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A critical study of novel physically structured lipid matrices composed of a homolipid from *Capra hircus* and theobroma oil

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Abstract

There is increasing interest in drug formulation using lipids. In this study, some physically structured lipid matrices were formulated and characterized for drug delivery applications. Lipid matrices containing a novel homolipid from *Capra hircus* (goat fat) and theobroma oil, at 25, 50 and 75% (w/w) concentration of the homolipid were formulated by fusion. The lipid matrices were subjected to some characterization procedures such as differential scanning calorimetry (DSC) to ascertain their supramolecular properties, small angle X-ray diffraction (SAXD), wide angle X-ray diffraction (WAXD), polarized light microscopy (PLM) and isothermal heat conduction microcalorimetry (IMC). The internal structures of some selected lipid matrices were also studied by freeze-fracture transmission electron microscopy (FFTEM). DSC results obtained indicated that goat fat has a pre-transition at $15.9 \pm 0.2\,^{\circ}C$ (after 1 week) and melts completely with two detectable melting peaks at 33.0 ± 0.2 and $49.9 \pm 0.1\,^{\circ}C$, and total enthalpy of 99.9 ± 2.5 mJ/mg determined after 6 weeks of preparation. The melting enthalpy of goat fat changed after 3 weeks but remained constant after 6 weeks while the melting enthalpy of the lipid matrix containing 50% (w/w) goat fat changed after 3 and 6 weeks. An increase in lower melting peak was observed in the lipid matrix containing 25% (w/w) goat fat after 6 weeks. WAXD and SAXD of the physically structured lipid matrices showed reflections of the different pure lipids but new interferences were detected in WAXD mostly between $2\theta = 17.5^{\circ}$ and $2\theta = 27.5^{\circ}$. PLM observation revealed the presence of Maltese crosses for the homolipid at 37 °C, which disappeared upon heating at 51.0 °C. PLM of the structured lipid matrix containing 25% (w/w) goat fat showed distinct crystal growth after 4 weeks among the admixtures. However, IMC studies did not reveal any change in recrystallization behaviour in this lipid matrix within 24 h. Analysis of the crystallization exotherms indicated that the lipid matrix containing 50% (w/w) goat fat showed unique crystallization kinetics and possessed the lowest Avrami exponent, while goat fat alone showed slight change within the first 45 min of isothermal crystallization. Physically structured lipid matrix containing 75% (w/w) goat fat possessed the lowest growth rate constant.

Keywords: Goat fat (homolipid); Theobroma oil; Physically structured lipid matrices; X-ray diffraction; Isothermal heat conduction microcalorimetry; Crystallinity; In situ crystallization; Crystallization kinetics

1. Introduction

Homolipids and heterolipids have gained renewed interests as excipients for different drug delivery systems. Lipid based formulations have been shown to enhance bioavailability of drugs administered orally (Sarkar, 2002; Hou et al., 2003; Gao et al., 2004). The reasons for the increasing interest in lipid based drug delivery systems are many-fold and include an improved understanding of the manner in which lipids enhance oral bioavailability and reduce plasma profile variability, better characterization of lipidic excipients, an improved ability to address the key issue of technology transfer and manufacture scale up (Stuchlík and Žák, 2001). Natural fats possess better biocompatibility and lower in vivo toxicity than semi-synthetic lipids (Kim et al., 2005). Mixtures of natural lipids may further improve on the individual properties of the constituent lipids. In addition, lipid mixtures may alter the crystal arrangement of the individual lipids after melting and solidification, a phenomenon, which may increase its drug holding

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capacity, as it is known that highly ordered crystalline lipid matrices lead to drug expulsion upon crystallization of the previously molten matrices. This is always the case when highly purified lipids are used in formulations (Radtke et al., 2005). It is envisaged that the two candidate lipids used in this study with different fatty acid locations in their triglycerides may crystallize into lose packing after melting, producing highly unordered lipid structures or disordered imperfect lipid matrix structure offering space for drug molecules and amorphous clusters of drugs.

Many procedures are used to characterize fats both as a single compound or in mixtures with other fats. Among the prominent ones are differential scanning calorimetry (DSC), small angle X-ray diffraction (SAXD), wide angle X-ray diffraction (WAXD), polarized light microscopy (PLM) and isothermal heat conduction microcalorimetry (IMC) (Schubert et al., 2005; Solís-Fuentes et al., 2005; Fonollosa et al., 2004; Kong et al., 2000). These analytical techniques were employed in the characterization of the homolipid and its admixtures with theobroma oil. Mixture of fats have been variously studied by different scientists (Solís-Fuentes and Dúran-de-Bazúa, 2003; Nyholm et al., 2003). The homolipid (goat fat) used in this study has been evaluated as a basis for drug delivery (Attama et al., 2000, 2003). Theobroma oil (cocoa butter) is obtained by hydraulic expression of cocoa nibs from *Theobroma cacao*. It solidifies in a number of different crystal forms depending on the conditions of cooling. Its combination with mango seed almond fat has been studied (Solís-Fuentes and Dúran-de-Bazúa, 2004). It was thus the objective of this study to formulate physical mixtures of these two natural lipids and fully characterize same for drug delivery applications.

2. Materials and methods

2.1. Materials

Theobroma oil (Cocoa butter) (Caesar & Loretz, Hilden, Germany) DAB 1999 grade with a melting point range of 30–35 °C, activated charcoal and bentonite (Merck, Darmstadt, Germany) were procured from their manufacturers and used without further purification. Goat fat was obtained from a batch processed in the laboratory of Department of Pharmaceutics, University of Nigeria, Nsukka.

2.2. Extraction and purification of goat fat

The procedure adopted followed the method used in an earlier study (Attama et al., 2003). Briefly, the adipose tissue of *Capra hircus* was collected immediately after slaughter, 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 solidified fat was thereafter manually removed and bleached/deodorized by passing it through a mixture of activated charcoal and bentonite (2:1) at 100 °C at a ratio of 10 g of the fat and 1 g of the column material.

2.3. Preparation of lipid matrices

Binary mixtures of goat fat and theobroma oil were prepared by fusion and contained 25, 50 and 75% (w/w) of goat fat. In each case, the lipids were weighed with an electronic balance (Type L2200P-xD2, Sartorius AG, Göttingen, Germany) and melted together at 60 °C on a hot plate (RCT basic, IKA® Staufen, Germany) and stirred until solidification.

2.4. Characterization of the lipid matrices

2.4.1. Differential scanning calorimetry (DSC)

Melting transitions and changes in heat capacity of the physically structured lipid matrices were determined using a calorimeter (DSC 220C) connected to a disc station (5200H, Seiko, Tokyo, Japan). Approximately 5 mg of each lipid matrix was weighed into an aluminium pan and sealed hermetically, and the thermal behaviour determined in the range of 10–80 °C at a heating rate of 5 °C/min. The temperature was held at 80 °C for 10 min and thereafter, cooled at the rate of 5 °C/min to 10 °C. Baselines were determined using an empty pan, and all the thermograms were baseline-corrected. The degree of crystallinity of each lipid matrix was determined by calculating the crystallinity index (CI) from the heat of fusion using Eq. (1), the modified equation of Freitas and Müller (1999).

$$\mathrm{CI}\,(\%) = \frac{\mathrm{enthalpy}_{\mathrm{LMad}}\,(\mathrm{J/g})}{\mathrm{enthalpy}_{\mathrm{TO}}\,(\mathrm{J/g})}\,100\,f_{\mathrm{TO}} \quad (1)$$

where $\mathrm{enthalpy}_{\mathrm{TO}}$ is the fusion enthalpy of the pure theobroma oil, f_{TO} a correction factor which takes into account the concentration of theobroma oil and $\mathrm{enthalpy}_{\mathrm{LMad}}$ is the fusion enthalpy of the lipid matrix admixture. DSC thermograms were obtained 1, 3 and 6 weeks after lipid matrices preparation. The transition temperatures were taken as the temperatures at the peak minimum of the endothermic transitions, while transition enthalpies were obtained by integration of the endothermic transitions within the temperature range where melting signals were detected using linear baselines.

2.4.2. Hot stage polarized light microscopy (PLM)

The mesomorphic structures of the lipid matrices were assessed with a Zeiss Type III photomicroscope (Model No. SIP 48560, Oberkochen, West Germany) using cross-polarizers and a wavelength (λ) plate. Thermal behaviour of the matrices between ambient temperature and 60 °C was studied with an FP82HT Hot Stage with FP 90 Central Processor from Mettler Toledo (Gießen, Germany) with a heating rate of 5 °C/min. Relevant images were digitalized using a camera attached to the photomicroscope (Olympus DP12, Japan) and a computer software (Olympus DP-Soft, version 3.2). Static crystallization of the previously molten structured lipid matrices was monitored at 20 °C in the polarized microscope after 24 h, 1 week and 4 weeks.

2.4.3. Wide angle X-ray diffraction (WAXD)

WAXD is a useful technique for the analysis of the short range order of mesomorphic systems. Wide angle X-ray studies

were done on all the lipid matrices using an X-ray generator (PW3040/60 X'Pert PRO, Fabr. DY2171, PANalytical, The Netherlands) connected to the tube (PW3373/00 DK 147726 Cu LFF) copper anode which delivered X-ray of wavelength, $\lambda = 0.1542$ nm at a high voltage of 40 kV and an anode current of 25 mA. WAXD measurements were taken with a Goniometer (PW3050/60 MPD-System, PANalytical, The Netherlands) from 3.0° to 33.0° in 0.015° steps (1 s per step). The repeat distance d, otherwise called the interlayer spacing, was calculated from the scattering angles θ, using Bragg's equation.

$$n\lambda = 2d \sin\theta \quad (2)$$

where n, a positive integer, is the order of the interference and λ is the wavelength of the X-ray. WAXD diffractograms were obtained 1, 7 and 12 weeks after lipid matrices preparation.

2.4.4. Small angle X-ray diffraction (SAXD)

This technique was used to analyse the long range order of the crystalline structure of the lipid matrices. Characteristic Cu Kα radiation with a wavelength of $\lambda = 0.1542$ nm was produced by an X-ray tube (PW2213/20, Feinfokus Cu-Anode) connected to a PW1730/10 generator (PANalytical, The Netherlands) and a camera (Kiessig-OED, Fa. M. Braun, Garching, Germany) was used to detect the small angle scattering. To reduce the Kβ radiation, the X-ray beam was passed through a nickel filter. Tube voltage was set to 40 kV with an anode current of 25 mA. Measurement time was set at 800 s for all the lipid matrices and SAXD reference systems. Specimens were filled into aluminium cubes and equilibrated for 10 min at 20 °C before taking the measurements. Data generated were processed using ASA-SAXD program (Fa. M. Braun, Garching, Germany). The interlayer spacing was thereafter calculated using Bragg's equation (Eq. (2)). SAXD diffractograms were obtained 1, 7 and 12 weeks after lipid matrices preparation.

2.4.5. Freeze-fracture transmission electron microscopy (FFTEM)

The FFTEM of the pure lipids and the physically structured lipid matrix containing 50% (w/w) goat fat were determined to further study the internal structures of the matrices. In a typical experiment, the lipid samples were shock-frozen in melting nitrogen at 63 K between two flat gold holders. The frozen samples were fractured at 173 K in a BAF 400 instrument (Balzers, D-Wiesbaden, Germany) and then shadowed with platinum/carbon (2 nm) at 45° and with pure carbon at 90° for replica preparation. After cleaning with chloroform–methanol mixture (1:1), the replicas on uncoated grids were fixed onto a sample holder and placed in the vacuum chamber of a transmission electron microscope (Leo 922, Leo D-Oberkochen, Germany), and viewed under low vacuum at 200 kV.

2.4.6. Isothermal heat conduction microcalorimetry (IMC)

Crystallization exotherms of the lipid matrices were obtained using a Thermal Activity Monitor® (TAM 2277, Thermometric AB, Jarfalla, Sweden). Two calorimeter units were installed and run concurrently. A 300 mg quantity of each lipid matrix was weighed into 3 ml glass ampoules, melted at 60 °C and equilibrated for 30 min at 20 °C, and the heat flow measured at the range of −3000 to 3000 μW. Heat flow signals were monitored by the Digitam Software (Thermometric AB, Jarfalla, Sweden). Data obtained from the IMC measurements were thereafter analysed to determine the isothermal crystallization kinetics of the lipid matrices. During crystallization from a melt, the extent of crystallization is usually thought to be related to time through an equation developed by Avrami and may be expressed in the following form (Avrami, 1939, 1940):

$$1 - X_t = \exp(-kt^n) \quad (3)$$

where X_t represents the degree of crystallinity at time t, k is the growth rate constant of isothermal crystallization and the exponent n, represents the nucleation mechanism and growth dimension. The value of n can be any positive integer between 1 and 4.

3. Results and discussion

3.1. Hot stage polarized light microscopy (PLM)

PLM is a useful tool in studying liquid to crystal transitions and vice versa, as well as liquid crystalline materials (Harroun et al., 2005). The PLM micrographs for 1 and 4 weeks observations are presented in Fig. 1. The structured lipid matrices exhibited different crystalline structures depending on the ratio of the individual lipids and their textures appeared to be qualitatively different across the range. A transition of the crystalline phase to a smectic mesophase was observed with increasing temperature for goat fat matrix, with Maltese crosses being detected at 37 °C (Fig. 1), indicating a concentric lamellar liquid crystal arrangement (Glombitza and Müller-Goymann, 2002; Fonollosa et al., 2004). Maltese crosses disappeared upon heating at 51.0 °C. However, on cooling from isotropic state, anisotropic texture was observed at 33 °C (Fig. 1), which reorganized to crystalline structures after 24 h. A particular lipid can form various liquid crystalline phases, where small changes in molecular or ambient properties can lead to morphology and phase changes (Luzzati, 1968).

During crystallization, the material goes through different stages beginning with nucleation and followed by unhindered growth and structure formation. Eventually, crystals start forming clusters that impinge upon each other as shown by PLM studied under static condition (Mazzanti et al., 2003). Goat fat after 24 h presented identifiable fan-like structures characteristic of either crystals or smectic mesophases. This texture became more pronounced after 1 and 4 weeks (Fig. 1). The structured lipid matrix containing 75% (w/w) goat fat had influence of theobroma oil indicated by a coarser texture compared to pure goat fat. There was further crystal growth after 1 and 4 weeks (Fig. 1). However, the fan-like appearance characteristic of goat fat was still present after 4 weeks. It however presented no Maltese crosses, which were characteristic of a concentric lamellar mesophase with increase in temperature.

In the PLM of physically structured lipid matrix containing 50% (w/w) goat fat micrographs, greater influence of theobroma oil was noticed by the low degree of crystallization after 24 h (figure not shown). More crystal growth occurred over time until 1 and 4 weeks, again with coarser texture than in the case of pure goat fat (Fig. 1). No characteristic mesophase was either detected as this lipid matrix passed from crystalline to isotropic phase and vice versa. On the other hand, the structured lipid matrix containing 25% (w/w) goat fat had an even more delayed crystallization possibly due to higher content of theobroma oil

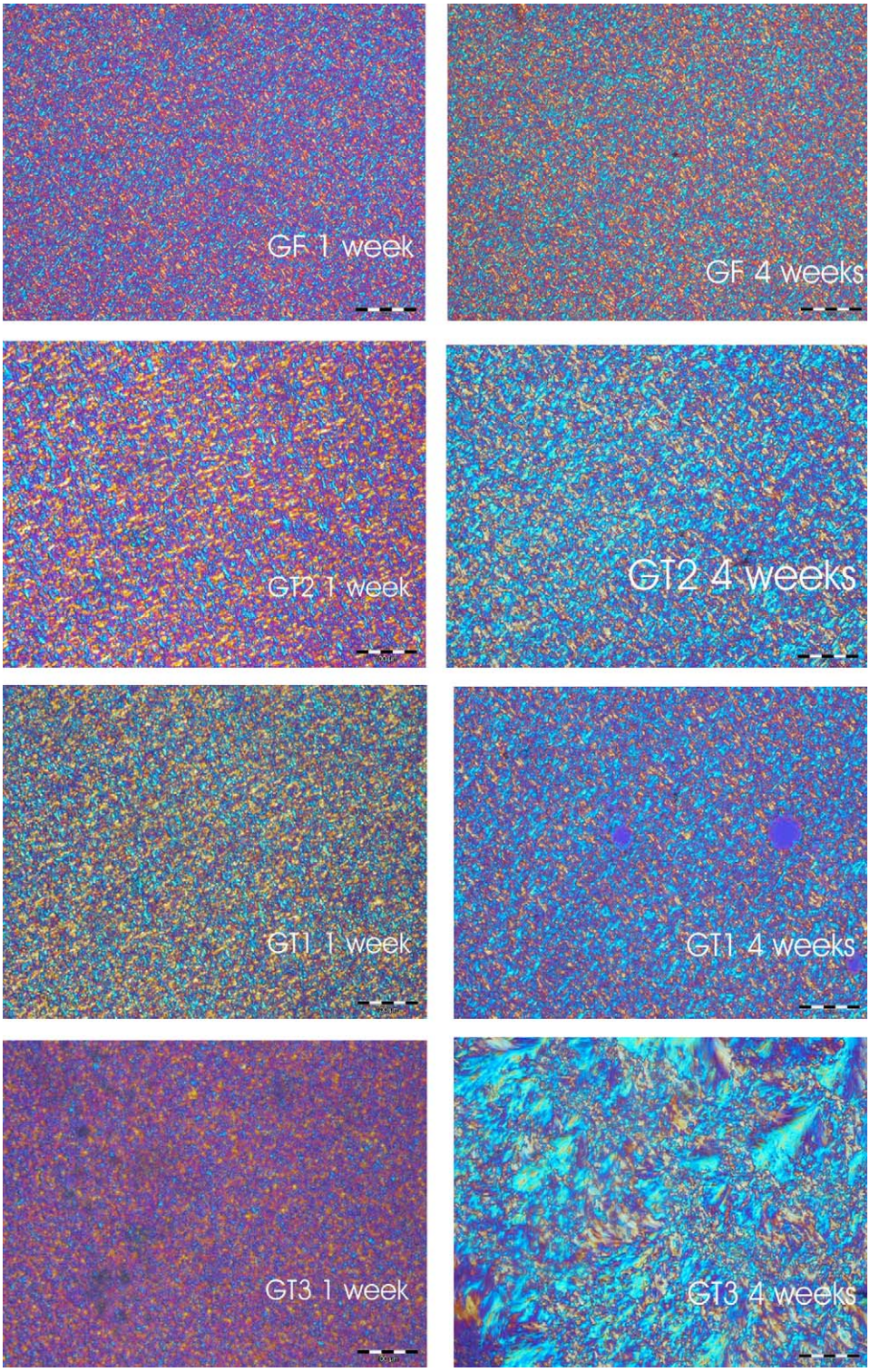

Fig. 1. Polarized light micrographs of the goat fat-theobroma oil lipid matrices after 1 and 4 weeks: GF (goat fat), GT2 (75%, w/w goat fat), GT1 (50%, w/w goat fat), GT3 (25%, w/w goat fat), TO (theobroma oil), GF-37 °C (goat fat showing Maltese crosses at 37 °C) and GF-33 °C (goat fat with anisotropic texture at 33 °C). Bar represents 100 μm.

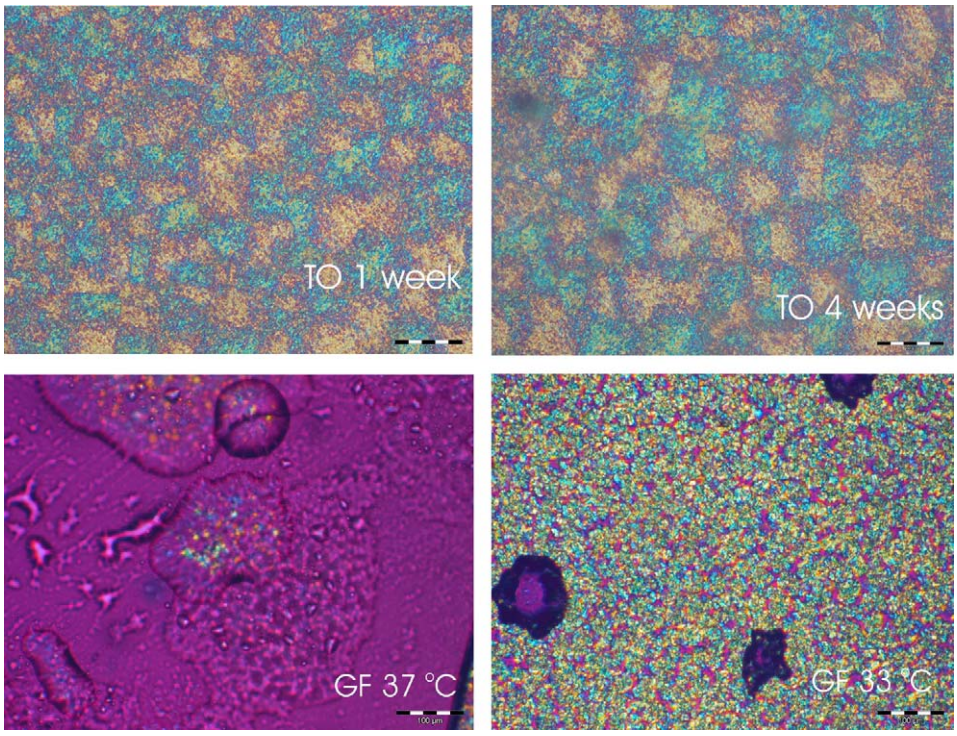

Fig. 1. (*Continued*).

compared with lipid matrix containing 50% (w/w) goat fat. Crystal growth was just noticeable after 24 h, and continued within 1 week with large crystals appearing within 4 weeks (Fig. 1). This may have accounted for the increase in CI detected for this lipid matrix after 6 weeks in DSC. Theobroma oil showed a noticeable clustered crystallization after 24 h, which grew into crystal aggregates after 1 week and further after 4 weeks to big granular crystals (Fig. 1). This behaviour has been noted for theobroma oil (Marangoni and McGauley, 2003; Toro-Vasquez et al., 2005). No mesophase formation was seen in theobroma oil as it passed from crystalline to isotropic phase and vice versa.

3.2. Differential scanning calorimetry (DSC) measurements

DSC was used to analyse the thermal behaviours of the lipids and the structured lipid matrices. In general, the thermotropic phase behaviour of a lipid matrix system is highly affected by the presence of guest molecules, and the related thermodynamic variables (melting temperature and enthalpy changes) depend on the nature of the interaction between the constituents. The DSC traces are presented in Fig. 2 representing the traces obtained after 6 weeks while Fig. 3a and b represent the changes in the thermodynamic variables of the lipid matrices for all the DSC measurements. The DSC traces for goat fat lipid matrix showed that it has a pretransition below room temperature at $15.9 \pm 0.2\,^{\circ}C$ which almost disappeared after 6 weeks. The traces also indicated two endothermic transitions for goat fat, which occurred at $30.5 \pm 0.2\,^{\circ}C$ after 1 week and at $33.0 \pm 0.2\,^{\circ}C$ after 6 weeks for the lower peak and at 49.8 ± 0.3, 51.1 ± 0.2 and $49.9 \pm 0.1\,^{\circ}C$ for the higher peak after 1, 3 and 6 weeks, respectively (Figs. 2 and 3a and b). Normally, the highest peak is attributed to a stable modification whereas the fraction responsible for the lower melting fraction is attributed to an unstable modification. According to PLM, however, the lower melting transition goes along with the formation of a smectic mesophase which has completely transformed into an isotropic melt at about 50–51 °C, independent of the storage time. With regard to melting enthalpy, there was a growth from 89.3 ± 2.5 mJ/mg after 1 week to 100.0 ± 1.9 mJ/mg after 3 weeks and it remained constant with a value of 99.9 ± 2.5 mJ/mg after 6 weeks (Fig. 3a). The increase in enthalpy confirms higher

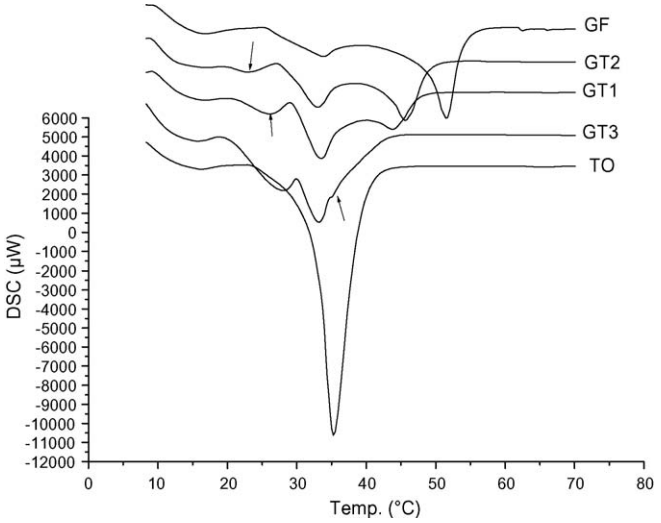

Fig. 2. DSC thermograms of the lipid matrices after 6 weeks: GF (goat fat), GT2 (75%, w/w goat fat), GT1 (50%, w/w goat fat), GT3 (25%, w/w goat fat) and TO (theobroma oil).

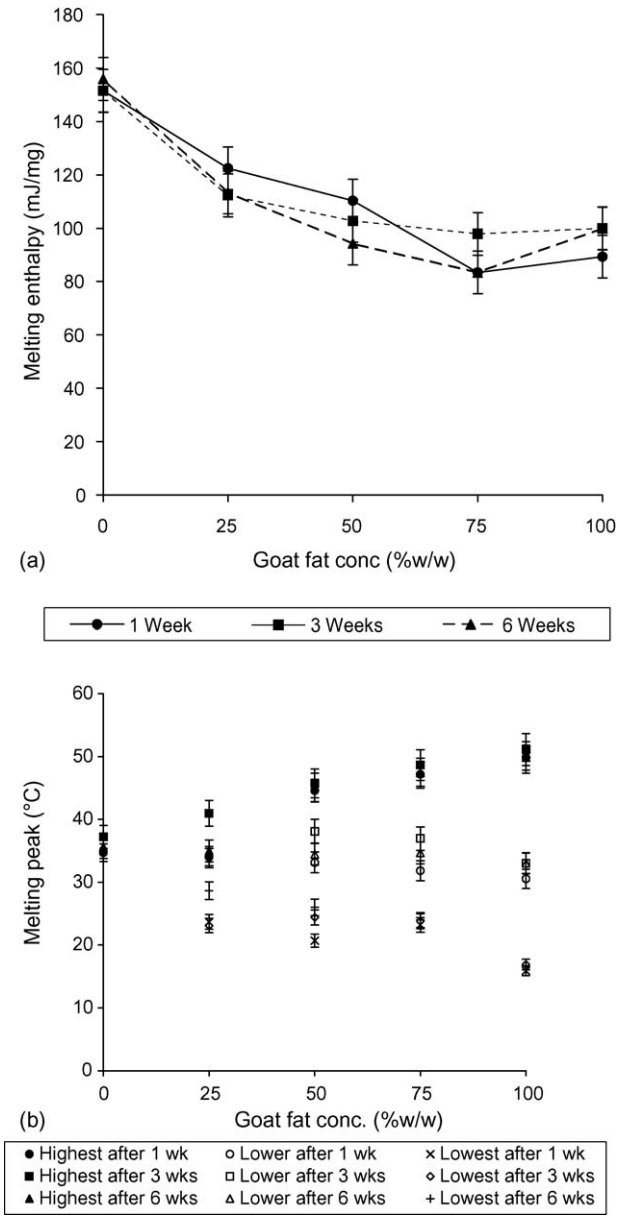

Fig. 3. (a) Melting enthalpy changes in the structured lipid matrices (mean ± standard deviation, $n = 3$). (b) Changes in the melting peaks of the structured lipid matrices (mean ± standard deviation, $n = 3$).

amounts of crystals upon storage due to delayed crystallization from fractions of a supercooled amorphous melt. Theobroma oil on the other hand, had a melting peak at 35.6 ± 0.2 °C after 6 weeks. This is in accordance with manufacturers specification and reported works (Solís-Fuentes and Dúran-de-Bazúa, 2004).

An earlier work carried out on goat fat showed that it had a melting point of 51 °C (Attama et al., 2003), and the above properties were not detected. The difference in the melting peak and the observed new properties was because of instrumentation as a more sensitive instrument was used in this study. The fatty acid composition of theobroma oil and animal fats (goat fat, tallow) are somewhat similar. They all have C16:0, C18:0 and C18:1 fatty acids. However, the locations of these fatty acids in their triglycerides differ. Theobroma oil is more homogeneous and melts sharply as against goat fat, which remains as liquid crystals (solid/liquid) over a wide temperature range indicated by its very broad endotherm compared with the endotherm of theobroma oil.

The physically structured lipid matrices composed of goat fat and theobroma oil presented characteristic thermograms with three different transitions (Fig. 2, see arrows). The thermally induced transitions of goat fat were remarkably affected by presence of theobroma oil. In contrast to the goat fat transition from crystalline to liquid crystalline which remained almost constant in all the lipid matrices, the highest transitions shifted to lower temperatures upon a decrease in goat fat concentration whereas an additional transition shifted from 23.2 ± 0.3 °C to higher temperature. Additional transitions occurred at 26.0 ± 0.2 and 23.2 ± 0.3 °C respectively for the structured lipid matrices containing 50 and 75% (w/w) goat fat, respectively, while the third transition for the lipid matrix containing 25% (w/w) goat fat was only noted as a shoulder which started at about 35.7 ± 0.2 °C (see arrow, Fig. 2), almost equal to the melting transition of pure theobroma oil. 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 later often lead to products of decreased in vivo tolerability (Kim et al., 2005). Structured lipid matrix containing 25% (w/w) goat fat presented the endotherms most closely together compared with other structured lipid matrices with melting transitions detectable at 27.9 ± 1.8, 34.1 ± 0.2 and 35.7 ± 0.2 °C as a shoulder after 6 weeks (Fig. 2). This suggests some modification of the lipid matrix, as well as the influence of the decrease in concentration of goat fat. The melting enthalpy of theobroma oil was much higher than that of goat fat (150 mJ/mg versus 100 mJ/mg). The melting enthalpies of the binary lipid matrices dropped upon storage except for the lipid matrix with 75% (w/w) goat fat. The lipid matrix with 25% (w/w) goat fat dropped after 3 weeks and remained constant after 6 weeks, while the lipid matrix containing 50% (w/w) goat fat showed a constant decrease in enthalpy ranging from 110.3 ± 4.0 to 102.0 ± 2.7 mJ/mg and 94.2 ± 3.6 mJ/mg after 1, 3 and 6 weeks respectively. This is reflected by a generally low crystallinity index (CI). CI values of 31.4%, 23.9% and 21.7% were obtained for the lipid matrices containing 25, 50 and 75% (w/w) goat fat respectively. The CI values reflected the theobroma oil concentration and shows that theobroma oil is more crystalline than goat fat. These findings suggest that goat fat and theobroma oil do not form lipid matrices of high crystallinity when the concentration of theobroma oil is higher in the mixture. It could thus be proposed that concentrations of goat fat higher than 50% (w/w) in theobroma oil would produce structured lipid matrices with low degree of crystalline order necessary for the increased drug loading as proposed in the new generation of lipid drug carrier system called nanostructured lipid carrier (Radtke et al., 2005).

3.3. Wide angle X-ray diffraction (WAXD) studies

Representative WAXD diffractograms and data of all the lipid matrices are presented in Fig. 4 and Table 1. Fig. 4 represents the determination after 12 weeks. In accordance with the liter-

Table 1
WAXD data for the lipid matrices containing theobroma oil and goat fat at different stages of storage

GF						GT2						GT1						GT3						TO					
1 week		7 weeks		12 weeks		1 week		7 weeks		12 weeks		1 week		7 weeks		12 weeks		1 week		7 weeks		12 weeks		1 week		7 weeks		12 weeks	
2θ (°)	d (Å)	2θ (°)	d (Å)	2θ (°)	d (Å)	2θ (°)	d (Å)	2θ (°)	d (Å)	2θ (°)	d (Å)	2θ (°)	d (Å)	2θ (°)	d (Å)	2θ (°)	d (Å)	2θ (°)	d (Å)	2θ (°)	d (Å)	2θ (°)	d (Å)	2θ (°)	d (Å)	2θ (°)	d (Å)	2θ (°)	d (Å)
												5.5	16.07	5.5	16.07			5.5	16.07	5.5	16.07	5.5	16.07	5.5	16.07	5.5	16.07	5.6	15.78
6.0	**14.73**	*6.0*	*14.73*	**6.0**	**14.73**	*6.0*	*14.73*	*6.0*	*14.73*	*6.0*	*14.73*	*6.0*	*14.73*	*6.0*	*14.73*	5.9	14.98	6.0	14.73	6.0	14.73	6.0	14.73						
				6.8	13.00	6.8	13.00	6.8	13.00	6.8	13.00	6.9	12.81	6.8	13.00	6.8	13.00	6.9	12.81	6.8	13.00	6.9	12.81	6.9	12.81	6.9	12.81	6.9	12.81
												8.2	10.78	8.2	10.78			8.3	10.65	8.2	10.78			8.3	10.65	8.3	10.65	8.3	10.65
9.9	8.94	10.0	8.85			10.0	8.85	10.1	8.76					10.0	8.85														
						10.9	8.12	10.9	8.12			10.9	8.12	10.9	8.12	10.9	8.12	11.0	8.04	10.9	8.12	10.9	8.12	10.9	8.12	10.9	8.12	11.1	7.97
												12.5	7.08	12.3	7.20			12.4	7.14	12.4	7.14			12.5	7.08	12.5	7.08	12.5	7.08
16.1	5.51	16.2	5.47			16.4	5.41	16.4	5.41	16.4	5.41	16.4	5.41	16.4	5.41	16.3	5.44	16.4	5.41	16.3	5.44	16.3	5.44	16.3	5.44	16.3	5.44	16.3	5.44
																				17.2	5.16	17.0	5.22	17.2	5.16	17.1	5.19		
17.8	*4.98*	17.9	4.96																										
																								18.3	4.85	18.3	4.85		
19.4	*4.58*	*19.4*	*4.58*	**19.3**	**4.60**	**19.4**	**4.58**	**19.4**	**4.58**	**19.4**	**4.58**	**19.3**	**4.60**	**19.3**	**4.60**	**19.3**	**4.60**	**19.3**	**4.60**	**19.3**	**4.60**	**19.3**	**4.60**	**19.3**	**4.60**	**19.3**	**4.60**	**19.3**	**4.60**
																								19.6	4.53	19.6	4.53		
20.7	**4.29**	**20.7**	**4.29**	**20.7**	**4.29**	**20.7**	**4.29**	**20.7**	**4.29**	**20.7**	**4.29**	**20.7**	**4.29**	**20.7**	**4.29**	*20.6*	*4.31*	*20.7*	*4.29*	*20.7*	*4.29*	*20.7*	*4.29*	20.8	4.27	20.8	4.27	20.9	4.25
21.3	**4.17**	*21.4*	*4.15*	**21.3**	**4.17**	**21.3**	**4.17**	**21.4**	**4.15**	**21.4**	**4.15**	*21.3*	*4.17*	**21.4**	**4.15**	*21.3*	*4.17*			*21.1*	*4.21*	*21.1*	*4.21*						
																								21.7	4.10	21.7	4.10		
																								22.0	4.04	22.0	4.04	22.0	4.04
				22.2	4.00	*22.2*	*4.00*	*22.2*	*4.00*	*22.3*	*3.98*	*22.3*	*3.98*	*22.3*	*3.98*	*22.2*	*4.00*	22.3	3.98	22.3	3.98	*22.3*	*3.98*						
																								22.7	3.92	22.7	3.92	22.7	3.92
						23.0	**3.87**	*23.0*	*3.87*	*23.0*	*3.87*	*23.0*	*3.87*	*23.0*	*3.87*	*22.9*	*3.88*	22.9	3.88	22.9	3.88	*23.0*	*3.87*	*23.0*	*3.87*	*23.0*	*3.87*	23.0	3.87
23.2	**3.83**	*23.3*	*3.82*	**23.2**	**3.83**	*23.3*	*3.82*	*23.2*	*3.83*	*23.3*	*3.82*																		
										23.7	3.75	23.6	3.77	23.6	3.77	23.6	3.77	23.6	3.77	*23.6*	*3.77*	*23.7*	*3.75*						
																								24.0	*3.71*	*24.0*	*3.71*	*24.0*	*3.71*
				24.2	3.68	24.2	3.68	24.2	3.68	24.3	3.66	24.2	3.68	24.2	3.68	24.2	3.68	24.2	3.68	24.2	3.68	*24.3*	*3.66*						
								24.8	3.59			24.9	3.58	24.8	3.59	24.9	3.58	24.9	3.58	24.9	3.58	24.9	3.58	25.0	3.56	25.0	3.56	25.0	3.56
																								25.4	3.51	25.3	3.52	25.4	3.51
																		26.4	3.38	26.3	3.39			26.5	3.36	26.5	3.36	26.6	3.35
																								28.7	3.11				

Key: High intensity (bold), medium intensity (italics), low intensity (underline) and weak intensity (normal). GF (goat fat), GT2 (75%, w/w, goat fat), GT1 (50%, w/w, goat fat), GT3 (25%, w/w, goat fat) and TO (theobroma oil).

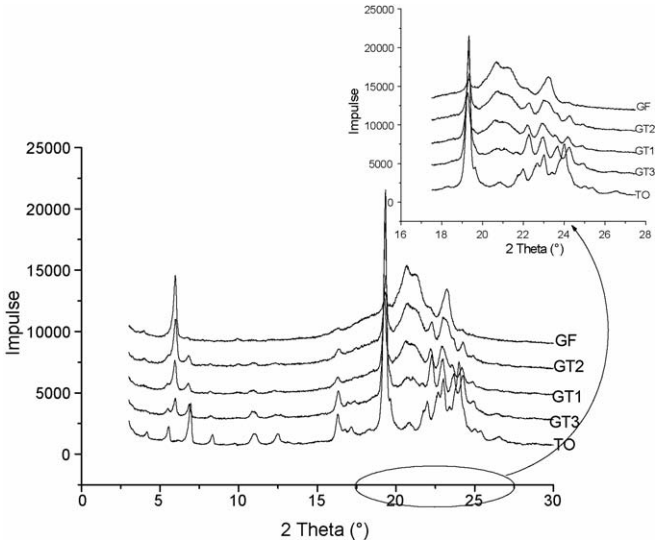

Fig. 4. WAXD diffractograms of the lipid matrices after 12 weeks: GF (goat fat), GT2 (75%, w/w goat fat), GT1 (50%, w/w goat fat), GT3 (25%, w/w goat fat) and TO (theobroma oil). Inset shows the magnified portion of the diffractograms between $2\theta = 17.5^\circ$ and $2\theta = 27.5^\circ$.

ature on triglyceride modifications (Solís-Fuentes et al., 2005; D'Souza et al., 1990; O'Brien, 1998) the four strong reflections presented by goat fat after 1 week at $2\theta = 6.0^\circ$, $d = 14.73$ Å; $2\theta = 20.7^\circ$, $d = 4.29$ Å; $2\theta = 21.3^\circ$, $d = 4.17$ Å and $2\theta = 23.2^\circ$, $d = 3.83$ Å could be assigned to β′, β′, α (or pseudo β′) and β′ modifications respectively, while the medium intensity reflection at $2\theta = 19.4^\circ$, $d = 4.58$ Å was attributable to β modification. After 7 weeks, the signal positions remained almost unchanged but were of lower intensities, which may suggest lowered crystallinity (Table 1). However, after 12 weeks, there was a minor increase in crystallinity evidenced by sharpening of the reflection at $2\theta = 21.3^\circ$, $d = 4.17$ Å without change in position (Fig. 4). There was also a slight shift in the position of the medium intensity reflection to $2\theta = 19.3^\circ$, $d = 4.60$ Å with increase in intensity indicating that it is in the β modification. This result shows that goat fat is present in α or β′ and β modifications, in which α and β′ modifications are metastable or that α and β′ transformation to the more stable β modification occurs over a long time (>12 weeks). Goat fat had broad WAXD reflections especially between $2\theta = 17.5^\circ$ and 25.0°. Broad WAXD reflections result from less crystal order and crystal defects. Theobroma oil presented a very strong intensity reflection at $2\theta = 19.3^\circ$, $d = 4.60$ Å, showing β modification, medium to low intensity reflections at $2\theta = 6.9^\circ$, $d = 12.81$ Å; $2\theta = 16.3^\circ$, $d = 5.44$ Å; $2\theta = 22.0^\circ$, $d = 4.04$ Å; $2\theta = 23.0^\circ$, $d = 3.87$ Å; $2\theta = 24.0^\circ$, $d = 3.71$ Å corresponding to different stable forms of theobroma oil (Marangoni and McGauley, 2003). Because of the noticeable interaction of the lipid matrices, the region between $2\theta = 17.5^\circ$ and 27.5° was magnified for clearer view (see inset in Fig. 4).

Lipid matrix containing 75% (w/w) goat fat had very high intensity reflections at $2\theta = 19.4^\circ$, $d = 4.58$ Å; $2\theta = 20.7^\circ$, $d = 4.29$ Å; $2\theta = 21.3^\circ$, $d = 4.17$ Å and $2\theta = 23.0^\circ$, $d = 3.87$ Å, medium intensity reflections at $2\theta = 6.0^\circ$, $d = 14.73$ Å; $2\theta = 22.2^\circ$, $d = 4.00$ Å; $2\theta = 23.3^\circ$, $d = 3.19$ Å and low intensity reflections at $2\theta = 16.4^\circ$, $d = 5.41$ Å and $2\theta = 24.2^\circ$, $d = 3.68$ Å after 1 week (Table 1). These reflections are characteristic for goat fat and theobroma oil. The positions of the reflections changed slightly with slight increase in intensity of the reflections at $2\theta = 22.2^\circ$, $d = 4.00$ Å and $2\theta = 24.2^\circ$, $d = 3.68$ Å and $2\theta = 6.8^\circ$, $d = 13.00$ Å after 7 and 12 weeks respectively (Table 1 and Fig. 4).

In the lipid matrix with 50% (w/w) goat fat, the influence of theobroma oil was more pronounced indicated by the highest intensity of the β-modification-connected reflection at $2\theta = 19.3^\circ$, $d = 4.60$ Å, and increased intensity of the other reflections characteristic for theobroma oil at $2\theta = 23.0^\circ$, $d = 3.87$ Å. Only minor shifts in reflection positions, changes in intensity and disappearance of some reflections were detected after 7 and 12 weeks (Table 1).

At 25% (w/w) of goat fat, high intensity reflections characteristic of both goat fat and theobroma oil still existed at their respective positions. However, there was a splitting of the reflection due to theobroma oil at $2\theta = 24.0^\circ$, $d = 3.71$ Å into two reflections of medium and low intensities detectable at $2\theta = 23.6^\circ$, $d = 3.77$ Å and $2\theta = 24.2^\circ$, $d = 3.68$ Å, respectively detectable throughout the study period with increase in intensity after 12 weeks (Table 1 and Fig. 4). The lattice spacings of these two reflections are very close to lattice spacings assigned to β′ modification (Solís-Fuentes et al., 2005). For this matrix, the reflection positions changed and intensities slightly increased after 12 weeks (Table 1). There was also a sharpening of the initially almost diffuse reflection due to goat fat at $2\theta = 20.7^\circ$, $d = 4.29$ Å and $2\theta = 21.1^\circ$, $d = 4.21$ Å with consequent increase in intensity after 12 weeks (Fig. 4). All these changes that occurred in this matrix containing 25% (w/w) goat fat may be due to higher content of theobroma oil which undergoes a high degree of polymorphic transformation. This result may indicate that the observed changes in intensity and wavelength correspond to a secondary crystallization step where metastable and loose crystals formed in the first step may become more stable and more compact (Okubo et al., 2005), or attain a different crystalline state. The structured lipid matrices had diffuse reflections at some points in their diffractograms pointing to the possible partially amorphous nature of these matrices.

3.4. Small angle X-ray diffraction (SAXD)

Analysis of small angle reflections ($2\theta < 10^\circ$) allows us to determine the long range order of the lattice while the reflections or diffuse bands in the high angle region provide information concerning the conformation of hydrocarbon chains (Luzzati and Tardieu, 1974). SAXD diffractograms are shown in Fig. 5, representing the determination after 12 weeks, while data obtained after 1, 7 and 12 weeks are presented in Table 2. Goat fat had one strong intensity peak at $2\theta = 2.0^\circ$, $d = 44.18$ Å and a weak intensity reflection at $2\theta = 6.1^\circ$, $d = 14.49$ Å. These two dominating Bragg reflections did not show integral higher order reflections. These reflections were also repeated after 7 and 12 weeks, but with a very weak additional signal appearing at $2\theta = 1.4^\circ$, $d = 63.11$ Å after 12 weeks (Fig. 5 and Table 2). This result indicates that goat fat has most of its matrices in 44.18 Å bilayer arrangement with small proportion in 63.11 Å. With

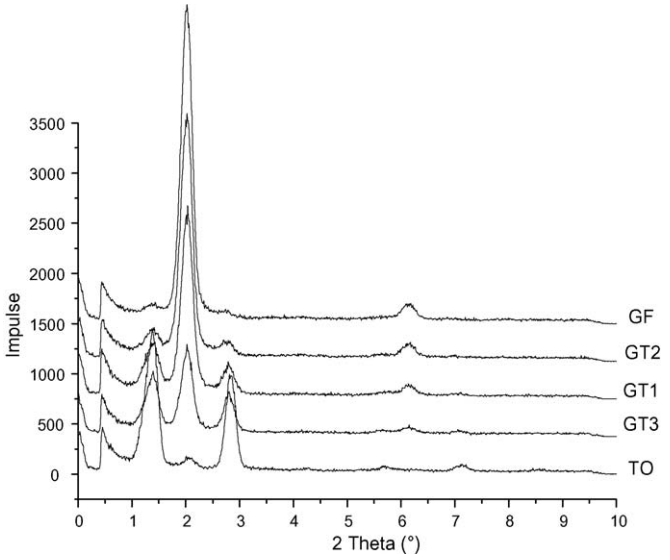

Fig. 5. SAXD diffractograms of the lipid matrices after 12 weeks: GF (goat fat), GT2 (75%, w/w goat fat), GT1 (50%, w/w goat fat), GT3 (25%, w/w goat fat) and TO (theobroma oil).

theobroma oil, two reflections were detected after 1 week, which roughly depicted lamellar arrangement. FFTEM micrographs confirmed the lamellar arrangement (Fig. 6). One of the two reflections for theobroma oil ($2\theta = 2.8°$, $d = 31.56$ Å) remained at the same position while the second reflection shifted to $2\theta = 1.4°$, $d = 67.96$ Å after 7 and 12 weeks (Fig. 5 and Table 2). There was increase in intensity of these two reflections especially after 12 weeks with the appearance of two very weak signals at $2\theta = 2.1°$, $d = 42.07$ Å (detected after 7 weeks) and $2\theta = 7.2°$, $d = 12.28$ Å.

The structured lipid matrices had reflections characteristic of both goat fat and theobroma oil throughout the study period. At 75% (w/w) goat fat concentration, two reflections at $2\theta = 2.0°$, $d = 44.18$ Å and $2\theta = 6.1°$, $d = 14.49$ Å were detected after 1 week (Table 2). This arrangement was however, interfered with by the appearance of additional two very weak reflections at $2\theta = 1.4°$, $d = 63.11$ Å and $2\theta = 2.8°$, $d = 31.56$ Å after 7 weeks with a subsequent very slight increase in intensity after 12 weeks (Fig. 5 and Table 2). This may suggest that majority of the matrices were in 44.18 Å bilayer arrangement and small fractions in 63.11 and 31.56 Å bilayer arrangements.

In the matrix containing 50% (w/w) goat fat, four reflections were detected after 1 week at $2\theta = 1.4°$, $d = 63.11$ Å, $2\theta = 2.0°$, $d = 44.18$ Å, $2\theta = 2.8°$, $d = 31.56$ Å and $2\theta = 6.1°$, $d = 14.49$ Å (Table 2). These reflections remained at the same positions but with slight increase in intensities of the two detected at $2\theta = 1.4°$, $d = 63.11$ Å and $2\theta = 2.8°$, $d = 31.56$ Å after 12 weeks (Fig. 5 and Table 2). The original lamellar arrangement detected for theobroma oil was modified and a greater proportion of the matrices in addition to 44.18 Å bilayer, were in 63.11 and 31.56 Å bilayer arrangement compared with the structured lipid matrix containing 75% (w/w) goat fat.

The reflections detected for the structured lipid matrix containing 25% (w/w) goat fat after 1 week at $2\theta = 1.3°$, $d = 67.96$ Å, $2\theta = 2.0°$, $d = 44.18$ Å and $2\theta = 2.8°$, $d = 31.56$ Å, with the highest reflection occurring at $2\theta = 2.0°$ (Table 2) also reflect a

Table 2
SAXD data for the lipid matrices containing theobroma oil and goat fat at different stages of storage

GF					GT2					GT1					GT3					TO				
1 week		7 weeks		12 weeks	1 week		7 weeks		12 weeks	1 week		7 weeks		12 weeks	1 week		7 weeks		12 weeks	1 week		7 weeks		12 weeks
2θ (°)	d (Å)	2θ (°)	d (Å)	2θ (°) d (Å)	2θ (°)	d (Å)	2θ (°)	d (Å)	2θ (°) d (Å)	2θ (°)	d (Å)	2θ (°)	d (Å)	2θ (°) d (Å)	2θ (°)	d (Å)	2θ (°)	d (Å)	2θ (°) d (Å)	2θ (°)	d (Å)	2θ (°)	d (Å)	2θ (°) d (Å)
				1.4 63.11			1.4	63.11	1.4 63.11	1.4	63.11	1.4	63.11	1.4 63.11	1.3	67.96	1.4	63.11	1.4 63.11	**1.3**	**67.96**	**1.4**	**63.11**	**1.4 63.11**
2.0	**44.18**	**2.0**	**44.18**	**2.0 44.18**	**2.0**	**44.18**	**2.0**	**44.18**	**2.0 44.18**	**2.0**	**44.18**	**2.0**	**44.18**	**2.0 44.18**	*2.0*	*44.18*	**2.0**	**44.18**	*2.0 44.18*			2.1	42.07	2.1 42.07
							2.8	31.56	2.8 31.56	2.8	31.56	2.8	31.56	2.8 31.56	2.8	31.56	2.8	31.56	2.8 31.56	2.8	*31.56*	*2.8*	*31.56*	*2.8 31.56*
6.1	14.49	6.1	14.49	6.1 14.49	6.1	14.49	6.1	14.49	6.1 14.49	6.1	14.49	6.1	14.49	6.1 14.49			6.1	14.49	6.1 14.49					7.2 12.28

Key: High intensity (bold), medium intensity (italics), low intensity (underline) and weak intensity (normal). GF (goat fat), GT2 (75%, w/w, goat fat), GT1 (50%, w/w, goat fat), GT3 (25%, w/w, goat fat) and TO (theobroma oil).

superposition of both goat fat and theobroma oil. The positions of two reflections remained unchanged after 7 and 12 weeks but with slight increase in intensity and shift of the reflection at $2\theta = 1.3–1.4^\circ$, $d = 63.11$ Å occurred. Due to intensities of the peaks, this structured lipid matrix may be said to have its matrices in 63.11, 44.18 and 31.56 Å layered arrangements. All the structured lipid matrices presented strong reflections at $2\theta = 2.0^\circ$, $d = 44.18$ Å characteristic for goat fat and medium to low reflections at $2\theta = 2.8^\circ$, $d = 31.56$ Å due to theobroma oil and $2\theta = 1.4^\circ$, $d = 63.11$ Å due to both pure lipids. The complexity of the patterns shown by the Bragg reflections of these structured lipid matrices showed that they possessed multiple layered arrangements. Due to chemical similarity of the triglycerides, the matrices are likely to form mixed crystals at least in part.

3.5. Freeze-fracture transmission electron microscopy (FFTEM)

The FFTEM micrographs are presented in Fig. 6. Theobroma oil showed lamellar microstructure with extended layers and sharp edges due to its crystalline state (Glombitza and Müller-Goymann, 2002). Goat fat on the other hand, also showed the typical layered texture of triglycerides. However, there were little sharp edges possibly due to liquid crystalline amounts. With the structured lipid matrix containing 50% (w/w) goat fat, the FFTEM microstructure appeared as a modification of the two component lipids as there was crystalline edges and layered structures.

3.6. Isothermal heat conduction microcalorimetry (IMC)

The IMC plots for the pure lipids and the structured lipid matrices are presented in Fig. 7. The result showed greater heat flow in theobroma oil than in goat fat in accordance with higher melting enthalpy of theobroma oil than that of goat fat. In goat fat, there was a slight change in the slope of the curve after about 45 min of isothermal crystallization. This may be due to slight decrease in rate of crystallization in context with crystal growth slowdown because of interactions between growing crystals. The lipid matrix containing 75% (w/w) goat fat showed a delayed crystallization indicated by initially high positive slope lasting for about 75 min, with a subsequent high negative slope indicative of spontaneous crystallization (Schubert et al., 2005).

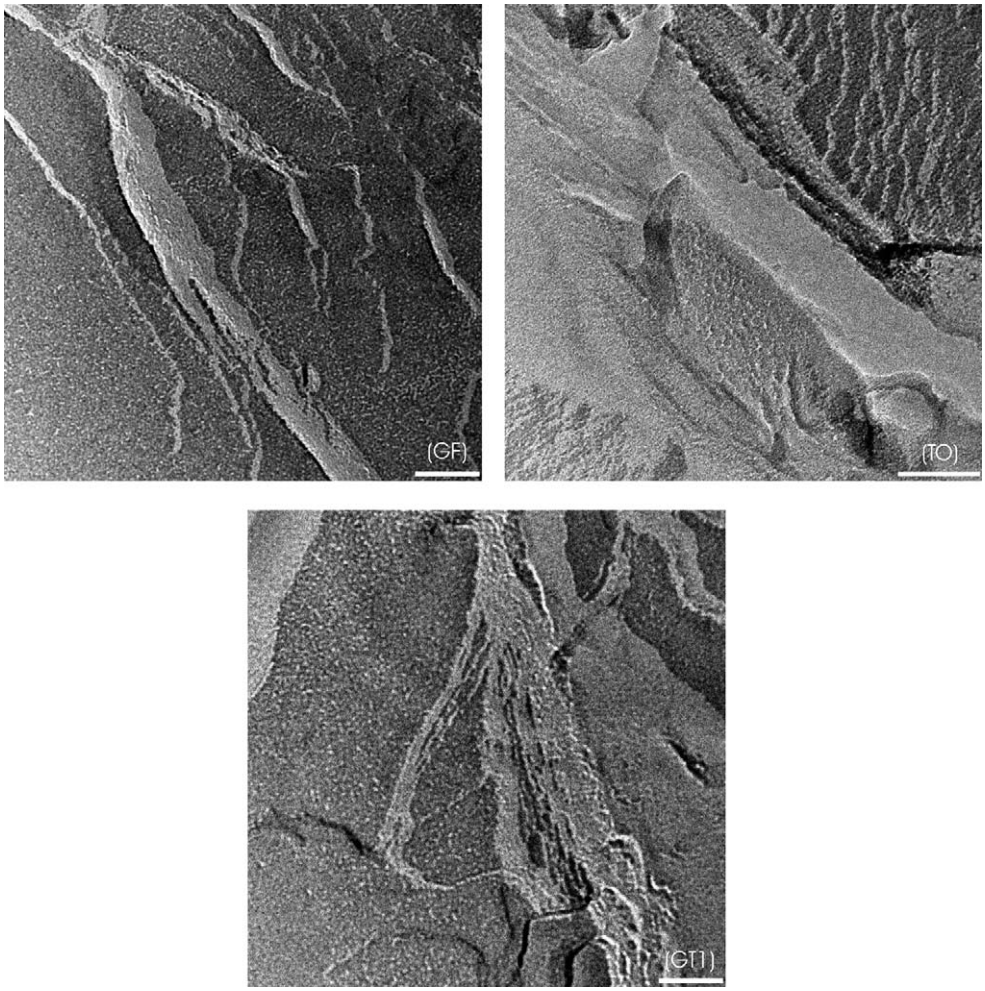

Fig. 6. Freeze-fracture transmission electron micrographs of the lipid matrices: goat fat (GF), theobroma oil (TO) and 50%, w/w goat fat (GT1). Bars represent 50 nm.

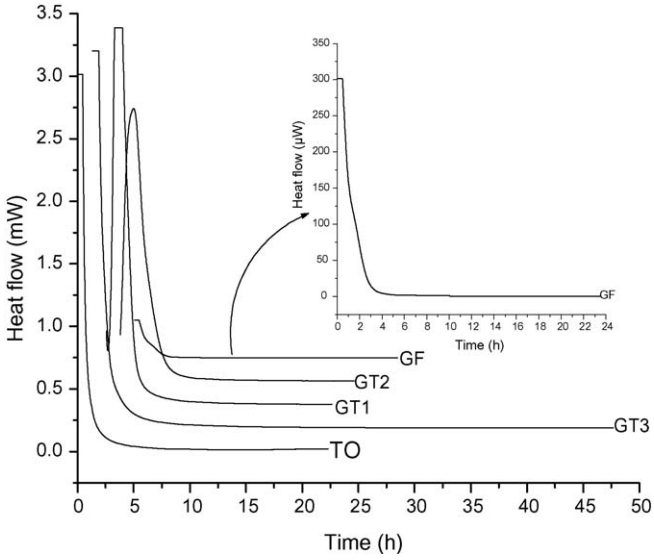

Fig. 7. Crystallization exotherms of the lipid matrices: GF (goat fat), GT2 (75%, w/w goat fat), GT1 (50%, w/w goat fat), GT3 (25%, w/w goat fat) and TO (theobroma oil). Inset shows the crystallization exotherm of goat fat.

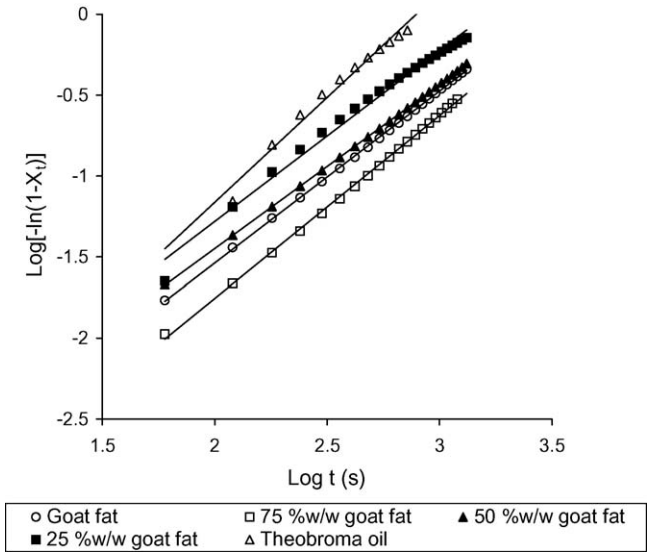

Fig. 8. Isothermal crystallization kinetic plots for the lipid matrices.

Similar observation was made for the lipid matrix containing 50% (w/w) goat fat. However, there was greater heat flow owing to higher content of theobroma oil. In this matrix also, there was a delayed crystallization lasting for about 15 min until a positive slope was obtained giving rise to the highest heat flow in all the lipid matrices. This may be due to some discrete spontaneous crystallization of part of the high melting fraction of goat fat present in the mixture. This crystallization process lasted for about 90 min. There was spontaneous crystallization of the lipid matrix containing 25% (w/w) goat fat and the study period was extended to 48 h to check if we could observe any immediate change that could give a clue to the PLM observation after 4 weeks (Fig. 1), but nothing was detected. IMC gives a short time study of the spontaneity and progress of crystallization process. In all the lipid matrices, the respective curves were asymptotic with time axis at later time indicating that the lipid matrices will attain ultimate crystallinity at infinite time.

Avrami equation (Eq. (3)) was further analysed to determine the crystallization kinetic parameters. The results are presented in Fig. 8 and Table 3. Fig. 8 shows the log–log plot from where the kinetic parameters presented in Table 3 were deduced. The result indicated that all the lipid matrices had good correlation coefficients ($r^2 > 0.9$) and n values close to 1, with the lipid matrix containing 75% (w/w) goat fat having the lowest growth rate constant (k value). Their low n values may suggest heterogeneous nucleation (Supaphol, 2001). Although the n values obtained in this study were fractional values and not integers as stipulated by Avrami equation, deviation of crystallization of a supercooled melt from Avrami equation is known to be a result of several factors generally attributed to the simplified assumptions made in the Avrami model, which include among others, constant radial growth rate, constant density and shape of the growing nuclei and no volume change during phase transformation or crystallization (Avrami, 1941). Because of these simple assumptions, the Avrami model leads to some fractional and high values of n that cannot be explained on any physical basis. Higher n values and non-linearities in the evaluation of Avrami model could in principle result from the simultaneous growth of two different types of spherulites, a single structure grown from two types of nuclei (homogeneous and heterogeneous) and a secondary crystallization neglected in the Avrami analysis. High n value (ca. 4) may correspond to three-dimensional spherulitic growth from homogeneous nuclei (Kong et al., 2000). Avrami equation is obeyed very well in the early stages of spontaneous crystallization process and that confirmed our choice of the time interval within the first 1440 s of spontaneous crystallization. The n values of the physically structured lipid matrices were closer to the n value of goat fat than to theobroma oil, signifying that goat fat may have had a greater influence on the nucleation mechanisms and growth dimensions of the structured lipid matrices from their melts. Table 3 also shows that the structured lipid matrix with 25% (w/w) goat fat had the highest growth rate constant compared with other lipid matrices. The structured lipid matrix containing 50% (w/w) goat fat which had the lowest n value with $r^2 = 1.000$ within the time interval studied, possessed the greatest heat flow despite the second highest growth rate constant. Hence, heterogeneous nucleation is most likely and sufficiently fast to yield small crystal sizes because of crystal growth limitations.

Table 3
Isothermal crystallization kinetic parameters determined within the first 1440 s of spontaneous isothermal crystallization

Lipid matrix	Parameter		
	n	k (s^{-1})	r^2
GF	1.062	2.18×10^{-4}	0.9997
GT2	1.129	9.68×10^{-5}	0.9992
GT1	1.015	3.33×10^{-4}	1.000
GT3	1.057	4.02×10^{-4}	0.9879
TO	1.298	1.75×10^{-4}	0.9808

Key: GF (goat fat), GT2 (75%, w/w, goat fat), GT1 (50%, w/w, goat fat), GT3 (25%, w/w, goat fat) and TO (theobroma oil).

4. Conclusions

This investigation reveals that goat fat crystallizes into different stable polymorphic forms, and also possessed intermediate liquid crystalline phase. The structured lipid matrices had characteristic properties depending on the ratio of the two individual lipids. DSC and XRD measurements suggested less ordered arrangements of the structured lipid matrices comprising mixtures of crystalline and amorphous portions. WAXD studies specifically revealed that goat fat and the structured lipid matrices had diffuse reflections at some points in their diffractograms pointing to the partly amorphous nature of these matrices. Analysis of the crystallization exotherm showed that the lipid matrix containing 75% (w/w) goat fat possessed the lowest growth rate constant (k value) among all the structured lipid matrices, but the n values of all the structured lipid matrices suggested that goat fat had more influence on the nucleation mechanism and growth dimension than theobroma oil. It is our thinking that the result of this study may have important implications for developing uses of lipid mixtures for novel drug delivery systems including solid lipid nanoparticles and nanostructured lipid carriers which are current research areas in drug delivery pharmaceutics.

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