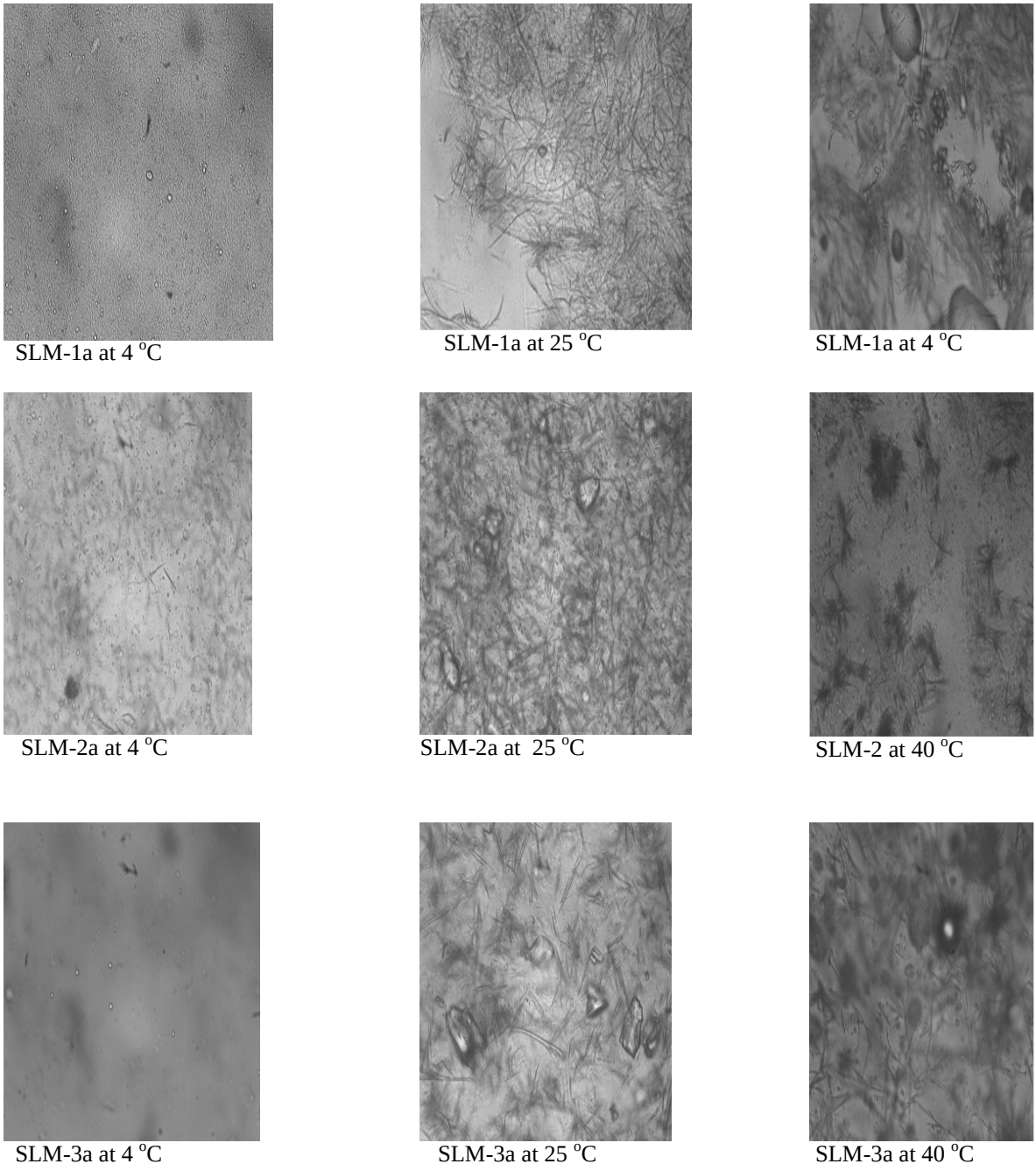


Fig. 7: Photomicrographs of the cimetidine-loaded SLMs formulated with different structured goat fat (GF) and tallow fat (TF) matrices under different storage temperatures.

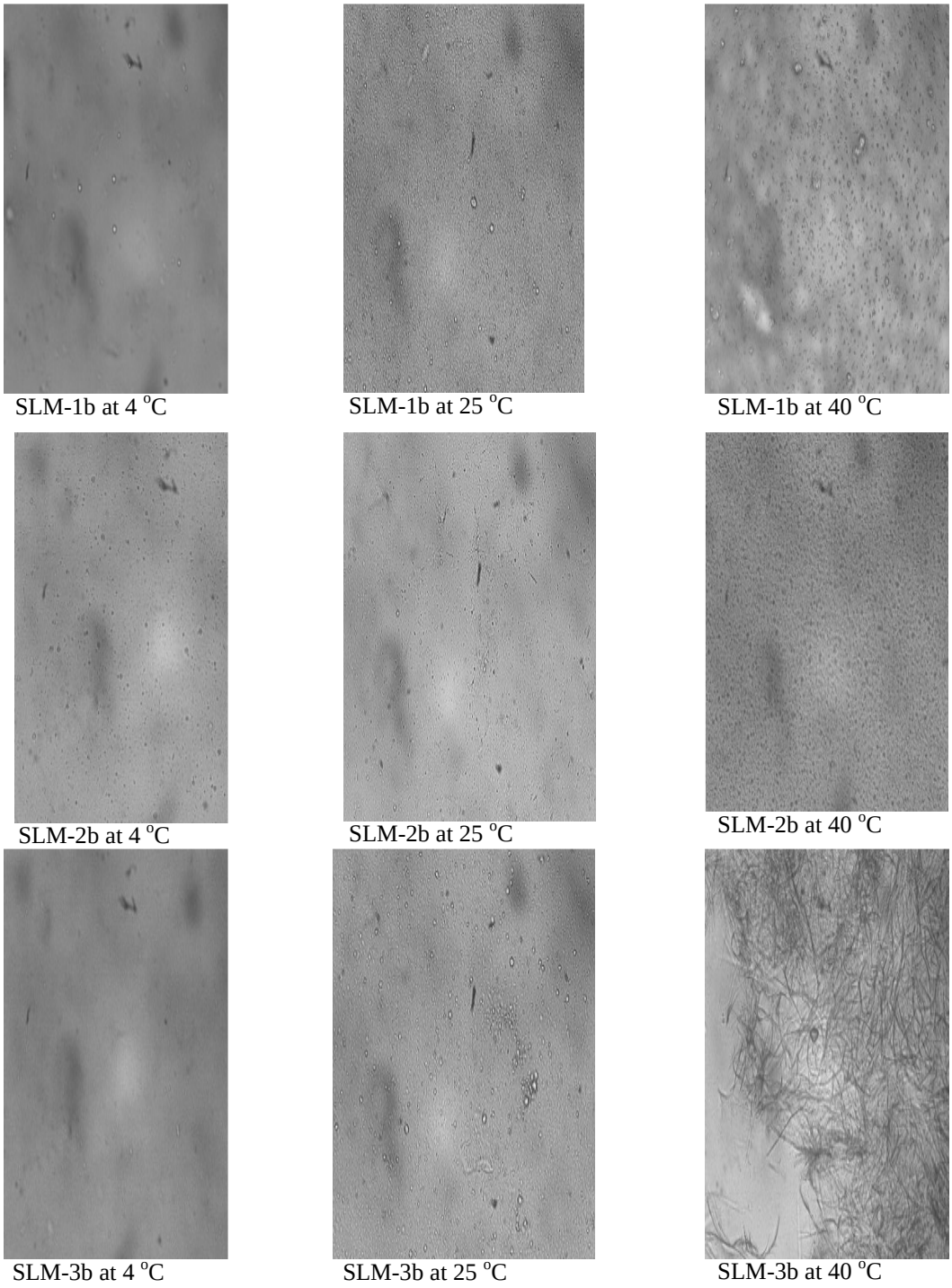


Fig. 8: Photomicrographs of the zero-cimetidine SLMs formulated with different structured goat fat (GF) and tallow fat (TF) matrices under different storage temperatures

Table 3: Properties of the cimetidine-loaded SLMs formulated with P90G-structured goat fat and tallow fat

Formulations	*Goat fat: Tallow fat	Drug composition (g) w/w	Av. particle sizes at different storage temperatures (µm)			Drug encapsulation efficiency (%)	Injectability (Gauge) at		
			4 °C	25 °C	40 °C		1 week	After 6 months	Aft mo
SLM-1	1:1	10.0	5.2	5.9	15.7	45.37 ± 9.26	27	25	18
SLM-2	1:2	10.0	1.20	42.9	14.0	40.30 ± 10.20	27	25	18
SLM-3	2:1	10.0	1.0	90.5	32.2	38.87 ± 3.90	27	25	18
SLM-4	1:1	0.0	1.0	1.8	2.6	-	27	25	18
SLM-5	1:2	0.0	0.7	4.8	0.9	-	27	25	18
SLM-6	2:1	0.0	-	5.5	1.7	-	27	25	18

*Goat fat: tallow fat refers to the lipid matrices containing 25 % P90G

Table 4: *In vivo* cimetidine release profile in ulcerated animal models

Formulations	Cimetidine Dose (mg/kg)	Mean ulcer index ± SEM	% ulcer inhibition
SLM 1:1	100	1.65 ± 1.42	76.18±5.2
SLM 1:1	0.0	6.00 ± 1.15[b]	13.0±1.23
SLM 1:2	100	1.75 ±1.70[b]	74.62±4.6
SLM 1:2	0.0	6.15 ± 1.30[a]	10.9±2.1
SLM 2:1	100	1.30 ± 1.0[a]	81.20±5.0
SLM 2:1	0.0	5.80 ± 0.92	15.9±1.5
CEMTAB	100	1.90 ± 1.1	72.50±2.5
DW	3.0 ml/kg	6.90 ± 1.40	0.00±0.0

Each value represents the mean (± SEM) of five observations.
NS= $p < 0.005$; [a]$p < 0.001$ Vs control; [b]$p < 0.01$

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-dose drug (200 mg or 400 mg) and encapsulating up to 10 % w/w of it in 5 % w/w of the P90G-structured goat-tallow fat matrices may lead to supersaturation coupled with the fact that the drug is slightly water soluble. So, the controlled delivery of this drug would not be easily possible in the investigated system. This is actually in agreement with an earlier observation (Muller *et al.*, 2000) that the amount of drug partitioning to the water phase will increase with the solubility of the drug in the water and will defeat the meaning of controlled release. It is therefore pertinent to say at this point that SLMs is a better drug delivery system for low dose poorly-soluble drugs.

Antiulcer properties of the cimetidine SLMs in ulcerated rats

After sacrificing the rats and opening their stomachs along the greater curvature, they were found to have developed ulcer on examination of their stomachs. A comparative result of the ulcer indices is shown in Table 4. The result of the *in vitro* drug release profile does not correlate with the *in vivo* performance. All the three cimetidine-loaded matrices performed creditably at inhibiting ulcer and were found to be much more superior in this regard to the commercial sample, CEMTAB®. The overall *in vivo* performance credited the 2:1 goat fat – tallow fat matrices as having some 81.20 % ulcer inhibition on the rats. This was followed by the 1:1 matrix which inhibited ulcer by 76.18 %. The 1:2 matrices that took the lead in the *in vitro* drug release profile became last in the *in vivo* assessment achieving an ulcer inhibition of 74.62 % although still superior to the commercial brand of cimetidine.

However, the possible mechanism of action of these systems aside from the drugs' intrinsic mechanism of action is based on their small particle sizes because such small sized particles are easily recognized by the immune system as danger signals from where they generally get internalized by antigen-sampling membranous (M) cells in intestinal Peyer's patches. These M cells (specialized epithelial cells) have a thinner glycocalyx and less organized microvilli than enterocytes and are known to internalize and transcytose particles to underlying lymphocytes and antigen-presenting cells (Clark *et al* 2001). It is noteworthy to recall that lymphocytes arise from stem cells in bone marrow and differentiate centrally into B-cells and T-cells (thymus) from where they move through the bloodstream to the peripheral lymphoid tissues – the lymph nodes, spleen, and lymphoid tissues associated with the mucosa, like the gut-associated lymphoid tissues such as tonsils, Peyer's patches, and appendix, which are sites of lymphocyte activation by antigens. Particles up to 10 μm in diameter can be internalized into Peyer's patches and particles less than 5 μm can be transported to draining lymph nodes and the spleen (Eldridge *et al* 1990). Lymph draining carries these particles from the tissues (extracellular fluid as lymph) via the afferent lymphatics vessels into the thoracic duct, which returns the lymph to the bloodstream by emptying into the left subclavian vein. Although the organization of the spleen is similar to that of a lymph node (like Peyer's patches), antigen or particles within the required dimension enters the spleen from the blood (via trabecular artery into the central arteriole from where they enter the marginal sinus and drain into a trabecular vein) rather than from the lymph.

Another side to this analogy is that exogenously administered triglycerides are digested by the action of pancreatic lipase/colipase digestive enzymes in the small intestine and absorbed into enterocytes. After

absorption, long-chain fatty acids or lipids are biosynthesized into triglyceride-rich lipoprotein particles (chylomicrons), which are secreted into intestinal lymph. The size of intestinal lipoproteins precludes their absorption into the blood capillaries, and therefore they are secreted into the lymph. Secondly, the cellular lining of the gastrointestinal tract is composed of absorptive enterocytes interspersed with membranous epithelial (M) cells. M cells that cover lymphoid aggregates, known as Peyer's patches, take up microparticles by a combination of endocytosis or transcytosis (Andrianov and Payne, 1998). The important characteristics of microparticles for their uptake are optimum size (10-100 nm), hydrophobicity, and surface charge (Swartz, 2001). Uptake of many colloidal polymeric carriers across the intestinal mucosa (Kreuter, 1991) has been shown to occur via Peyer's patches or isolated lymphoid follicles after oral administration (Florence *et al.*, 1995). In addition to the size of these SLMs within one week of preparation, their hydrophobic surface, imparted by phosphatidylcholine, might have influenced the SLM uptake by Peyer's patches (Cavalli *et al*., 2003).

Stability studies

The lipid microparticulate dispersions stored at 40 ^{o}C showed rapid aggregation within 1 month of storage. This was especially pronounced in the drug-loaded samples (Fig. 7). Although the storage at this temperature could not be continued due to epileptic power supply, the dispersions underwent sedimentation, and a significant increase in particle size was observed in all samples. In contrast, the dispersions stored at 4 - 6 ^{o}C remained stable, with only a slight change in particle size upon 6 months storage. Storage of the microparticles at 4 ^{o}C did not affect the intact spherical and smooth surfaces of the microparticles as well as the drug loading. However, the dispersions stored at room temperature generally showed gross particle growth during the period of storage (Fig. 7). This is partly because of high amount of crystals due to delayed crystallization from fractions of the cooled amorphous melt. Yet this does not preclude the use of the microparticulate dispersion for oral drug delivery of cimetidine investigated since strict limit in particle size and particle stability may be overlooked. The cimetidine–containing SLMs (Fig. 7) prepared from structured goat and tallow fats showed instability in all samples stored at 25 and 40 ^{o}C. Generally, the samples stored at 40 ^{o}C exhibited the worst instability in all the SLMs (40 ^{o}C > 25 ^{o}C > 4^{o}C). The zero-cimetidine samples (Fig. 8) did not show serious instability as observed with the drug-loaded samples. Summarily, it could be said that the SLMs were best stored at 4 ^{o}C.

Syringeability studies

All formulations remained uniformly dispersed at room temperature, within 1 week of preparation. These formulations could also be pushed through syringe with a 27- needle (27 G). This suggests that the microparticles could be potential drug carriers for parenterally-intended actives. Generally upon storage, the syringeability of the SLMs varied through 25-18 G over 6 months but remained stable for a 12 month study period. However, relating the storage temperature to the syringeability, it was found that the SLMs stored at 4 ^{o}C retained the injectability of 27 G

regardless of the duration of storage suggesting that 4 ^{o}C is the best storage temperature for the SLMs.

CONCLUSION

SLMs indeed serve as microcarriers for drug delivery. The cimetidine SLMs achieved high *in vivo* antiulcer effects especially the SLMs prepared with the 2:1 tallowated P90Gylated-goat fat matrices which surpassed the commercial sample CEMTAB® probably due to the small size of the microparticles which may have enhanced their uptake by the specialized epithelial cells (M-cells) of the intestinal Peyer's patches. However, according to BCS, class III drugs which exhibit high solubility but low permeability has cimetidine as a good representative. But cimetidine is a high dose drug and is slightly soluble in water and since partitioning to the water phase increased with its solubility in water, the meaning of controlled release was defeated. Also, with the encapsulation efficiency of less than 50 % and that the *in vitro* release study does not correlate with what happens *in vivo*, we state here, therefore, that cimetidine was not a good drug candidate for delivery in SLMs in this regard. Low dose poorly water-soluble drugs especially those belonging to class II of the BCS would be better delivered as SLMs since their solubilities are rather enhanced in the structured lipids. This is evident in some of our recent works (Nnamani *et al.*, 2010 a, b.; Nnamani, 2010).

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