

# Development, in vitro and in vivo evaluations of novel lipid drug delivery system of *Newbouldia laevis* (P. Beauv.)

Nanobiomedicine

Volume 3: 1–17

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DOI: 10.1177/1849543516673445

nab.sagepub.com



Chukwuebuka Umeyor<sup>1</sup>, Emmanuel Anaka<sup>1</sup>,  
Franklin Kenechukwu<sup>2</sup>, Chinazom Agbo<sup>2</sup>, and Anthony Attama<sup>2</sup>

## Abstract

*Newbouldia laevis* (P. Beauv.) is a tropical rainforest plant used in traditional folk medicine for the treatment of malaria, cough, joint pains, stomach ache, oedema and inflammation. The main thrust of this research work was to study the analgesic/anti-nociceptive properties of *N. laevis*-loaded solid lipid microdispersions. *N. laevis* leaves were extracted using ethanol, and the extract was formulated into solid lipid microdispersions using lipid matrix comprising a rational blend of Precirol<sup>®</sup> ATO 5 and Softisan<sup>®</sup> 154. Characterization of the solid lipid microdispersions include determination of morphology, particle size, pH, thermal property, encapsulation efficiency percentage and analgesic/anti-nociceptive property. The results obtained showed that the particles were spherical with sizes ranging from 40 µm to 125 µm. The solid lipid microdispersions maintained a stable pH within the acidic region of 5–6 with insignificant variations ( $p > 0.05$ ) over a period of 90 days. Thermal analysis showed that *N. laevis* was entrapped in the lipid matrix used for the formulations. Solid lipid microdispersions recorded a maximum encapsulation efficiency up to 88.1%. *N. laevis*-loaded solid lipid microdispersions also produced good analgesic/anti-nociceptive property comparable with the standard diclofenac potassium. *N. laevis*-loaded solid lipid microdispersions showed good analgesic/anti-nociceptive effect and could be used in the treatment and management of pain.

## Keywords

Solid lipid microdispersions, Precirol<sup>®</sup> ATO 5, Softisan<sup>®</sup> 154, analgesic/anti-nociceptive, phytomedicine

Date received: 10 March 2016; accepted: 14 September 2016

## Introduction

The use of herbs by man for therapeutic purposes started right from the development of human culture. Until the discovery and introduction of modern therapeutics, various plant parts have been utilized by man in the form of decoctions, macerations, teas, concoctions and so on, for the treatment, management and cure of various ailments. For many centuries, these crude drugs and their folkloric uses have been handed down from one generation to another, and in the past millennium, reports abound of the screening of extracts of these medicinal plants for possible use in the alleviation of various health challenges.<sup>1</sup> These waves of research findings have made it imperative for formulation scientists to embark on the formulation, characterization

<sup>1</sup>Nanomedicines and Drug Delivery Research Group, Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria

<sup>2</sup>Drug Delivery and Nanomedicines Research Group, Department of Pharmaceutics, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka, Enugu State, Nigeria

### Corresponding Author:

Chukwuebuka Umeyor, Nanomedicines and Drug Delivery Research Group, Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka 422001, Anambra State, Nigeria.  
Email: ec.umeyor@unizik.edu.ng



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and delivery of these 'green' medicines for possible translation to clinical use, in line with the recent advocacy on green initiatives to save our planet, as well as be at the frontiers of providing novel and standardized medicines for clinical applications.

Lipid-based drug delivery systems (LBDDSs) have been reported to modulate the physico-pharmacological properties of drugs due to their propensity to alter the pharmacokinetic and biodistribution profiles of drugs. This modulation leads to enhanced bioavailability of drugs encapsulated in the lipid matrix due to increased absorption of the bioactive components through different mechanisms to modify their release. LBDDSs also affect the intestinal environment, stimulate the lymphatic transport of released drugs and interact with enterocyte-based transport. This has made LBDDS an attractive alternative for the delivery of both hydrophilic and lipophilic drugs.<sup>2,3</sup> The use of solid lipids in LBDDS imparts very good stability profile to the formulation and maximizes the rate and extent of drug dissolution. Also, it has the ability to control as well as sustain the release properties of an incorporated drug by lowering its mobility within the solid lipid core.<sup>4</sup> Solid lipid microdispersions (SLMs) are LBDDS prepared using a uniform blend of lipid matrices, surfactant and water and encapsulating a hydrophilic or lipophilic drug for improved absorption upon administration. SLMs have been shown to protect encapsulated drug against chemical degradation, provide sustained release and ensure longer shelf life of the active drug. Generally, they are biocompatible and biodegradable, physicochemically stable and could be produced at a relatively cheap cost. They are widely accepted as an important and promising alternative drug delivery system because they are endowed with several advantages of different traditional drug carrier systems, while avoiding some of their disadvantages.<sup>5-7</sup>

Most phytochemicals from plants are very water soluble, for example, glycosides, flavonoids and so on, and this hydrophilicity limits their effectiveness because they are poorly absorbed with low bioavailability when they are applied topically or administered orally.<sup>8</sup> As a result of this drawback, encapsulating these phytoconstituents using lipid-based drug carriers has been found to greatly enhance their bioavailability with faster and improved systemic absorption.<sup>9</sup> In a study, lipid vesicles of boswellic acid were developed using phosphatidylcholine, and anti-inflammatory activity evaluation showed a clear increase in the activity of boswellic acid with a notable increase in absorption.<sup>10</sup> Recently, *Usnea barbata* carbon dioxide-supercritical extract was formulated as a lipid emulsion with improved stability and antibacterial activity profiles.<sup>11</sup> These reports support the need to develop dosage forms of natural products using lipid-based carrier systems.

*Newbouldia laevis* (P. Beauv.) belongs to the *Bignoniaceae* family and is commonly known as smooth *Newbouldia* or boundary tree. In Nigeria, it is known with different names among different communities: 'Aduruku', 'Ogirisi',

'Ikhimi' and 'Akoko' by the Hausa, Igbo, Bini and Yoruba communities, respectively. In Africa, *N. laevis* is widely used in folk medicine for the treatment of malaria, cough, stomach aches and pains, toothache, breast cancer and constipation. In south-east and Midwest Nigeria, the plant is well known for the treatment of inflammation, oedema, septic wounds, eye problems and sexually transmitted infections (STIs).<sup>12-15</sup> Phytochemical screening of the plant has revealed the abundance of alkaloids, phenylpropanoids, flavonoids, tannins and glycosides in its leaf, root and flowers.<sup>16,17</sup> Aqueous and ethanolic leaf extracts of the plant have been reported to increase the frequency of spontaneously contracting tissues and directly stimulate uterine contractions in albino rats.<sup>18</sup> Similarly, ethanolic extracts of the leaves and stem of *N. laevis* have been reported to possess antioxidant activity in diabetic rats.<sup>19</sup>

Since the fundamental importance of using plant-based medicines is their relative safety compared with synthetic drugs, and affordability of treatment, the objectives of this study were to formulate SLMs encapsulating *N. laevis* leaves extract and to study its analgesic/anti-nociceptive property.

## Materials and methods

### Materials

The following materials were obtained directly from their manufacturers and used without further purification: Softisan<sup>®</sup> 154, Kolliphor<sup>®</sup> 188 (BASF SE, Ludwigshafen, Germany), Precirol<sup>®</sup> ATO 5 (Gattefossé, Saint-Priest Cedex, France), ethanol (Sigma-Aldrich, Germany), diclofenac potassium (Healthy Life Pharma, Mumbai, Maharashtra, India). Distilled water was collected from an all-glass still. All other reagents used were analytical grade and used as supplied.

### Collection and extraction of *N. laevis*

Fresh leaves of *N. laevis* were harvested from a thick bush at Agulu, Anaocha Local Government Area, Anambra State, Nigeria, in June 2014, were authenticated by a taxonomist in our institution and given the voucher number N.A.U.H. no. 22, and the specimen was deposited in the herbarium of the Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka. The leaves were dried under shade for 14 days and milled using a cutter mill (JCT Thakur, Hoshiarpur, Punjab, India). Powdered *N. laevis* leaves (400 g) were extracted by continuous Soxhlet extraction using ethanol, with the extractor connected to a cooling chamber. The residue was discarded and the extract was concentrated using a rotary evaporator (Stuart, Barloworld Scientific, Essex, UK) under reduced pressure to yield a dark, green mass labelled as *N. laevis* extract (NLE), which was stored in an airtight plastic container until required.

### Screening for secondary metabolites

Phytochemical assay of the crude ethanolic extract of *N. laevis* leaves was carried out in order to ascertain the presence or absence of secondary metabolites in the extract using standard conventional protocols.<sup>20,21</sup>

### Acute toxicity study

White albino rats were maintained at standard housing conditions, and the animals were fed with standard pellets (Vital Feeds, Nigeria) and water *ad libitum* during the experiment. Ethical clearance for this study was obtained from the Animal Ethics Committee of the Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka, following animal experimental protocols in accordance with the European Community Guidelines (European Economic Community (EEC) Directive of 2010; 2010/63/EEC). A total of 25 rats grouped into I–V and comprising five rats per group were used for this study. Rats were fasted for 24 h prior to administration of NLE. Groups I, II, III and IV were administered 250, 500, 1000 and 2000 mg kg<sup>-1</sup> body weight single dose of NLE, respectively, by oral gavage, while the control group V received normal saline (10 mL kg<sup>-1</sup>). Feeding of rats resumed immediately after NLE administration, and the lethal dose (LD<sub>50</sub>) of the plant extract was calculated following standard methods.<sup>22</sup> Furthermore, time-dependent cage-side clinical observations for toxicity signs such as paw licking, motor activity, tremors, convulsions, spasticity, salivation, diarrhoea, writhing and change in skin colour were made 2 weeks post-administration. Body weights were also measured shortly before the administration of NLE and weekly thereafter.

### Preparation of lipid matrix

The lipid matrices consisting ratios of 1:1, 1:2 and 2:1 mixtures, respectively, of Precirol<sup>®</sup> ATO 5 and Softisan<sup>®</sup> 154 were prepared by fusion.<sup>20</sup> Briefly, the lipids were weighed out using an electronic balance (Pyrex, Germany), and melted together at 70°C in a thermoregulated water bath and stirred until solidification to get lipid matrices, labelled as 'PreciSoft' lipid matrix.

### Formulation of solid lipid microdispersions

The hot-melt homogenization method<sup>23</sup> was adapted in the formulation of NLE-loaded SLMs according to the quantities of ingredients shown in Table 1. In each case, the PreciSoft matrix was melted at 70°C, and the surfactant aqueous phase containing Kolliphor<sup>®</sup> 188 at the same temperature was added to the molten lipid matrix with gentle stirring with a homogenizer (Stuart) at 2000 r min<sup>-1</sup> for 20 min to produce hot primary emulsions, which were collected in beakers. Unloaded SLMs that served as negative control were also formulated. Each batch of SLMs was lyophilized using a freeze dryer

**Table 1.** Composition of *Newbouldia laevis*-loaded SLMs.<sup>a</sup>

Batch	Softisan <sup>®</sup> 154 (%w/w)	Precirol <sup>®</sup> ATO 5 (%w/w)	Kolliphor <sup>®</sup> 188 (%w/w)	Distilled water (qs)	NLE (%w/w)
X1	2.5	2.5	2.0	100	1.0
X2	2.5	2.5	2.0	100	3.0
X3	2.5	2.5	2.0	100	5.0
X4	2.5	2.5	2.0	100	—*
Y1	1.7	3.3	2.0	100	1.0
Y2	1.7	3.3	2.0	100	3.0
Y3	1.7	3.3	2.0	100	5.0
Y4	1.7	3.3	2.0	100	—*
Z1	3.3	1.7	2.0	100	1.0
Z2	3.3	1.7	2.0	100	3.0
Z3	3.3	1.7	2.0	100	5.0
Z4	3.3	1.7	2.0	100	—*

SLMs: solid lipid microdispersions; NLE: *Newbouldia laevis* extract.

<sup>a</sup>For each batch, X1–X3, Y1–Y3 and Z1–Z3 contain *Newbouldia laevis*, while X4, Y4 and Z3 contain no *Newbouldia laevis*.

\*Unloaded.

(Yorco, York Scientific, Ghaziabad, Uttar Pradesh, India) and the lyophilized samples were stored in airtight plastic containers until future use.

### Determination of percentage recovery

The lyophilized SLMs were weighed to get the yield of SLMs formulated per batch. The percentage (%) yield was calculated using the formula

$$\text{Percentage (\%)} \text{ recovery} = \frac{W_1}{W_2 + W_3} \times 100 \quad (1)$$

where  $W_1$  is the weight of the SLMs formulated (g),  $W_2$  is the weight of the drug added (g) and  $W_3$  is the weight of the lipids used and surfactant (g).

### Morphology and time-resolved particle size analysis

A weighed amount of SLMs (50 mg) from each batch was dispersed in distilled water and smeared on a microscopic slide using a glass rod. The mixture was covered with a cover slip and viewed with a Hund<sup>®</sup> binocular photomicroscope (Weltzlar, Germany) attached with a digital camera (Moticam, China) at a magnification of 100×. Several microparticles were counted and their sizes recorded ( $n = 100$ ). A mean of particles sizes was calculated as the representative size for a batch. This was done 24 h and 60 days post-formulation.

### Time-dependent pH study

A weighed amount of SLMs (50 mg) from each formulation was dissolved in 50 mL of distilled water in a 250-mL beaker. This dispersion was used to determine the pH of each sample in triplicates using a pH meter (Jenway 3505,

East Norwalk, Connecticut, USA). This was done at different time intervals of 24 h, 30 days and 90 days.

### Thermal analysis

Melting transitions and changes in heat capacity of Precirol<sup>®</sup> ATO 5, Softisan<sup>®</sup> 154, PreciSoft lipid matrices, and loaded SLMs were determined using a differential scanning calorimeter (Netzsch DSC 204 F1, Germany). One milligram of each batch was weighed into aluminium pan, hermetically sealed and the thermal behaviour was determined in the range 50°C–350°C under a 20 mL min<sup>-1</sup> nitrogen flux at a heating rate of 10°C min<sup>-1</sup>. The thermal property of the NLE was also determined in the range 50°C–350°C. The baselines were determined using an empty pan, and all the thermograms were baseline corrected.

### Determination of encapsulation efficiency

About 10 mg of each batch of the SLMs was dispersed in 100 mL of distilled water in a 100-mL volumetric flask. The dispersion was allowed to equilibrate for 48 h at room temperature (25°C ± 2°C), centrifuged at 4000 rpm and filtered. The filtrate was adequately analysed for crude drug extract content spectrophotometrically (Jenway UV/Vis 6505) at 250 nm. This was done in triplicates for each batch. The amount of drug encapsulated in the microparticles was calculated with reference to a standard Beer's plot for NLE. Encapsulation efficiency (EE %) was calculated using the following formula

$$\text{Encapsulation efficiency(\%)} = \frac{\text{Actual drug content}}{\text{Theoretical drug content}} \times 100 \quad (2)$$

### Determination of drug loading capacity

Drug loading capacity (DLC) estimates the ratio between the entrapped Active Pharmaceutical Ingredient (API) and total weight of the lipids. DLC was determined using the following relationship

$$\text{DLC (\%)} = \frac{W_a}{W_l} \times 100 \quad (3)$$

where  $W_l$  is the weight of lipid used in the formulation and  $W_a$  is the actual amount of NLE encapsulated in the SLMs.

### Pharmacodynamic studies

Mature mice were maintained at standard housing conditions, and the animals were fed with standard pellets (Vital Feeds) and water *ad libitum* during the experiment. All animal experimental protocols were carried out in accordance with guidelines of the Animal Ethics Committee of the Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka, Nigeria, and European Union (EU) Directive 2010/63/EU for animal experiments.

**Table 2.** Phytochemical constituents of *Newbouldia laevis* extract.

Secondary Metabolite	Presence
Alkaloids	+
Saponins	–
Tannins	++
Flavonoids	++
Steroids	–
Terpenoids	–
Cardiac glycosides	++
Proteins	–

+: moderately present (about 50%); ++: abundantly present (70% and above); –: absent.

### Determination of analgesic/anti-nociceptive property

Analgesic activity was tested in mice using the hot plate method.<sup>24</sup> Mature mice of either sex (150–200 g) were divided into 10 groups of five mice per group. NLE-loaded SLMs equivalent to 250 mg were administered orally to test groups. The control groups received normal saline 10 mL kg<sup>-1</sup>, while the reference group received 10 mg kg<sup>-1</sup> of diclofenac potassium. Mice were placed on hotplate maintained at 55°C ± 1°C and the reaction latency (seconds) for licking of hind paw or jumping was recorded. Recordings were taken before treatment with the different drugs and at 30, 60, 90 and 120 min post-treatment. Results were expressed as the difference between the baseline reaction latency and the reaction latency at each time interval.

### Statistical analysis

Statistical analysis was carried out using SPSS version 14.0 (SPSS Inc., Chicago, Illinois, USA). All experiments were performed in replicates ( $n = 3$ ) for validity of statistical analysis. Results were expressed as mean ± standard deviation. Analysis of variance and Student's *t*-tests were performed on the data sets and differences were considered significant for  $p < 0.05$ .

## Results

### Secondary metabolites screening

The result of the phytochemicals screening is presented in Table 2. The result indicates the absence of saponins, steroids, terpenoids and proteins. Alkaloids were moderately present (about 50%), while tannins, flavonoids and cardiac glycosides were abundantly present (70% and above).

### Acute toxicity test

Acute toxicity study is used to determine LD<sub>50</sub> value, which is a useful indicator of the safety margin and dose range at which a drug can be used such that there is no harmful or lethal effect on the animal. The result of the test

**Table 3.** Effect of NLE on body weights of rats.<sup>a</sup>

Time	Group I (g)	Group II (g)	Group III (g)	Group IV (g)	Group V(g)
Day 1	195 ± 0.25	205 ± 1.50	198 ± 1.08	185 ± 1.83	178 ± 1.20
Day 7	197 ± 1.12	203 ± 1.10	196 ± 1.23	183 ± 0.23	174 ± 2.05
Day 14	199 ± 0.52	204 ± 0.67	199 ± 2.45	184 ± 0.94	177 ± 1.67

NLE: *Newbouldia laevis* extract.

<sup>a</sup>All values are expressed as mean ± standard deviation.

**Table 4.** Percentage recovery of *Newbouldia laevis*-loaded SLMs.

Batch	Amount recovered (g)	Percentage yield
X1	6.33	86.3
X2	7.29	88.0
X3	8.25	89.2
X4	6.44	92.0
Y1	6.61	88.0
Y2	8.27	90.0
Y3	8.41	90.8
Y4	6.41	92.8
Z1	7.07	88.4
Z2	9.41	91.7
Z3	11.00	94.1
Z4	6.10	85.7

SLMs: solid lipid microdispersions.

showed that the plant extract was generally safe even at a high dose of 2.5 g kg<sup>-1</sup>, and no mortalities were recorded in the study animals. Furthermore, there was no significant ( $p > 0.05$ ) change in body weights (Table 3) and clinical behaviour of rats throughout the period of the study.

### Percentage recovery of SLMs

The percentage recovery of SLMs post-formulation is shown in Table 4. For the 'X' batches, X1, X2 and X3 gave percentage yields of 86.3%, 88.0% and 89.2%, respectively. In the 'Y' batches, Y1, Y2 and Y3 produced percentage yields of 88.0%, 90.0% and 90.8%, respectively, while within the 'Z' batches, Z1, Z2 and Z3 gave percentage yields of 88.4%, 91.7% and 94.1%, respectively. However, X4, Y4 and Z4 yielded 92.0%, 92.8% and 85.7%, respectively.

### Morphology and time-resolved particle size

Morphology of the SLMs is presented as photomicrographs in Figures 1 to 6, while time-resolved particle sizes are presented in Table 5. The photomicrographs of representative SLMs showed stable and spherical microparticles evenly distributed throughout the microscopic images of the NLE-loaded SLMs, while the photomicrographs of unloaded SLMs showed irregularly shaped particles. After 60 days, drug-loaded 'X' batches recorded sizes ranging from 74.2 ± 5.9 μm to 75.1 ± 9.0 μm. Also, drug-loaded 'Y' batches recorded sizes ranging from 53.6 ±

7.0 μm to 66.5 ± 5.2 μm after 60 days. Similarly, drug-loaded 'Z' batches gave sizes ranging from 59.2 ± 9.3 μm to 89.0 ± 4.4 μm after 60 days. After 60 days, unloaded SLMs recorded size ranges of 52.0 ± 4.4–125.1 ± 3.2 μm across all batches.

### Time-dependent pH studies

The result of the pH of SLMs studied over time is presented in Table 6. From the table, 'X' batches of SLMs formulated with 1:1 lipid matrix had pH range of 6.03 ± 0.1–6.79 ± 0.2 after 90 days. 'Y' batches formulated with 1:2 lipid matrix had pH ranging from 5.03 ± 0.4 to 6.77 ± 0.4, whereas 'Z' batches formulated with 2:1 lipid matrix had their pH ranging from 5.43 ± 0.2 to 6.87 ± 0.1.

### Thermal analysis

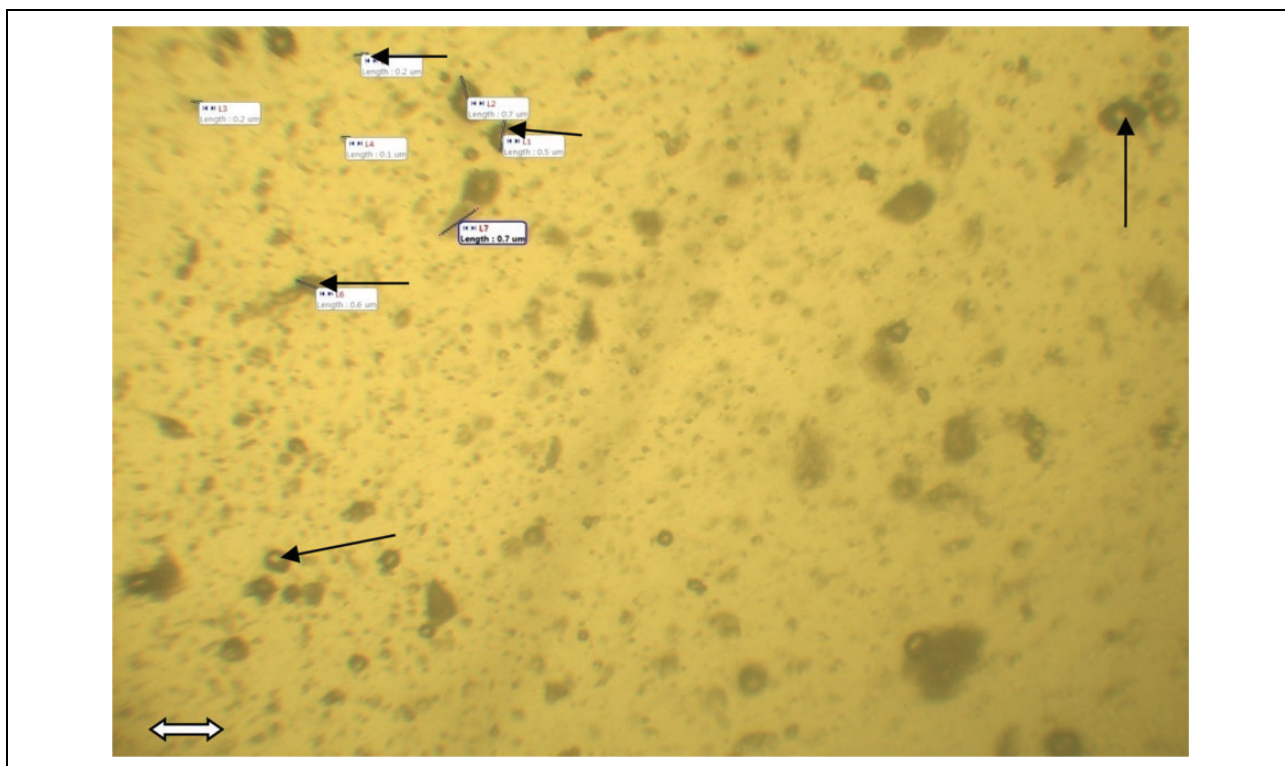
The result of the thermal analysis is presented in Figures 7 to 16. The differential scanning calorimeter (DSC) thermogram of Softisan<sup>®</sup> 154 showed melting peak of 59°C with enthalpy of -8.873 mW mg<sup>-1</sup>, while the thermogram of Precirol<sup>®</sup> ATO 5 showed melting peak of 69.2°C with enthalpy of -24.31 mW mg<sup>-1</sup>. SLMs formulated from the lipid matrices produced using these lipids gave DSC thermograms with lower enthalpies, indicating low crystallinity and high drug entrapment.

### EE (%) and DLC

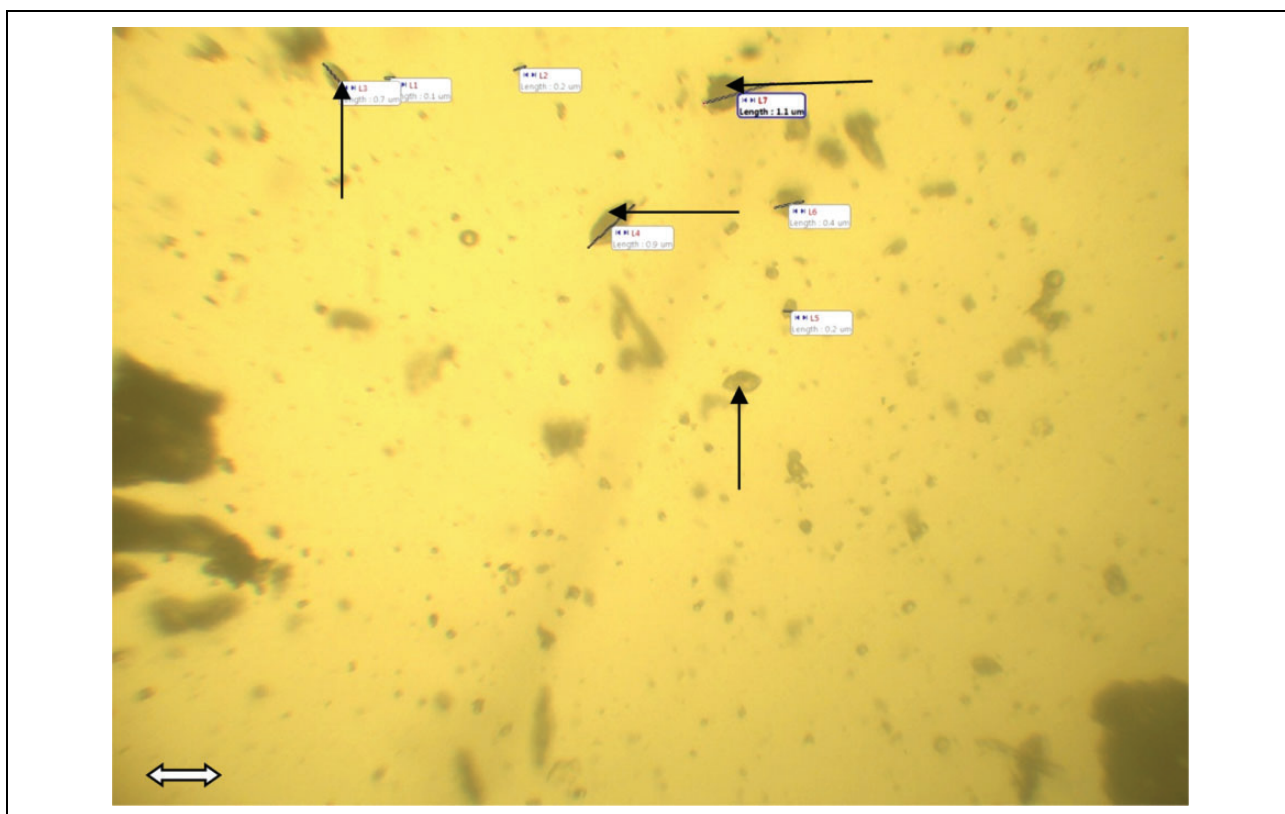
Results of the EE % and DLC of the SLMs are shown in Table 7. From the table, EE % decreased with the increase in drug loading with maximum EE % recorded for 'X' (1:1) batches, 'Y' (1:2) batches and 'Z' (2:1) batches at 77.2%, 81.0% and 88.1%, respectively, at 1% w/w of NLE. Conversely, DLC increased with the increase in drug loading with maximum DLC per 100 g of lipid recorded for 'X', 'Y' and 'Z' batches at 20.0, 28.0 and 34.0 g, respectively, at 5% w/w of NLE.

### Analgesic/anti-nociceptive property

The result of analgesic/anti-nociceptive study is presented in Table 8. From the result, analgesic/anti-nociceptive activity of the SLMs was comparable with the basal values after 30 min but were enhanced 60 min and above when the pain reaction time was greatly increased. The delayed

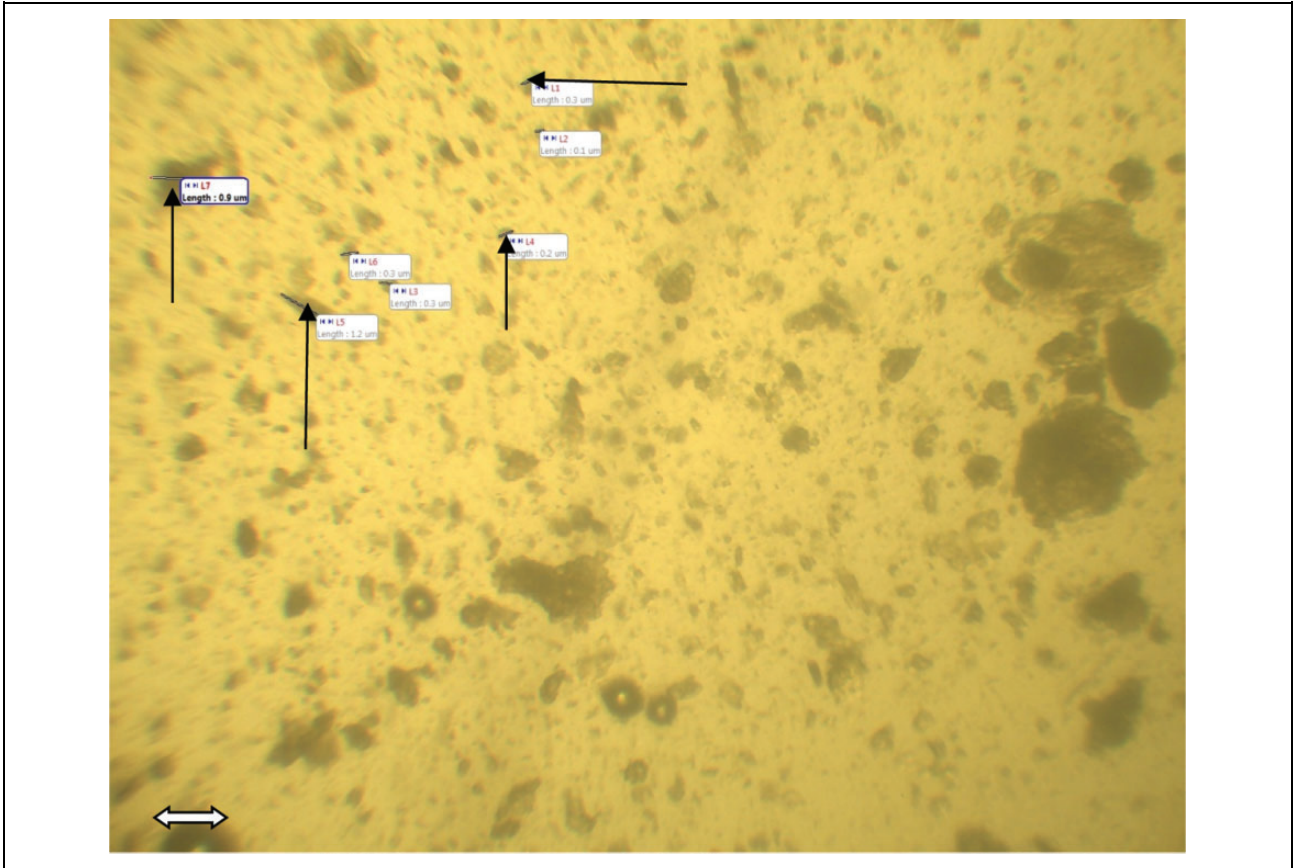


**Figure 1.** Photomicrograph of batch X1 showing spherical *Newbouldia laevis*-loaded SLMs (bar:  $\times 100$ ). SLMs: solid lipid microdispersions.

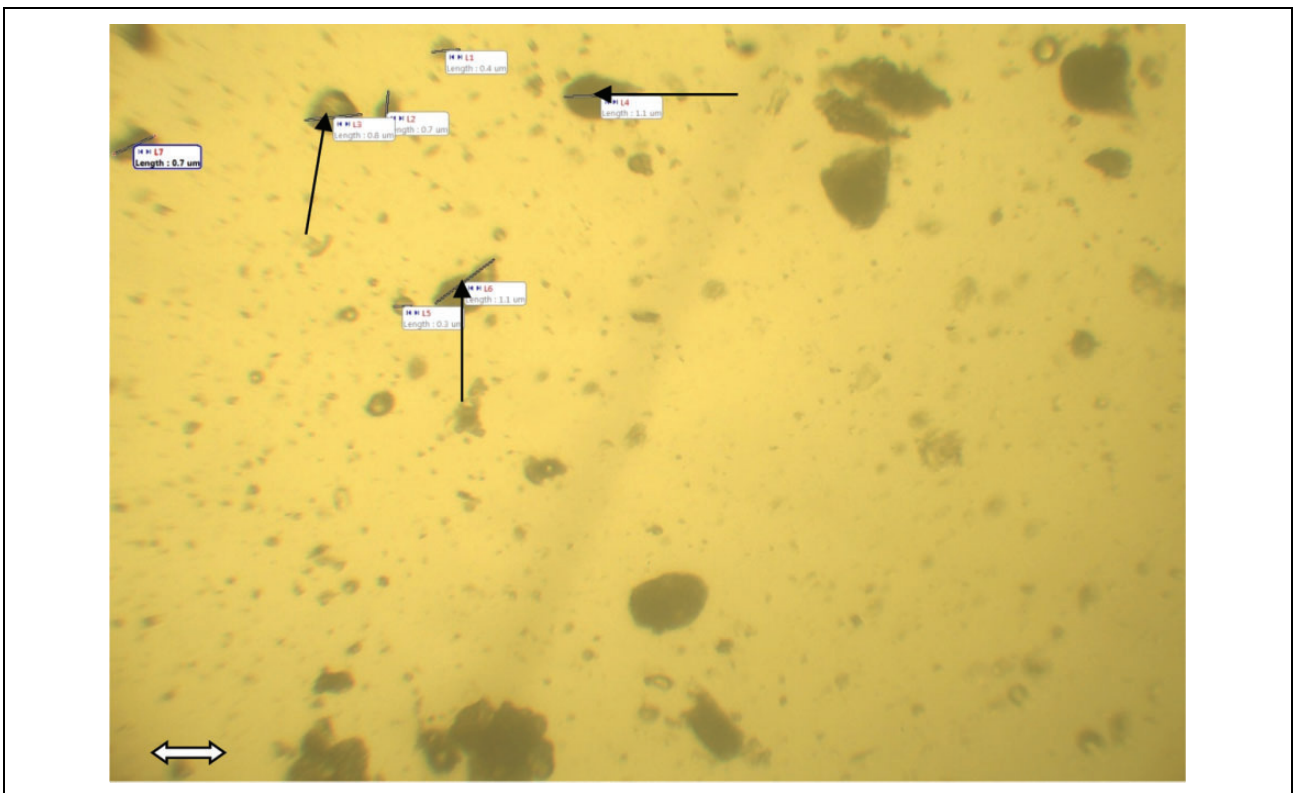


**Figure 2.** Photomicrograph of batch X4 showing spherical, unloaded SLMs (bar:  $\times 100$ ). SLMs: solid lipid microdispersions.

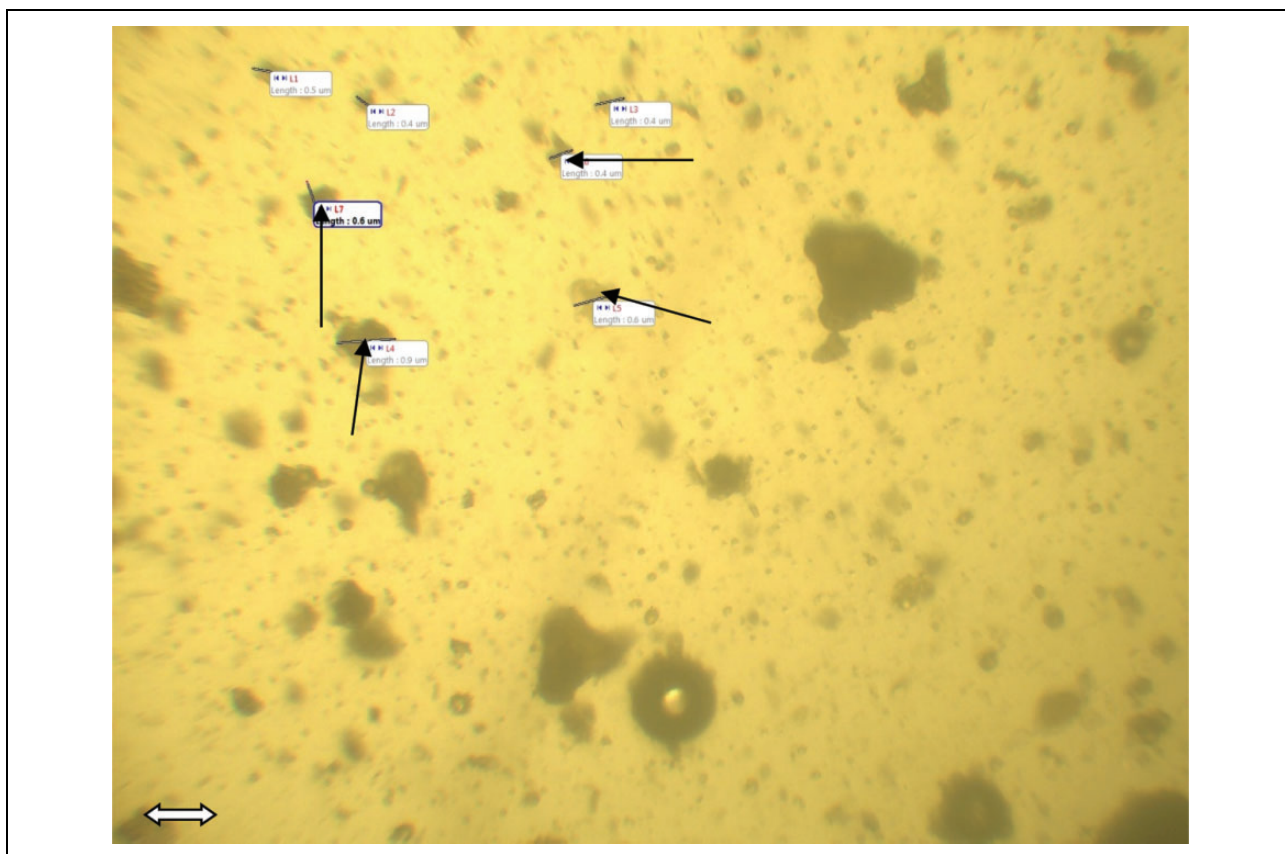




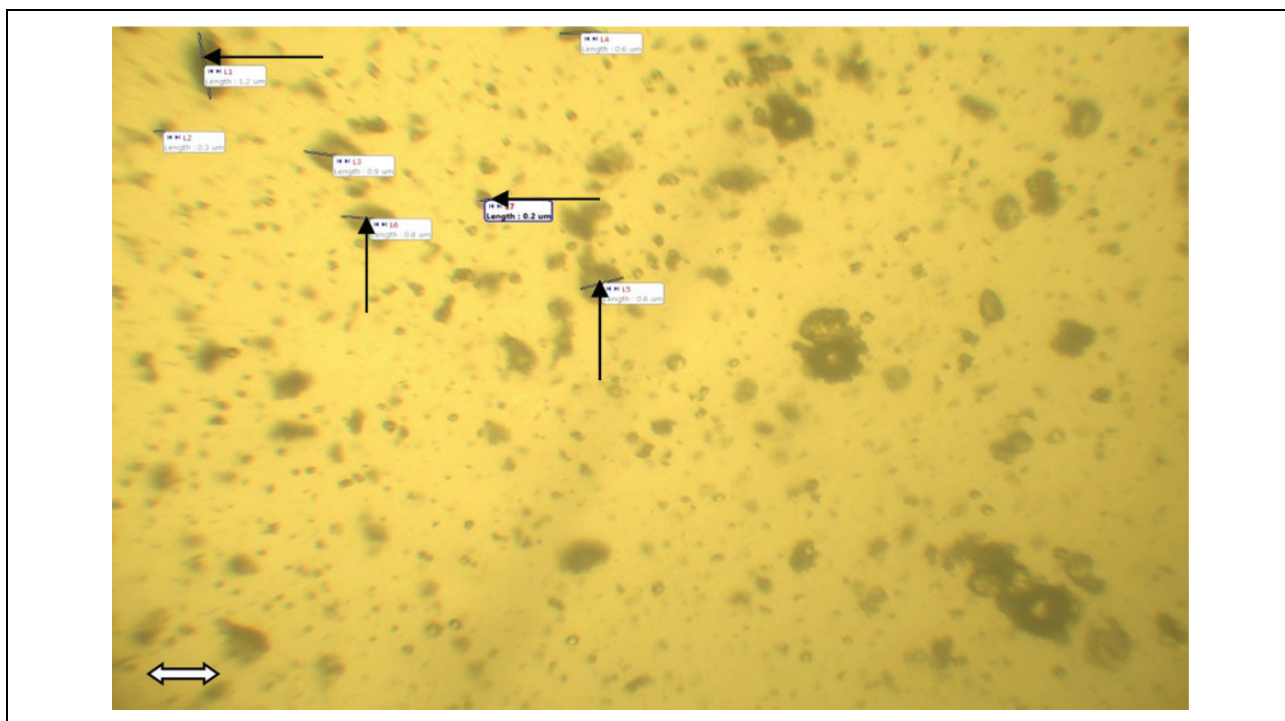
**Figure 3.** Photomicrograph of batch Y1 showing spherical *Newbouldia laevis*-loaded SLMs (bar:  $\times 100$ ). SLMs: solid lipid microdispersions.



**Figure 4.** Photomicrograph of batch Y4 showing spherical, unloaded SLMs (bar:  $\times 100$ ). SLMs: solid lipid microdispersions.



**Figure 5.** Photomicrograph of batch Z1 showing spherical *Newbouldia laevis*-loaded SLMs (bar:  $\times 100$ ). SLMs: solid lipid microdispersions.



**Figure 6.** Photomicrograph of batch Z4 showing spherical, unloaded SLMs (bar:  $\times 100$ ). SLMs: solid lipid microdispersions.



**Table 5.** Time-resolved particle size analysis.

Batch	Particle size ( $\mu\text{m} \pm \text{SD}$ )* after 24 h	Particle size ( $\mu\text{m} \pm \text{SD}$ )* after 60 days
X1	43.0 $\pm$ 2.5	74.2 $\pm$ 5.9
X2	51.4 $\pm$ 6.4	74.5 $\pm$ 3.6
X3	61.4 $\pm$ 3.7	75.1 $\pm$ 9.0
X4	40.0 $\pm$ 7.5	125.1 $\pm$ 3.2
Y1	47.1 $\pm$ 7.0	53.6 $\pm$ 7.0
Y2	57.1 $\pm$ 5.5	54.1 $\pm$ 6.8
Y3	73.0 $\pm$ 4.0	66.5 $\pm$ 5.2
Y4	41.4 $\pm$ 8.5	70.0 $\pm$ 2.4
Z1	54.3 $\pm$ 9.5	59.2 $\pm$ 9.3
Z2	65.7 $\pm$ 5.5	66.6 $\pm$ 1.3
Z3	77.5 $\pm$ 6.0	89.0 $\pm$ 4.4
Z4	51.4 $\pm$ 7.7	52.0 $\pm$ 4.4

SD = standard deviation.

\*n = 3.

**Table 6.** pH analysis of *Newbouldia laevis*-loaded SLMs.

Batch	pH (after 24 h) <sup>a</sup>	pH (after 30 days) <sup>a</sup>	pH (after 90 days) <sup>a</sup>
X1	6.45 $\pm$ 0.1	6.45 $\pm$ 0.2	6.44 $\pm$ 0.5
X2	6.12 $\pm$ 0.1	6.14 $\pm$ 0.9	6.41 $\pm$ 0.3
X3	6.03 $\pm$ 0.1	6.04 $\pm$ 0.5	6.05 $\pm$ 0.3
X4	6.73 $\pm$ 1.3	6.79 $\pm$ 0.1	6.79 $\pm$ 0.2
Y1	6.35 $\pm$ 2.3	6.37 $\pm$ 0.3	6.36 $\pm$ 0.1
Y2	6.05 $\pm$ 0.3	6.06 $\pm$ 0.3	6.04 $\pm$ 0.9
Y3	5.92 $\pm$ 0.7	5.92 $\pm$ 0.1	5.03 $\pm$ 0.4
Y4	6.77 $\pm$ 0.4	6.73 $\pm$ 0.6	6.73 $\pm$ 0.2
Z1	6.63 $\pm$ 0.1	6.63 $\pm$ 0.4	6.61 $\pm$ 1.2
Z2	5.61 $\pm$ 0.2	5.63 $\pm$ 0.1	5.70 $\pm$ 0.9
Z3	5.43 $\pm$ 0.5	5.43 $\pm$ 0.2	5.64 $\pm$ 0.5
Z4	6.82 $\pm$ 0.1	6.87 $\pm$ 0.1	6.86 $\pm$ 0.3

<sup>a</sup>Values are expressed as mean  $\pm$  standard deviation; n = 5

sensation to pain observed was reflective of that produced by the standard drug (positive control), diclofenac potassium, and the effect was sustained throughout the study period.

## Discussion

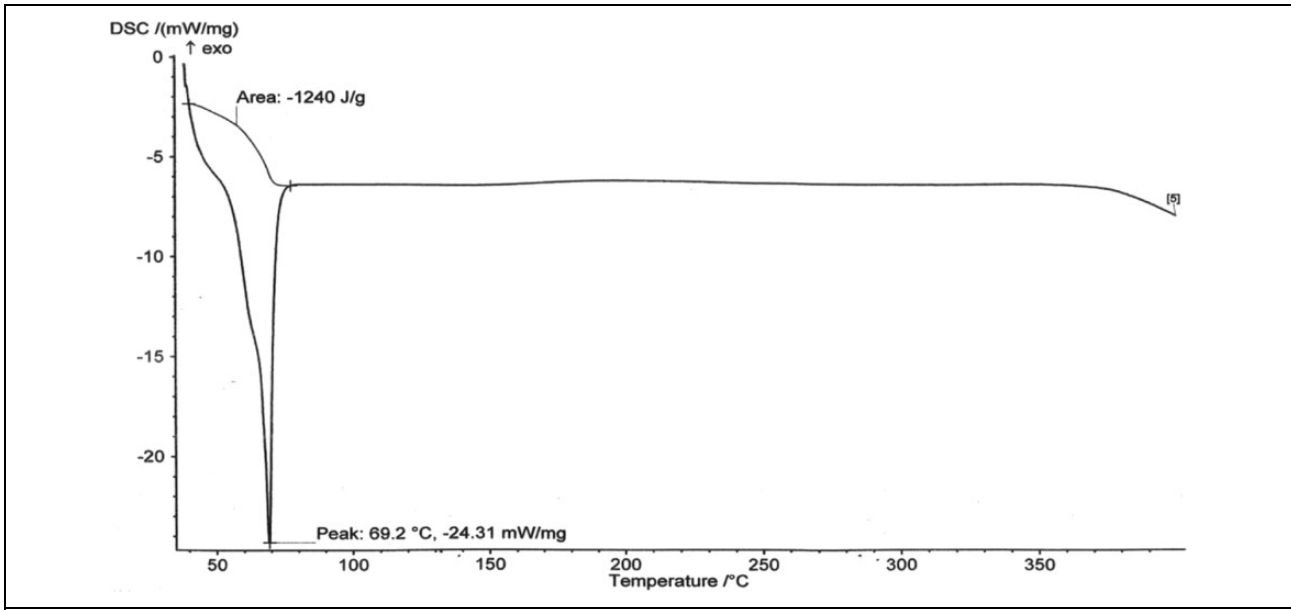
Findings from the screening for secondary metabolites were in agreement with earlier reports.<sup>16,17</sup> The presence or abundance of secondary metabolites has been attributed to the nature of soil where the plant grows and the prevailing microclimate conditions.<sup>25</sup> This result indicates that the medicinal values of the plant could be due to the presence of one or more of these metabolites. This could be verified from fractionation, isolation and identification or characterization of compounds present in the crude extract, formulating using an appropriate drug delivery system and studying its pharmacotherapeutic potentials.

The result of the acute toxicity study indicates that the extract has a very high safety margin on administration and

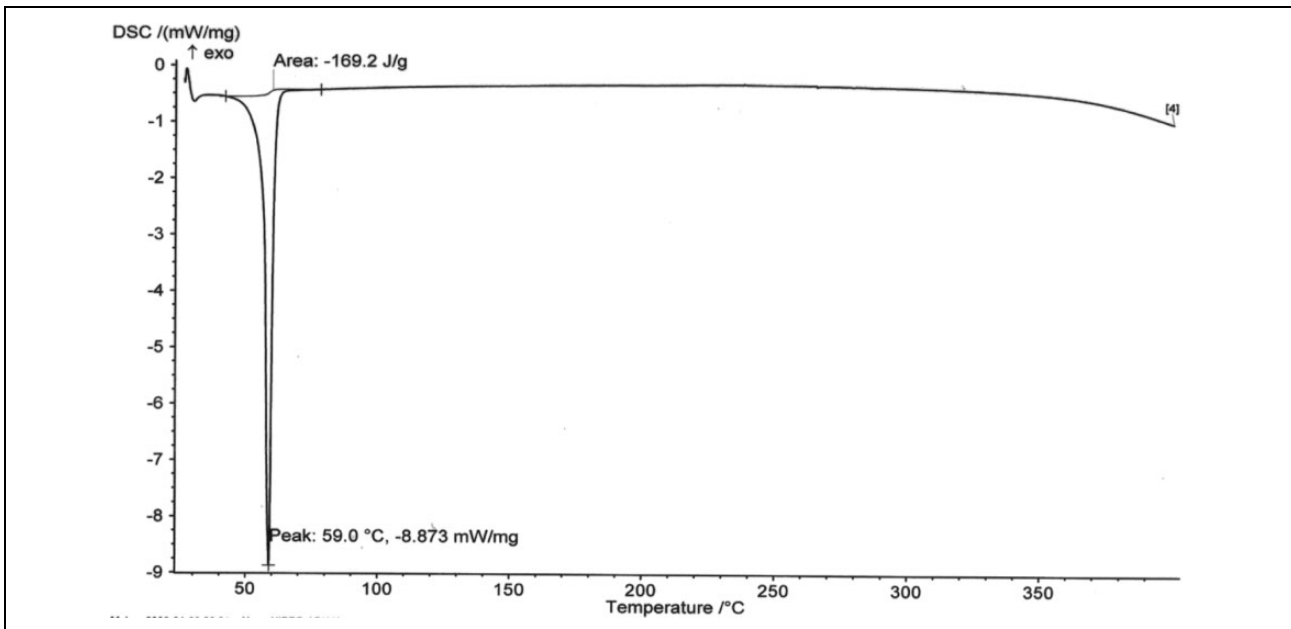
remained non-toxic to the rats. It was also observed that the animals remained healthy as there were no unusual clinical changes in locomotor activity or behaviour, and no ataxia and no signs of intoxication were observed. This indicates that all the animals tolerated the extract. Furthermore, there was no significant ( $p > 0.05$ ) difference in the rate of food and water consumption between the study and control groups of animals, and this might be responsible for the insignificant ( $p > 0.05$ ) changes in the body weight of the rats. The implication of these indicators is that the in vivo administration of any formulation of *N. laevis* may not be harmful to the biological system.

From the table of percentage recovery of SLMs, it could be seen that very good amounts of SLMs were recovered for all the batches. This indicates that hot melt homogenization method is reliable in the formulation of SLMs.<sup>21,26</sup> Within the loaded batches, there is an increase in SLMs recovery with the increase in NLE loading, for example, SLMs recovered increased from 86.3% (X1) to 88.0% (X2) and then to 89.2% (X3). This means that the amorphous lipid matrices created imperfections on melting leading to increased drug entrapment. Across batches, it could be observed that SLMs recovery was higher for the 'Y' batches formulated with 1:2 ratio of the lipid matrix. This means that at the ratio of 1:2 lipid matrix, there were extensive rearrangements in the crystalline structure of the lipids leading to high solubilization of NLE. The high recovery obtained for the unloaded SLMs batches (X4, Y4 and Z4) might be due to poor drying of the formulations, as the recovered particles were somewhat sticky and viscous in appearance. However, none of the batches recovered was up to 100%. This could be due to losses from weighing, transference, filtration and drying.

When particles of SLMs are viewed edge-on using a photomicroscope, the particles as shown on the photomicrographs appear to be two-dimensional, and the spherical shape of the SLMs may not be fully appreciated. However, all the NLE-loaded SLMs appeared spherical, smooth and stable. After lyophilization, some batches of the SLMs were free-flowing, while batches that retained certain level of moisture were viscous with retarded flow. After 24 h post-formulation, varying sizes of particles were obtained, and these variations could be due to factors such as concentrations of lipid matrices and surfactant used. It was observed that the particle size increased with the increase in drug loading. Therefore, drug loading produced significant ( $p < 0.05$ ) increase in average particle sizes of the SLMs, and batches with the highest drug loading produced the largest particle sizes due to increased drug concentration entrapped in the lipid matrix, for example, X1 loaded with 1% w/w NLE produced average particle size of  $42.86 \pm 2.5 \mu\text{m}$ , while X3 loaded with 5% w/w NLE produced particles with average size of  $61.43 \pm 3.7 \mu\text{m}$ . Also, Z1 loaded with 1% w/w NLE produced average particle size of  $54.29 \pm 9.5 \mu\text{m}$ , while Z3 loaded with 5% w/w NLE produced an average particle size of  $77.5 \pm 6.0 \mu\text{m}$ . The



**Figure 7.** DSC thermogram of Precirol<sup>®</sup> ATO 5. DSC: differential scanning calorimeter.

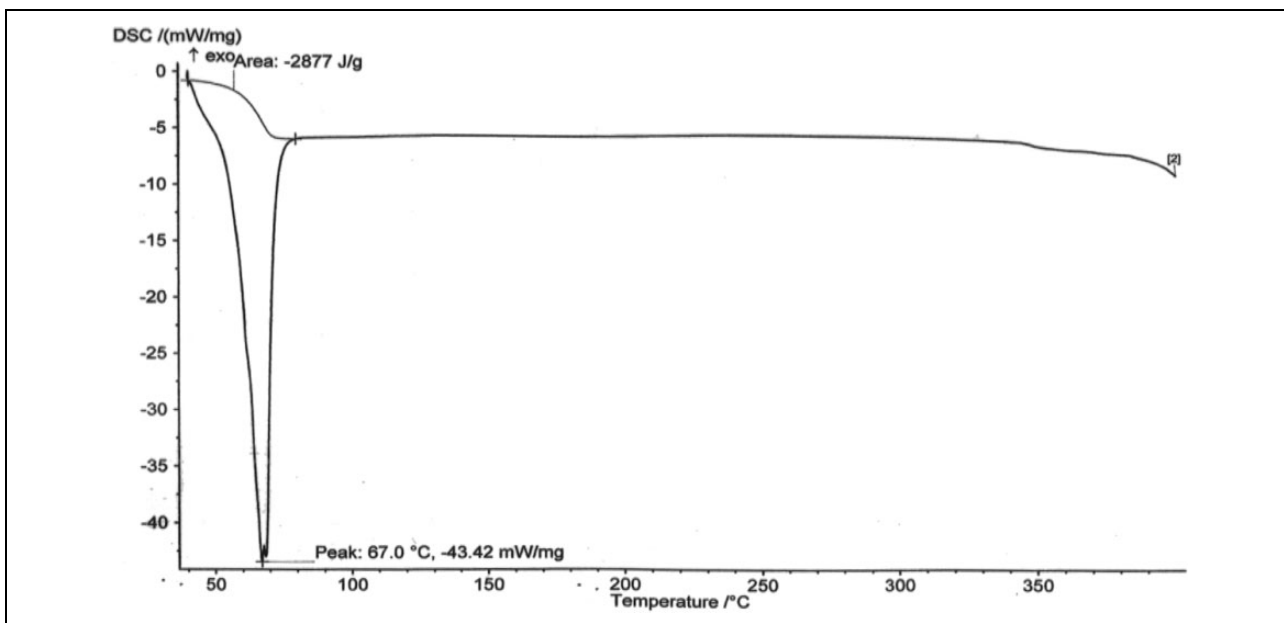


**Figure 8.** DSC thermogram of Softisan<sup>®</sup> I54. DSC: differential scanning calorimeter.

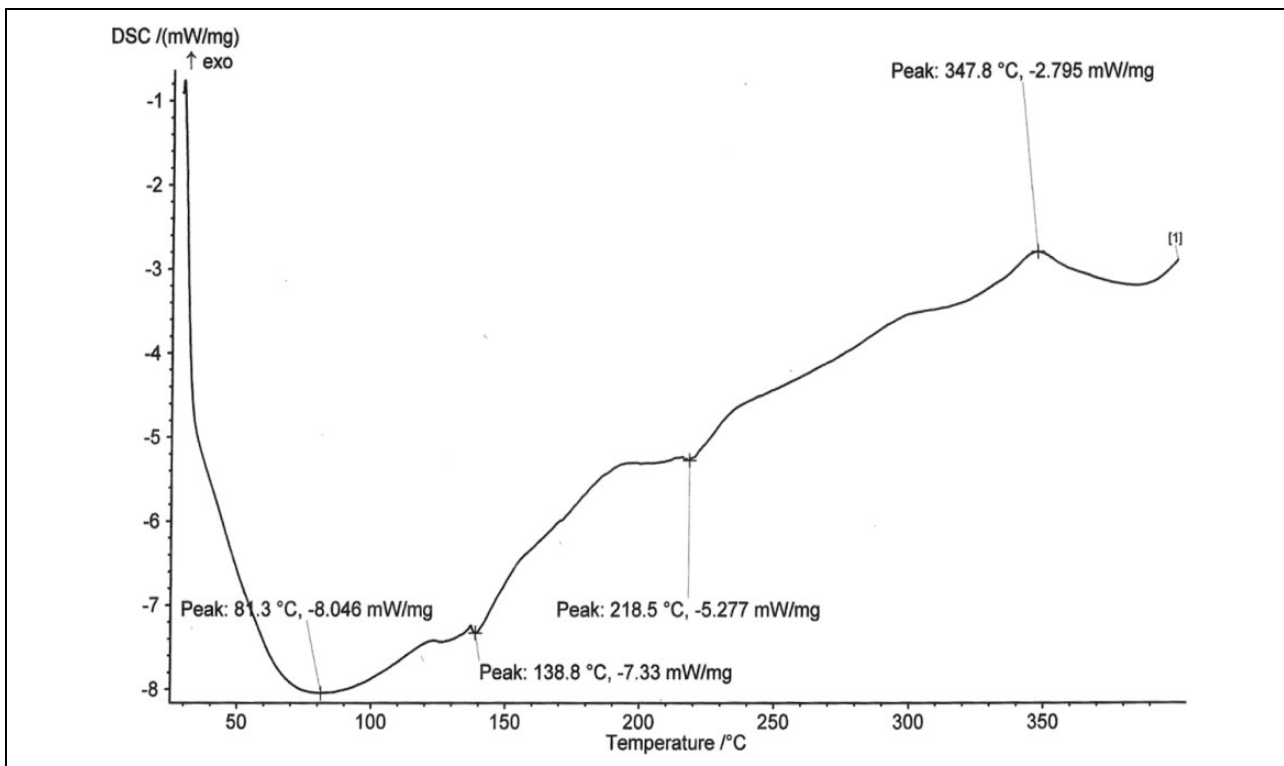
same scenario was observed when the SLMs were viewed after 60 days. However, a comparison of the particle sizes across batches depicted slight ( $p > 0.05$ ) increases in particle sizes after storage. This could be due to particulate aggregation in the formulation due to Ostwald ripening leading to greater particle size. It could also be due to the effect of temperature variations ( $25 \pm 2^\circ\text{C}$ – $27 \pm 2^\circ\text{C}$ ) during storage as SLMs are better stored in cool place.

A very good knowledge of the pH of maximum stability of a drug or its stability profile is important, as the

information will enable a formulator to adapt reliable and acceptable protocols in the formulation of a drug.<sup>21</sup> From the result, the pH values of all the SLMs ranged between  $5.03 \pm 0.4$  and  $6.87 \pm 0.1$ , which implies that the formulation is slightly acidic, hence will be most likely absorbed in acidic environment of the gastrointestinal tract. The acidic pH recorded by the SLMs could be due to the presence of free fatty acids in the lipid matrices. There was no significant ( $p > 0.05$ ) change in pH throughout the duration of study. This shows that the formulations were stable and



**Figure 9.** Representative DSC thermogram of lipid matrix (Softisan<sup>®</sup> 154 and Precirol<sup>®</sup> ATO 5 1:1). DSC: differential scanning calorimeter.

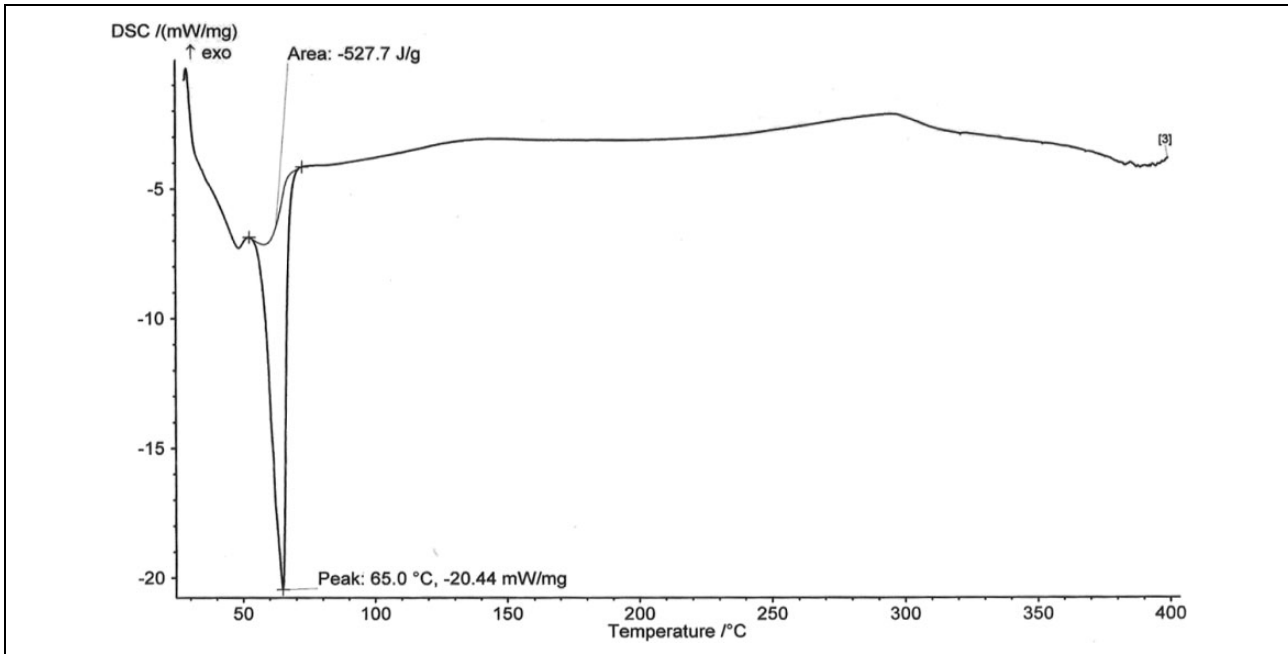


**Figure 10.** DSC thermogram of *Newbouldia laevis* extract. DSC: differential scanning calorimeter.

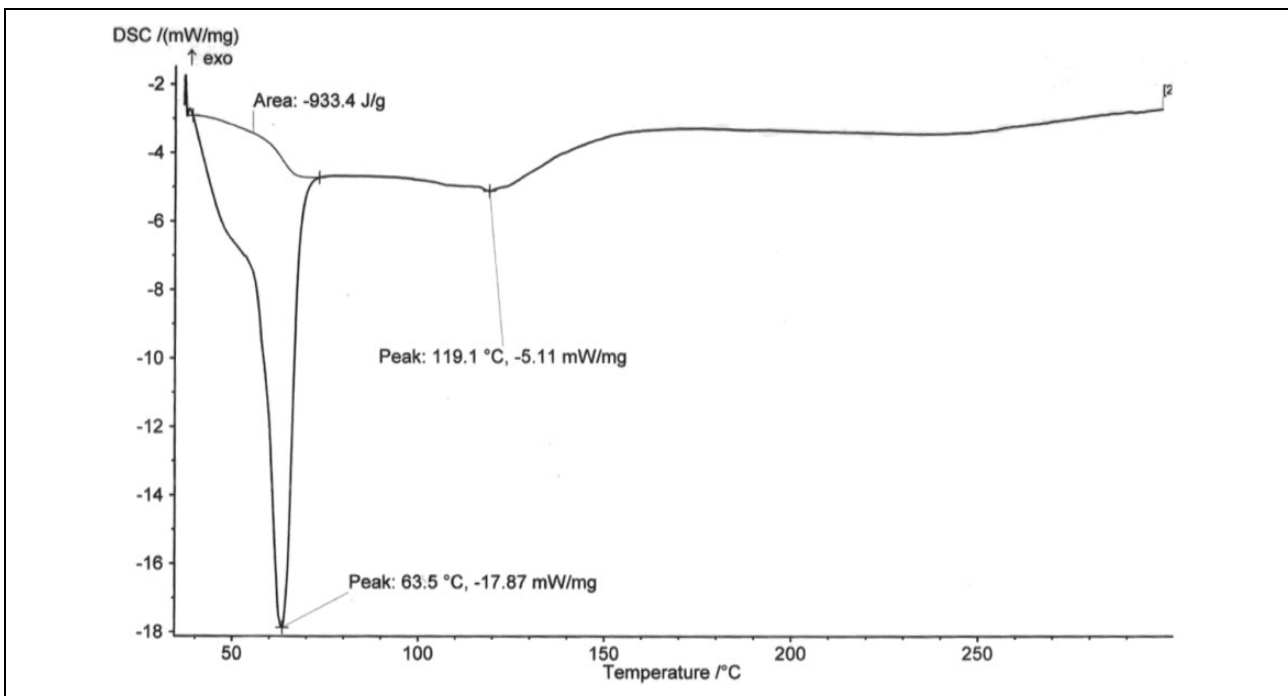
showed no signs of degradation. Furthermore, it was observed that pH decreased with the increase in NLE loading; this implies that NLE could be acidic or caused the release of free fatty acid in the NLE-loaded SLMs, hence the observed reduction in pH of the SLMs. This is because the unloaded SLMs (X4, Y4 and Z4) did not exhibit such

decline in pH as confirmed by their steady-state pH throughout the study period.

The DSC thermogram of Softisan<sup>®</sup> 154 showed an endothermic melting peak of 59°C with enthalpy  $-8.873 \text{ mW mg}^{-1}$ , while the DSC thermogram of Precirol<sup>®</sup> ATO 5 showed an endothermic melting peak of 69.2°C with



**Figure 11.** DSC thermogram of SLMs loaded with 1% w/w of *Newbouldia laevis* extract based on 1:1 lipid matrix. DSC: differential scanning calorimeter; SLMs: solid lipid microdispersions.

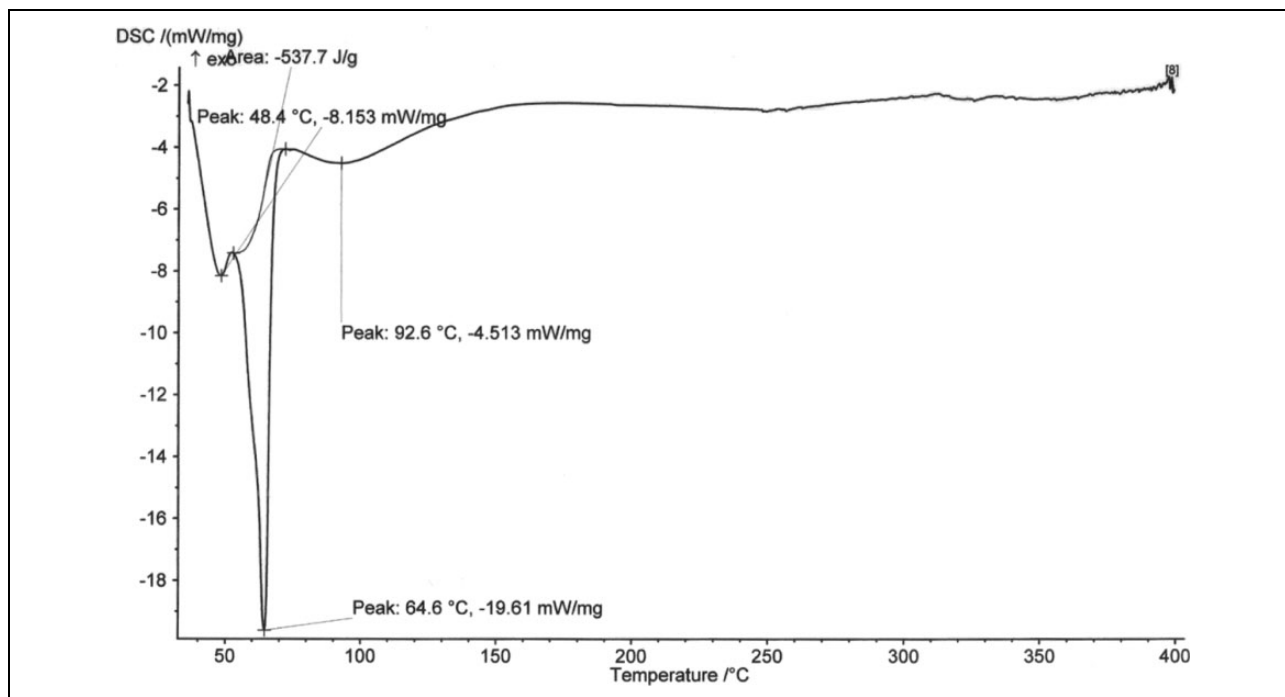


**Figure 12.** DSC thermogram of SLMs loaded with 5% w/w of *Newbouldia laevis* extract based on 1:1 lipid matrix. DSC: differential scanning calorimeter; SLMs: solid lipid microdispersions.

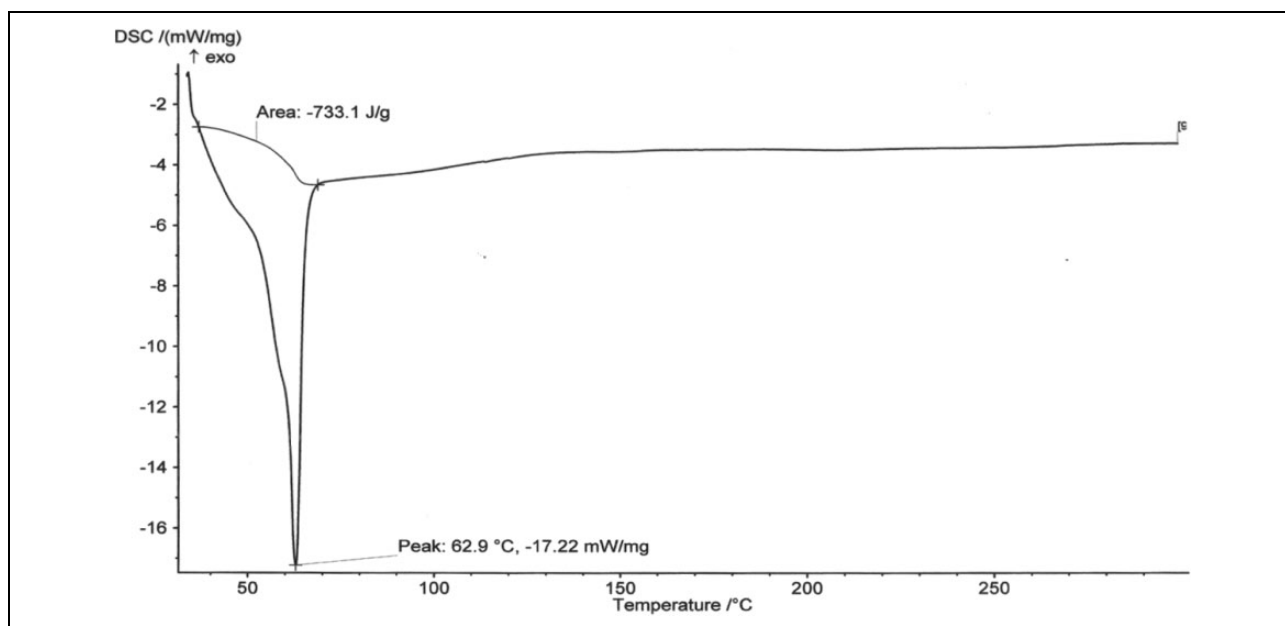
enthalpy  $-24.31 \text{ mW mg}^{-1}$ . These values correspond to the literature values of the melting points of Precirol<sup>®</sup> ATO 5 and Softisan<sup>®</sup> 154 with a slight variation, which could be due to sensitivity of the DSC instrument. When these two lipids were blended, the resulting PreciSoft lipid matrix produced the following endothermic melting peaks and enthalpies:  $67^\circ\text{C}$  and

$-43.42 \text{ mW mg}^{-1}$ ,  $66.7^\circ\text{C}$  and  $-61.08 \text{ mW mg}^{-1}$  and  $64.7^\circ\text{C}$  and  $-39.87 \text{ mW mg}^{-1}$  (at 1:1, 1:2 and 2:1 Softisan<sup>®</sup> 154 and Precirol<sup>®</sup> ATO 5), respectively. The single endothermic melting peaks and low enthalpies produced indicate reduced crystallinity and rearrangement for drug localization due to carbon chain adjustments from the interaction of saturated even-





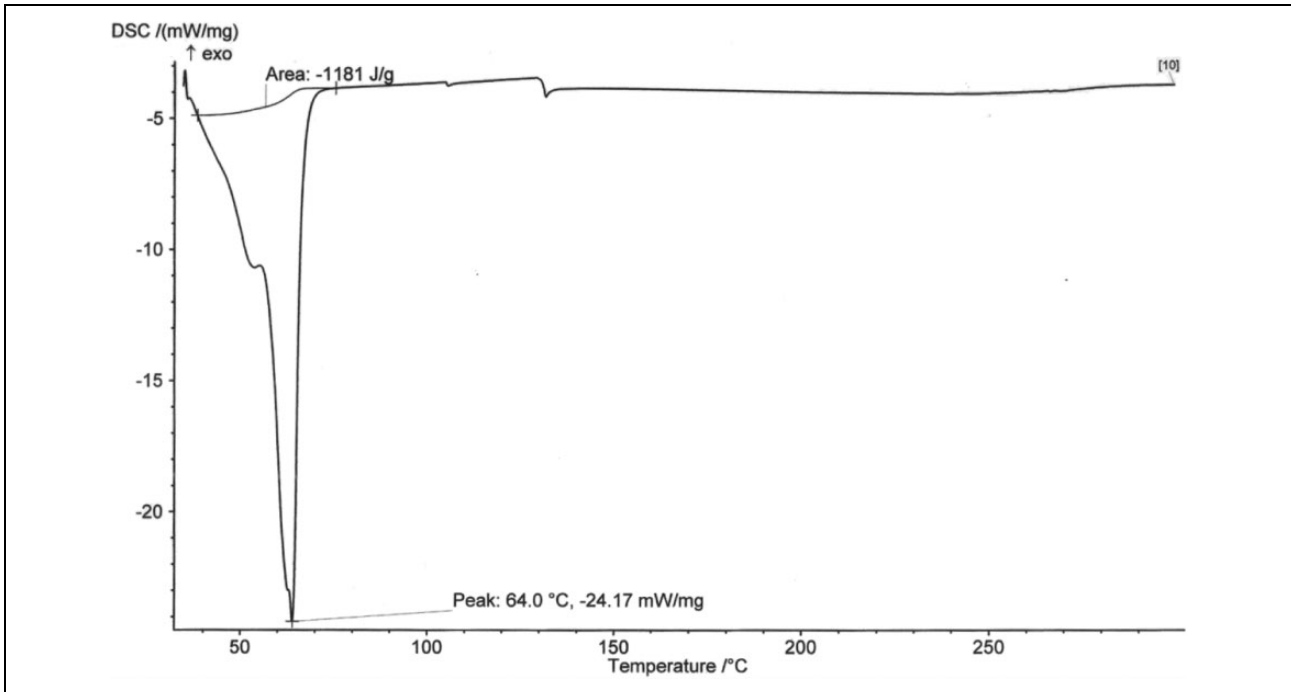
**Figure 13.** DSC thermogram of SLMs loaded with 1% w/w *Newbouldia laevis* based on 1:2 lipid matrix. DSC: differential scanning calorimeter; SLMs: solid lipid microdispersions.



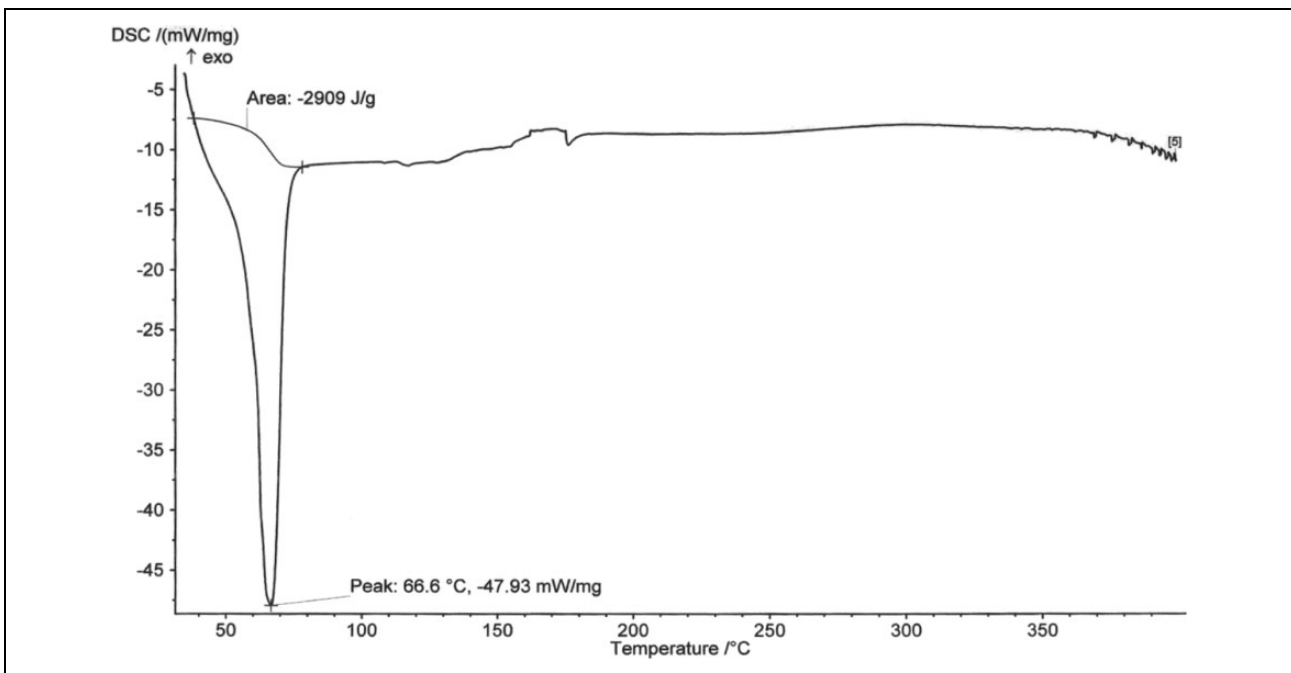
**Figure 14.** DSC thermogram of SLMs loaded with 5% w/w *Newbouldia laevis* based on 1:2 lipid matrix. DSC: differential scanning calorimeter; SLMs: solid lipid microdispersions.

numbered, unbranched and natural fatty acids of Softisan<sup>®</sup> 154 and mixture of mono-, di- and triglycerides of palmitic acid and stearic acid in Precirol<sup>®</sup> ATO 5. This presents an important alternative to lipid modification using chemical methods because the latter lead to the production of lipids with decreased in vivo compatibility. DSC thermogram of crude

NLE produced three endothermic peaks at 81.3°C, 218.5°C and 138.8°C with corresponding enthalpies of  $-8.046$ ,  $-5.277$  and  $-7.33$  mW mg<sup>-1</sup>. It also showed an exothermic melting peak at 347.8°C with enthalpy of  $-2.795$  mW mg<sup>-1</sup>. When NLE was loaded into the lipid matrices to formulate SLMs (Figures 11 to 16), it produced microparticles with low melting



**Figure 15.** DSC thermogram of SLMs loaded with 1% w/w *Newbouldia laevis* based on 2:1 lipid matrix. DSC: differential scanning calorimeter; SLMs: solid lipid microdispersions.



**Figure 16.** DSC thermogram of SLMs loaded with 5% w/w *Newbouldia laevis* based on 2:1 lipid matrix. DSC: differential scanning calorimeter; SLMs: solid lipid microdispersions.

peaks and enthalpies, for example, X1, Y1 and Z1 from 1:1, 1:2 and 2:1 lipid matrices produced the following melting peaks: 65, 64.6 and 64°C with the corresponding enthalpies: -20.44, -19.61 and -24.17 mW mg<sup>-1</sup>. This signifies high drug loading, despite the crude character of NLE. The implication of this finding is that there might be the possibility of a high

concentration of NLE made bioavailable in the biological system following administration, and this will be useful in order to initiate any meaningful pharmacotherapeutic event. However, an in vivo release study would clearly prove this.

EE is an essential characteristic of SLMs, which indicates their propensity to entrap and accommodate active

**Table 7.** Analyses of encapsulation efficiency and drug loading capacity.

Batch	Theoretical drug content (g)	Actual drug content (g)	Encapsulation efficiency (%)	Drug loading capacity (g API/100 g lipid)
X1	1.0	0.8	77.2	16.0
X2	3.0	0.9	29.6	18.0
X3	5.0	1.0	20.7	20.0
Y1	1.0	0.8	81.0	16.0
Y2	3.0	1.3	43.8	26.0
Y3	5.0	1.4	27.5	28.0
Z1	1.0	0.9	88.1	18.0
Z2	3.0	1.5	48.5	30.0
Z3	5.0	1.7	34.6	34.0

API: Active Pharmaceutical Ingredient.

**Table 8.** Analgesic/anti-nociceptive effect.

Group	Dose (mg)	Basal values (min)	30 min	60 min	90 min	120 min
X1	250	4.0 ± 0.5	5.0 ± 0.2	5.1 ± 0.2	6.0 ± 0.3	6.1 ± 1.2
X2	250	4.0 ± 0.9	4.3 ± 0.6	10.0 ± 1.2	9.6 ± 0.1	10.1 ± 0.6
X3	250	4.0 ± 1.2	5.2 ± 0.1	10.1 ± 0.5	8.5 ± 1.0	10.0 ± 0.2
Y1	250	5.1 ± 1.1	5.2 ± 1.3	6.0 ± 0.1	7.0 ± 0.3	8.2 ± 1.9
Y2	250	4.1 ± 0.3	4.6 ± 1.0	5.3 ± 0.9	6.0 ± 1.0	10.3 ± 0.5
Y3	250	3.0 ± 0.1	4.1 ± 0.3	5.1 ± 0.5	5.7 ± 0.7	6.1 ± 0.3
Z1	250	2.9 ± 0.2	3.2 ± 0.1	5.7 ± 0.5	5.7 ± 0.1	5.7 ± 0.1
Z2	250	4.0 ± 0.4	4.1 ± 0.3	4.9 ± 0.2	5.2 ± 0.8	7.0 ± 1.2
Z3	250	3.0 ± 0.1	4.0 ± 0.2	5.0 ± 1.1	4.0 ± 0.8	5.0 ± 1.4
Diclofenac potassium	10 mg kg <sup>-1</sup>	2.0 ± 0.7	4.9 ± 0.2	5.7 ± 1.0	12.0 ± 0.1	11.0 ± 0.3
Normal saline	10 mL kg <sup>-1</sup>	3.1 ± 0.1	3.2 ± 0.4	2.8 ± 1.0	3.4 ± 0.7	3.1 ± 0.2

drugs. From the table, the actual drug content increases with the increase in drug loading for all the batches. The order of increase across the batches is X < Y < Z. Again, across batches, the EE % decreased with the increase in drug loading giving maximum EE % for lipid matrices 1:1, 1:2 and 2:1 as 77.2%, 81.0% and 88.1%, respectively. This can be explained from the fact that each of the lipid matrix used for the formulation of the SLMs has reached its maximum level of drug solubilization and entrapment, for example, in batches X1, X2 and X3 formulated using 1:1 lipid matrix, increasing drug loading from 1% w/w to 3% w/w led to a decrease in EE % from 77.2% to 29.6%. This was also observed in SLMs formulated using lipid matrices 1:2 and 2:1. Furthermore, batches Z1, Z2 and Z3 formulated with lipid matrix 2:1 produced the highest EE % compared with the 'X' and 'Y' batches formulated with 1:1 and 1:2 lipid matrices. The high encapsulation efficiency in 'Z' batches is a clear indication that they produced the highest imperfections and disorderliness, which entrapped the highest amounts of NLE in comparison to batches X and Y.<sup>27</sup> This implies that *N. laevis* would be readily available systemically for the control of inflammation upon administration, and this would be higher for batch 'Z' formulations than batches 'X' and 'Y'. On the other hand, DLC shows the amount of encapsulated drug relative to the amount of lipids used in the formulation. From Table 6, DLC increased with the increase in drug loading. The highest DLC was obtained at 5% w/w of NLE

for all the batches, giving the maximum DLCs as 20, 28 and 34 g of NLE per 100 g of lipid for 1:1, 1:2 and 2:1 lipid matrices. This result shows that the 'Z' batches produced the highest DLC, and this is a confirmation of the highest EE % obtained with the 'Z' batches.

From the result of the analgesic/anti-nociceptive study of the SLMs, it could be seen that all the batches of SLMs have good anti-nociceptive effect comparable with the standard drug, diclofenac potassium. The relative values of the anti-nociceptive action of the formulations when compared to the basal value indicate an increased delayed sensation to pain of heat due to an increase in duration of response of the mice to stimulus (heat). However, this relativity lasted for 30 min; but from 60 min, there was enhanced delay in sensation to pain of heat, which lasted for another 60 min. This may be due to increased absorption of the *N. laevis* in vivo in the presence of the lipid carrier. This is because earlier reports have shown that drugs incorporated in lipid matrices have better absorption than unencapsulated drugs.<sup>18</sup> The pain reaction time increased with time as a result of delay in reaching pain threshold in the test animals. The anti-nociceptive action of the formulations was higher for the 'X' batches followed by the 'Y' batches, while the 'Z' batches produced the least pain reaction time. The high anti-nociceptive effect produced by the 'X' batches could mean that NLE was released in substantial amount from the 1:1 lipid matrix into the study mice producing a high pain threshold. The

delayed pain threshold was similarly produced and sustained throughout the duration of the study across all the batches. The observed effect could be due to the ability of the SLMs encapsulating the drug to permeate into the systemic circulation of the rats to produce a soothing effect caused by pain. The lipophilic surface of the SLMs might have influenced the release of the drug through the narrow pores of the lipid matrices into the skeletal muscles of the rats, and thus reducing pain. This confirms the ethnomedicinal use of *N. laevis* as an analgesic/anti-nociceptive agent. However, fractionation, isolation and physicochemical characterization of the active principles of the plant would assist in the identification of the component responsible for the analgesic/anti-nociceptive action of the plant. Also, the formulation of the pure phytochemical as a lipid drug delivery system such as SLMs could potentiate its analgesic/anti-nociceptive effect and could stake good clinical claims.

## Conclusions

This study has shown that the application of SLMs could be extended to the delivery of phytomedicines and other natural products with high EE and loading. A careful blend of saturated even-numbered, unbranched, natural fatty acids, for example, Softisan<sup>®</sup> 154, and mixture of mono-, di- and triglycerides, for example, Precirol<sup>®</sup> ATO 5, produces imperfect lipid matrix for active drug delivery and drug localization. NLE-loaded SLMs exhibited good analgesic/anti-nociceptive effect comparable with diclofenac potassium. This should engineer the development of phytomedicines into dosage forms using novel drug delivery technologies like SLMs. Further research into *N. laevis* is highly advocated because of the need to isolate, characterize and formulate pure phytochemicals from *N. laevis* for the management of analgesia.

## Acknowledgement

The authors thank BASF SE, Ludwigshafen, Germany, for the kind gift of Softisan<sup>®</sup> 154 and Kolliphor<sup>®</sup> 188. The authors also appreciate Gattefossé, Saint-Priest Cedex, France, for providing Precirol<sup>®</sup> ATO 5 used in this study free of charge.

## Authors' note

The authors alone are responsible for the content and writing of the article.

## Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

## Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article.

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