African Journal of Pharmaceutical Research and Development
Available online at https://ajopred.com
Vol. 16 No.2; pp. 76-87 (2024)

Original Research Article

PREPARATION OF LIPID-BASED FORMULATIONS USING LOCAL BEESWAX SOURCED FROM HONEYCOMBS

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ABSTRACT

Solid lipids used in formulation of novel drug delivery systems in most developing countries are sourced from abroad and are quite expensive. Wax from honeycombs is readily available, and is even disposed as waste, but can be utilized as a solid lipid for the formulation of different lipid-based drug delivery systems. This study aims to formulate and compare the features of the solid lipid nanoparticle (SLNs) and nanostructured lipid carrier (NLCs) delivery systems of quinine hydrochloride made with local beeswax (that is beeswax from locally sources honeycomb waste) and other solid lipids sourced from abroad. Local beeswax was first purified by hot bath method. Solidified reverse micellar solutions (SRMS) were prepared by fusion method using Phospholipon®90H and either local beeswax, Softisan®154, foreign beeswax, or stearic acid, separately. SLNs and NLCs of quinine made with SRMS of each of these solid lipids were formulated using hot homogenization method. These SLNs and NLCs were characterized. The average particle size of SLNs and NLCs made with local beeswax was 93.60 ± 4.44 nm and 112.80 ± 3.89 nm, respectively, and was not significantly ($p > 0.05$) different from the sizes of formulations made from other solid lipids (SLNs: 96.64 to 118.9 ± 4.13 nm and NLCs: 99.68 ± 6.75 to 117.30 ± 4.60 nm). The particles were monodispersed having PDI ranging from 0.281±0.054 to 0.308±0.055. Encapsulation efficiency and loading capacity were generally poor, especially among the SLN formulations. However, SLNs made with local beeswax and stearic acid were able to encapsulate quinine, whereas foreign beeswax and Softisan®154 had zero encapsulation. This study reveals that the features of the SLNs and NLCs made with local beeswax where highly comparable with those formulated with the imported solid lipids. Therefore, local beeswax can serve as a suitable affordable and available alternative to other imported solid lipids for formulating different lipid drug delivery systems.

ARTICLE INFO

Received 14 May, 2024
Accepted 11 July, 2024
Published 16 July, 2024

KEYWORDS

Nano-structured lipid carriers,
Local beeswax,
Solid lipids,
Solid lipid nanoparticles,
Solidified reverse micellar solutions

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https://doi.org/10.59493/ajopred/2024.2.8 ISSN: 0794-800X (print); 1596-2431 (online)

INTRODUCTION

Researchers have continued to seek ways to enhance conventional formulations, either by improving efficacy or safety, in order to better patients' compliance, hence, the current focus on novel drug delivery systems. Lipid-based drug delivery systems have received much attention, mainly due to their property of being biocompatible and biodegradable, since most lipids are obtained from natural sources unlike polymers. Here in our faculty (Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka), most of the researches that are based on formulating lipid drug delivery systems rely on solid lipids imported from outside the country. This is also the case in most developing countries of the world. More than 70 % of the researchers in our institution (University of Nigeria, Nsukka) use imported/ foreign lipids such as Softisan®154 [1], beeswax [2,3], stearic acid [4,5], Compritols® [4,5] etc. Most times, these lipids are obtained as samples, and in small quantities from manufacturers abroad. Hence, the inventiveness of the researcher is restricted, since such study must be designed based on the quantity of lipid, gifted. When more is required, then, it must be bought. This may not be economical for both the researcher, and for the cost of the final product. This has led to focus on locally available and sourced solid lipids. Previous researchers have used locally sourced solid lipids such as *Capra aegagrus hircus* (goat) fat, dika wax, Tallow fat etc. in formulation of different drug delivery systems [6,11] . However, the use of honeycomb for the preparation of drug delivery systems has not been tried.

Beeswax, is a natural fat obtained from the honeycomb of the insect, *Apis mellifera*. It contains mainly esters of higher fatty acids (C_{16} to C_{36}) and has been comprehensively studied and applied in many pharmaceutical formulations by researchers in our institution. The beeswax is also imported from abroad [6], yet, here in Nigeria, it is a waste product obtained after honey extraction. We therefore decided to try utilizing the locally extracted beeswax for formulating drug delivery systems. Hence, in this study, beeswax was obtained locally from honeycomb, purified, and then used for the formulation of NLCs and SLNs containing quinine hydrochloride. Other foreign solid lipids (Softisan®154, foreign Beeswax, and Stearic acid), which are extensively used for the formulation of lipid-based drug delivery systems were also used for the formulation of NLCs and SLNs of quinine hydrochloride. The features of these NLCs and SLNs formulated with foreign solid lipids and local beeswax were compared in order to determine if locally sourced beeswax, can serve as a suitable, affordable and more available alternative to these foreign solid lipids. To the best of our knowledge, this will be the first research where locally sourced beeswax was used for the formulation of lipid drug delivery systems.

MATERIALS AND METHODS

The following materials were used: Quinine hydrochloride (purity ≥ 95%) (Sigma-Aldrich, England, UK), Isopropyl alcohol, Ethanol, Phospholipon®90H (Phospholipid GmbH, Koln, Germany), Beeswax, Polysorbate 80 (Sigma-Aldrich, England, UK), Stearic acid (Sigma-Aldrich, England, UK), Softisan®154 (Schuppen Condea Chemie GmbH, Germany), Medium-chain triglyceride (IOI Oleo GmbH, Witten, Germany), and Distilled water (Department of Physics & Astronomy, University of Nigeria, Nsukka). All other reagents were of analytical grade and used without further purification.

Purification of Beeswax

Beeswax was extracted from honeycombs obtained in Nsukka, Nigeria. The raw large lumps of beeswax were size reduced by cutting into smaller pieces. Impurities and debris contained in the beeswax were removed by boiling it in water half its weight for 30 mins, when the beeswax completely melted. The water was allowed to cool, and the wax separated from the water which now contained the impurities by sieving with a clean muslin. The wax obtained was subjected to the process again for further purification. After cooling at room temperature, the wax was separated from the aqueous phase [12,13]. The beeswax was stored at room temperature and referred to in this study as local beeswax.

Preparation of Solidified Reverse Micellar Solution (SRMS)

The lipid matrices were prepared by fusion method, by melting and mixing Phospholipon® 90H and either local beeswax, foreign beeswax, Softisan®154, or stearic acid, separately. P90H and each of the solid lipids were mixed using a ratio of 1:2, respectively. The excipients were weighed out using an analytical balance (Adventurer®, Ohaus, China) to ensure precise measurements. The lipids were melted individually in an oil bath set at 70^{o}C ± 2 ^{o}C, and P90H was gradually added in each case and stirred [1]. The solidified matrices were carefully stored in plastic containers for future use.

Preparation of Solid Lipid Nanoparticles

The lipid matrix was melted at 70^{o}C. This was then loaded with a pre-determined quantity of the drug according to the specifications in Table 1. The surfactant, Polysorbate®80, was dissolved appropriately in the aqueous phase and heated to the same temperature as the lipid phase. Both phases were thoroughly mixed using a homogenizer (Ultra-Turrax, T18 Basic, IKA Germany) at 15000 rpm for 15 minutes. The emulsion was allowed to cool at 27 ± 1^{o}C. SLNs containing different lipids were formulated using the method above. Various formulations of SLNs containing the drug (loaded) and SLNs without the drug (unloaded) were prepared using the design in Table 1.

Preparation of Nanostructured Lipid Carriers

The lipid matrix was melted at 70^{o}C and the liquid lipid (medium-chain triglyceride) was added to it. This was then loaded with a predetermined quantity of the drug according to the specifications in Table 1. The surfactant, Polysorbate®80,

was dissolved appropriately in the aqueous phase and heated to the same temperature as the lipid phase. Both phases were thoroughly mixed using a homogenizer (Ultra-Turrax, T18 Basic, IKA Germany), at 15000 rpm for 15 minutes. The emulsion was allowed to cool at 27 ± 1°C. NLCs containing different lipids were formulated using the method above. Multiple batches of NLCs containing quinine (loaded) and NLCs without the drug (unloaded) were prepared by applying the design in Table 1.

Particle Size and Particle Size Distribution Analysis

A serial dilution of SLNs and NLCs from each batch was performed by diluting them 100-fold with distilled water. Exactly 100 microliters were taken and analyzed using the dynamic light scattering system (Malvern® Zetasizer ZSP, UK). The average particle sizes and size distribution were obtained. The representative size of each batch was determined by calculating the mean of the particle sizes [4].

Fourier-transform Infrared (FT-IR) Analysis

The FT-IR spectroscopy was performed using a Shimadzu 8400S FTIR Spectrophotometer (Shimadzu, Tokyo, Japan). The spectra were obtained within the wavelength range of 4000–400 cm^{-1}. A dropper was used to place a drop of sample from each batch of SLN and NLC formulations on sodium chloride plates. Another sodium chloride plate was placed on it for a film to be formed. The plates were placed into the sample holder, positioned within the optical pathway. The resulting spectra were recorded [14].

Time-dependent pH Stability Studies

Using a pH meter (pHep® Hana instrument, Italy), the pH of the formulations was determined. This was conducted at various time intervals: immediately after formulation, 3 weeks after formulation, and 6 weeks after formulation, with the samples being stored at room temperature to evaluate the effect of storage on stability. This was replicated for all batches [4].

Determination of Encapsulation Efficiency

The Beer's plot for quinine was determined through evaluation in distilled water at a predetermined wavelength of 300 nm. A 10 mg quantity of a pure sample of quinine was weighed into a beaker containing 50 ml of distilled water. It was dissolved, and the solution was made up to the 100 ml mark and allowed to stand for 5 minutes. The mixture was then filtered through Whatman No. 1 filter paper. Serial dilutions were carried out from the stock solution, and their absorbance was obtained spectrophotometrically (Jenway 6405 spectrophotometer, UK). A 1 ml quantity of each drug-loaded formulation was withdrawn and introduced into the test tube, and a 10-fold serial dilution was carried out on each. The absorbance reading was obtained at a wavelength of 300 nm. The drug concentrations were calculated with reference to the Beer's plot already obtained. The encapsulation efficiency of the SLNs and NLCs was determined employing centrifugation method. The different SLN and NLC preparations (1 ml each) were centrifuged at 14,000 rpm for 5 minutes and the supernatant withdrawn. Appropriate dilution was done and the solutions analysed by UV-spectrophotometer at a wavelength of 300 nm [15]. The absorbance of the supernatant was recorded, and the encapsulation efficiency of each preparation was calculated using Equation (1) below:

$$\text{E.E (\%)} = \frac{\text{Actual Drug Content} - \text{Drug in the supernatant}}{Actual\ Drug\ content} \times 100 \text{----------Equation 1}$$

Determination of the Drug-loading Capacity

Drug loading capacity (DLC) was evaluated using the following formula (Equation 2):

$$\text{DLC} = {}^{W_1}/_{W_2} \times 100 \text{----------Equation 2}$$

where W_1 is the amount of API entrapped in the formulation and W_2 is the weight of the lipid added to the formulation [15].

***In vitro* Drug Release**

In the *in vitro* drug release studies, the formulations were studied in phosphate buffer (pH 7.4), with the temperature and speed of rotation of the medium maintained at 37 ± 2°C and 100 rpm, respectively. A volume of 2 ml from each formulation was measured and transferred into a polycarbonate dialysis membrane (MWCO 6000-8000, Spectrum Labs, Breda, Netherlands) that had been pre-treated by immersion in distilled water for 24 hours before use. The dialysis membrane, which contained either SLNs or NLCs, was tightly fastened using a thread that could withstand high temperatures. Subsequently, it was immersed in a 200 mL medium. At specified time intervals, 5 ml of the dissolving liquid was taken out, and the absorbance was measured at a wavelength of 300 nm using a UV spectrophotometer (model 6405, Jenway, UK). The quantity of drug released at specific time intervals was determined using Beer's plot as a reference. Exactly 5 ml of new medium was introduced following each withdrawal, in order to maintain a consistent volume of the dissolution medium, as well as maintain sink condition [3].

Statistical Analysis

Microsoft Excel and STATA 11 software package were used for all analyses and the results obtained were stated as mean ± standard deviation. One-way ANOVA with duplication was applied for comparisons made between groups, and the significant differences in the mean values were evaluated. Differences were considered statistically significant for $p<0.05$ [16].

RESULTS

Particle Size and Particle Size Distribution

The result of the particle size and particle size distribution of the SLN and NLC formulations is presented in Table 2. This table provides information about different formulations of quinine. The mean particle size (Z-average) of the SLNs had minimum and maximum values of 93.60 ± 4.44 nm and 118.9 ± 4.13 nm, respectively, while that for the NLCs were 99.68 ± 6.75 nm and 117.3 ± 4.60 nm, respectively. Both SLN and NLC formulation had low polydispersity index values (closer to 0) indicating uniform particle size distributions. The PDI of the SLNs ranged from 0.281 ± 0.054 to 0.308 ± 0.055, while the NLC formulations ranged from 0.287 ± 0.078 to 0.305 ± 0.065. There was no significant ($p < 0.05$) difference between the average particle sizes and PDI of locally sourced beeswax and other lipids.

Fourier-transform Infrared (FT-IR) Analysis

The results of the FT-IR analysis are displayed in Table 3. The FT-IR spectra analysis of the lipid matrices and quinine revealed no significant interaction between the lipids used and the drug, implying that the lipids were compatible with quinine, and no new compound was formed. The prominent peaks identified in the FT-IR spectra of the formulation components were also present in the FT-IR spectra of the SLNs and NLCs. Nevertheless, a number of negligible peaks were not observed in the lipid matrix spectra, possibly due to overlapping caused by hydrogen bonding.

The FT-IR spectrum of Softisan®154 showed characteristic peaks at 2915.6 cm^{-1}, 2849.5 cm^{-1}, 2956.3 cm^{-1}, 1737.4 cm^{-1}, and 1172.9 cm^{-1} representing O-H bond stretching (carboxylic acid), C-H bond stretching, O-H bond stretching (alcohol), C=O bond stretching and C-O bond stretching respectively. That for Phospholipon® 90H showed peaks at 3371.5 cm^{-1}, 2956.8 cm^{-1}, 1195.0 cm^{-1}, 2917.0 cm^{-1}, 2850.0 cm^{-1}, 1091.9 cm^{-1}, 1728.5 cm^{-1} and 966.5 cm^{-1} representing N-H bond stretching, O-H bond stretching (alcohol), C-O bond stretching, O-H bond stretching (carboxylic acid), C-H bond stretching, P-O bond stretching, C=O bond stretching and C=C bond bending respectively. The spectrum for the Foreign Beeswax had peaks at 3321.1 cm^{-1}, 3160.8 cm^{-1}, 1851.2 cm^{-1}1431.3 cm^{-1} and 1107.0 cm^{-1} representing O-H bond stretching (alcoholic), O-H bond stretching (carboxylic acid), C-H bond bending, O-H bond bending (carboxylic acid) and C-O bond stretching respectively. That for Local Beeswax had similar peaks with the foreign beeswax at 2847.7 cm^{-1}, 2809.0 cm^{-1}, 1283.6 cm^{-1}, 1699.7 cm^{-1}, and 1318.8 cm^{-1}, representing O-H bond stretching (carboxylic acid), C-H bond stretching, C-O bond stretching, C=O bond stretching and O-H bond bending (carboxylic acid). That for Stearic Acid had peaks at 3373.2 cm^{-1}, 2847.7 cm^{-1}, 1699.7 cm^{-1}, 1080.9 cm^{-1}, and 760.4 cm^{-1}, representing O-H bond stretching (alcoholic), O-H bond stretching (carboxylic acid), C=O bond stretching, C-O bond stretching and C-H bond bending. The pure quinine spectrum had characteristic peaks at 3300.2 cm^{-1}, 2927.9 cm^{-1}, 1852.2 cm^{-1}, 1618.4 cm^{-1}, 1238.9 cm^{-1} representing N-H bond stretching, O-H bond stretching (alcoholic), C-H bond bending, C=C bond stretching and C-N bond stretching respectively.

Time-dependent pH Stability Studies

Since pH can significantly impact the stability and integrity of formulations, this study was carried out to identify the pH ranges where the formulations remained stable and to determine if the formulations maintained their stability and effectiveness over time. The results of the pH study of the SLNs and NLCs of locally sourced beeswax and that of other lipids are presented in Figure 1 (a to d). The formulations had nearly similar pH ranges after 3 and 6 weeks. The pH of the NLCs were within the range of 4.6 to 5.3 (after 3 weeks) and 4.1 to 5.3 (after 6 weeks) and that of the SLNs were 4.7 to 5.3 (after 3 weeks) and 4.3 to 5.2 (after 6 weeks). However, after 11 weeks, the SLNs had higher pH range of 4.6 to 5.9, compared to that of the NLCs, 4.5 to 5.5.

Drug Content Analysis

In order to ensure consistency and efficacy, this test was carried out and the results presented in Figure 2. All SLN and NLC formulations had a consistent drug concentration of 15.56 µg/ml, except formulations containing Softisan® 154 which had drug concentration of 13.33 µg/ml. These were close to the theoretical drug content, which was 15.5 µg/ml. There was a loss of about 2 µg/ml of drug in the Softisan® 154 formulations.

Encapsulation Efficiency and Drug-loading Capacity

Table 4 illustrates the encapsulation efficiency and loading capacity values as percentages for each batch of SLNs and NLCs. Overall, all batches of drug-loaded SLMs exhibited consistent encapsulation efficiencies. The NLC formulation with Softisan (Q6) exhibited a notably elevated encapsulation efficiency of 52.82 %, while the NLC that included the foreign beeswax (Q4) showed the lowest encapsulation effectiveness, measuring at 37.93%.

In vitro Drug Release

Figure 3(a and b) displays the outcomes of the quinine's release in phosphate buffer during *in vitro* testing. After 8 hours, the stearic acid SLN and NLC exhibited the lowest and slowest drug release percentages, measuring 41.05 % and 40.37 % respectively. Q3 and Q1 made with S154, and local beeswax exhibited the highest *in vitro* drug release of 100 % and 98 % respectively.

DISCUSSION

The Z-average is a metric that quantifies the average size of particles in a sample, taking into account their size distribution weighted by their intensity. The result showed that the quinine-loaded NLC formulations had higher mean particle sizes (99.68±6.75 to 117.30±4.60 nm) while that of the SLNs was (93.60±4.44 to 118.9±4.13 nm). Among the SLNs, the

Table 1: Content of SLN and NLC Formulations of the loaded and unloaded batches

Batches	Drug-delivery system	Quinine (% w/w)	SRMS (% w/w)	Liquid lipid (% v/v)	Polysorbate® 80 (% w/w)	Distilled water (ml)
Unloaded	SLN	-	5	-	2	50
Loaded	SLN	1.5	5	-	2	50
Unloaded	NLC	-	3	2	2	50
Loaded	NLC	1.5	3	2	2	50

Key: *SLN: Solid Lipid Nanoparticles; NLC: Nanostructured Lipid Carriers; SRMS: Solidified reverse micellar solutions*

Table 2: Particle size and size distribution of SLN and NLC drug formulations

Formulations	Batches	Z- average (d nm)	Polydispersity index
SLN	Q1	93.60 ± 4.44	0.281 ± 0.054
	Q3	96.64 ± 7.16	0.284 ± 0.064
	Q5	118.90 ± 4.13	0.308 ± 0.055
	Q7	96.64 ± 7.36	0.284 ± 0.069
NLC	Q2	112.80 ± 3.89	0.302 ± 0.065
	Q4	99.68 ± 6.75	0.287 ± 0.078
	Q6	115.90 ± 4.01	0.305 ± 0.065
	Q8	117.30 ± 4.60	0.303 ± 0.071

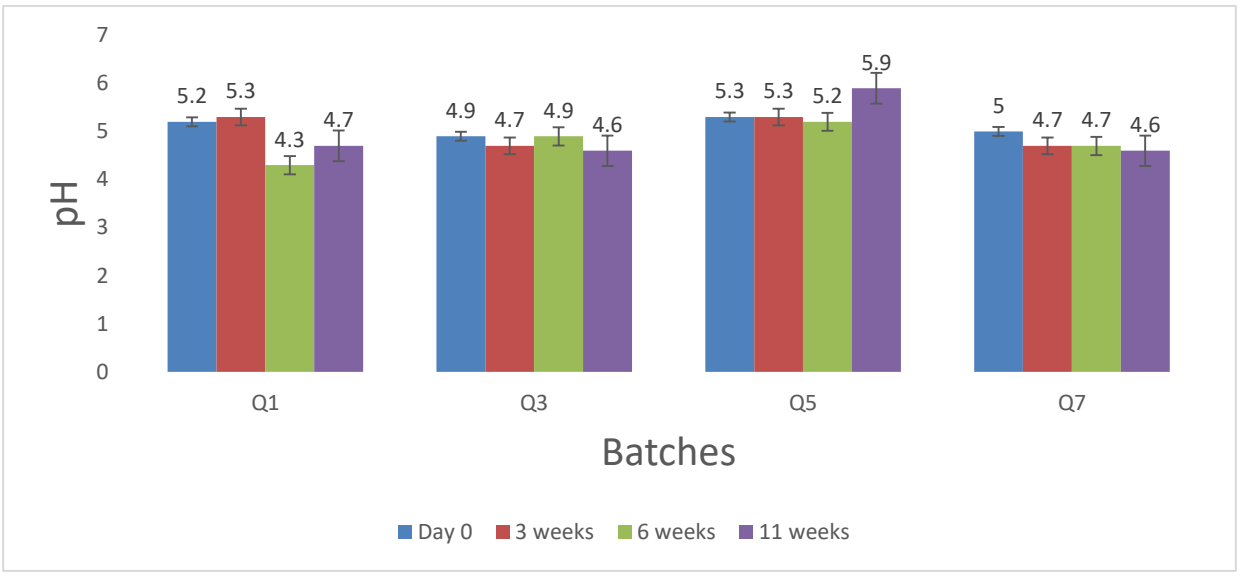
Key: *SLN: Solid Lipid Nanoparticles; NLC: Nanostructured Lipid Carriers; Q1: Local beeswax with quinine SLN; Q2: Local beeswax with quinine NLC; Q3: Foreign beeswax with quinine SLN; Q4: Foreign beeswax with quinine NLC; Q5: Softisan with quinine SLN; Q6: Softisan with quinine NLC; Q7: Stearic acid with quinine SLN; Q8: Stearic acid with quinine NLC*

Table 3: FT-IR profiles of major formulation excipients and the drug

Materials	Principal peak (cm^{-1})	Type of bond
Softisan® 154	2915.6	O-H bond stretching (Carboxylic acid)
	2849.5	C-H bond stretching
	2956.3	O-H bond stretching (Alcohol)
	1737.4	C=O bond stretching
	1172.9	C-O bond stretching
Phospholipon® 90H	3371.5	N-H bond stretching
	2956.8	O-H bond stretching (Alcohol)
	1195.0	C-O bond stretching
	2917.0	O-H bond stretching (Carboxylic acid)
	2850.0	C-H bond stretching
	1091.9	P-O bond stretching
	1728.5	C=O bond stretching
	966.5	C=C bond bending
Foreign Beeswax	3321.1	O-H bond stretching (Alcohol)
	3160.8	O-H bond stretching (Carboxylic acid)
	1851.2	C-H bond bending
	1431.3	O-H bond bending (Carboxylic acid)
	1107.0	C-O bond stretching
Local Beeswax	2847.7	O-H bond stretching (Carboxylic acid)
	2809.0	C-H bond stretching
	1283.6	C-O bond stretching
	1699.7	C=O bond stretching
	1318.8	O-H bond bending (Carboxylic acid)
Stearic Acid	3373.2	O-H bond stretching (Alcohol)
	2847.7	O-H bond stretching (Carboxylic acid)
	1699.7	C=O bond stretching
	1080.9	C-O bond stretching
	760.4	C-H bond bending

Quinine	3300.2	N-H bond stretching
	2927.9	O-H bond stretching (Alcohol)
	1852.2	C-H bond bending
	1618.4	C=C bond stretching
	1238.9	C-N bond stretching

a)



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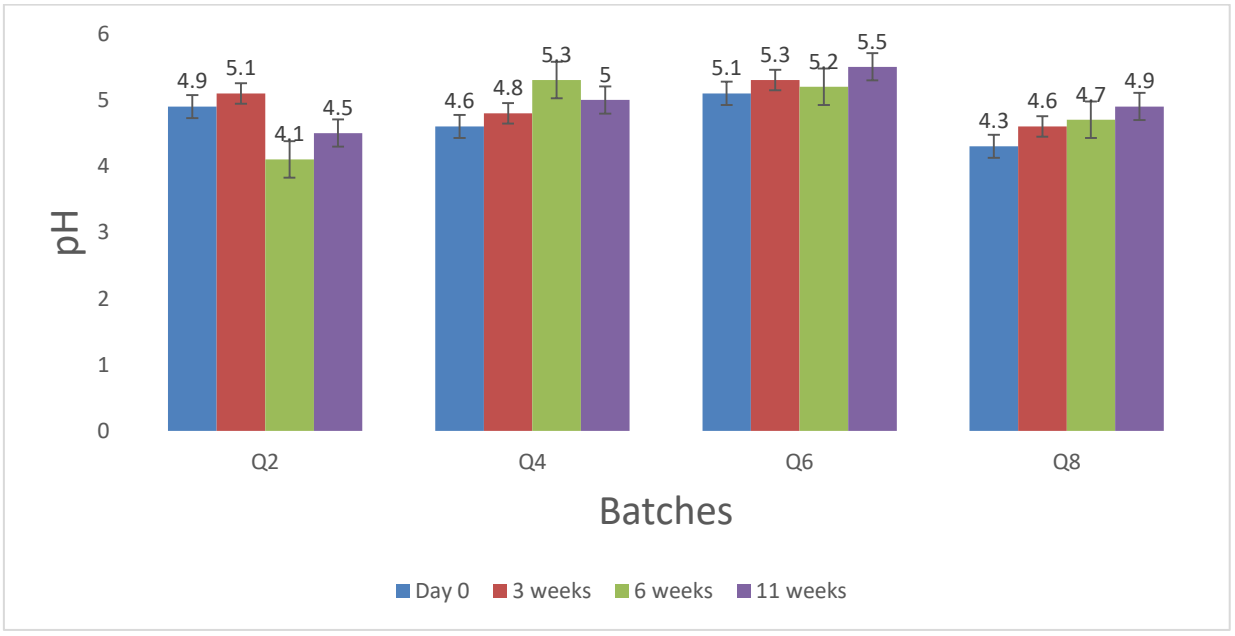

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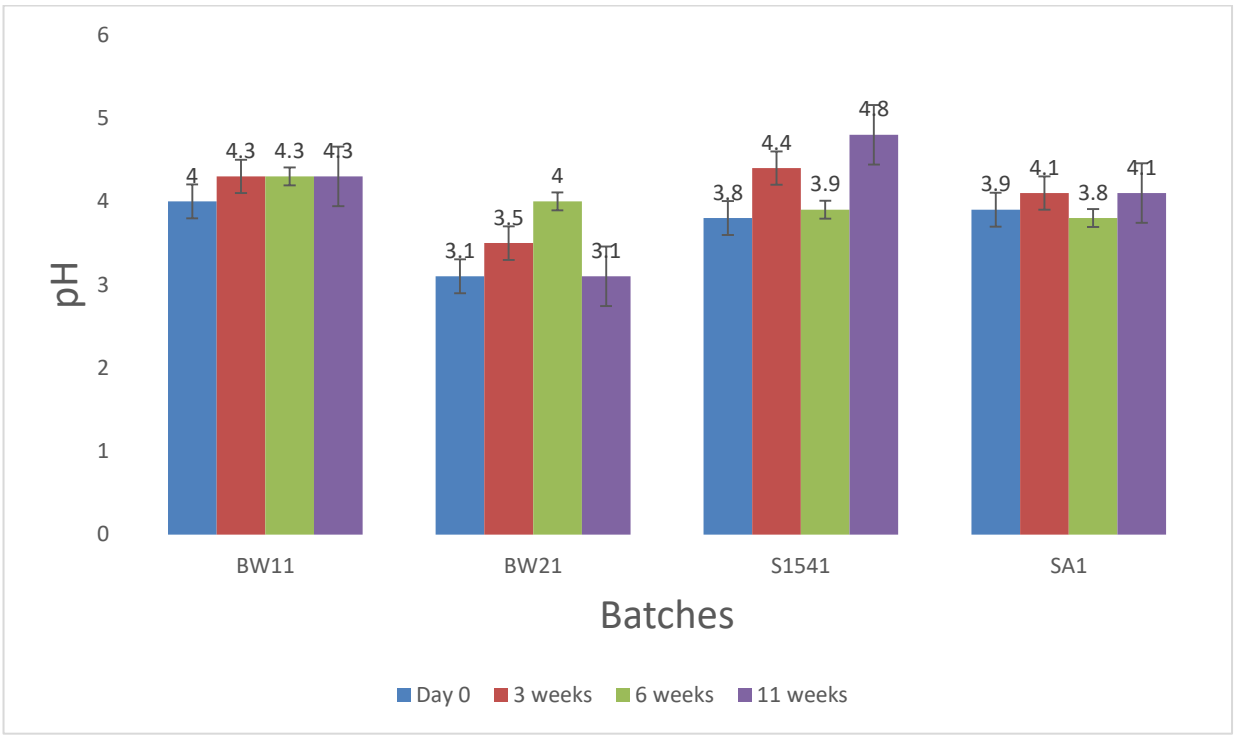

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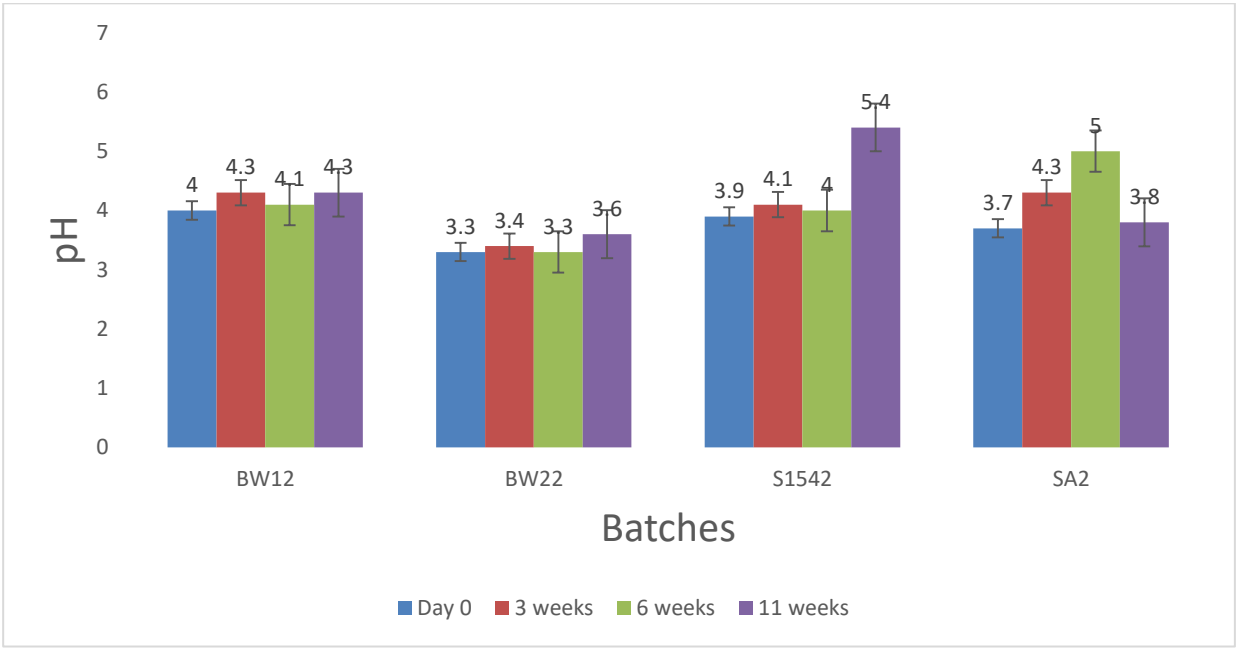
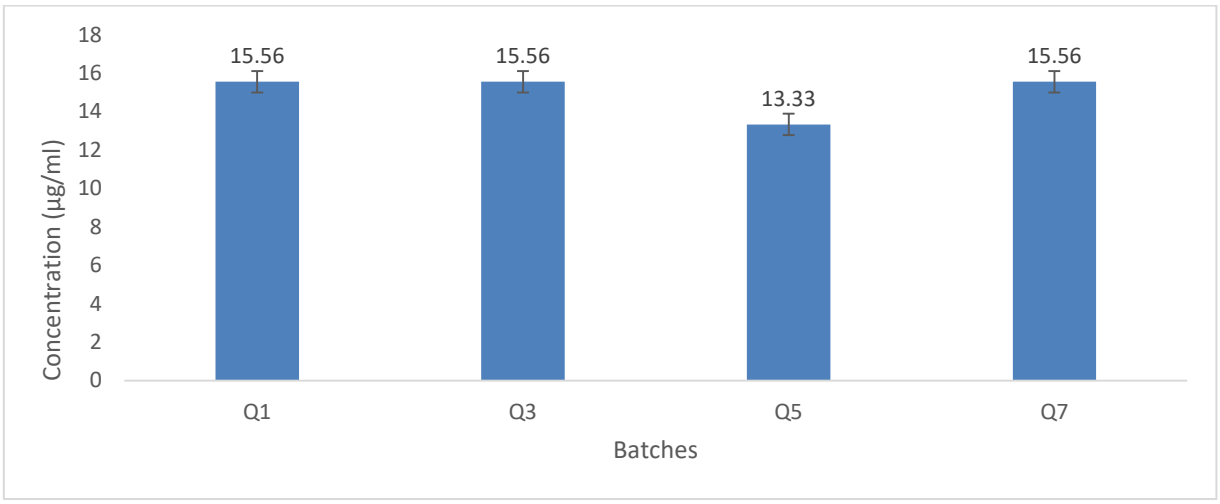


Figure 1: Time-dependent pH analysis of (a) SLNs containing quinine (b) NLCs containing quinine (c) SLNs not containing quinine and (d) NLCs not containing quinine.

Key: *BW11: Local beeswax SLN; BW12: Local beeswax NLC; BW21: Foreign beeswax SLN; BW22: Foreign beeswax NLC; S1541: Softisan SLN; S1542: Softisan NLC; SA1: Stearic acid SLN; SA2: Stearic acid NLC; Q1: Local beeswax with quinine SLN; Q2: Local beeswax with quinine NLC; Q3: Foreign beeswax with quinine SLN; Q4: Foreign beeswax with quinine NLC; Q5: Softisan with quinine SLN; Q6: Softisan with quinine NLC; Q7: Stearic acid with quinine SLN; Q8: Stearic acid with quinine NLC*



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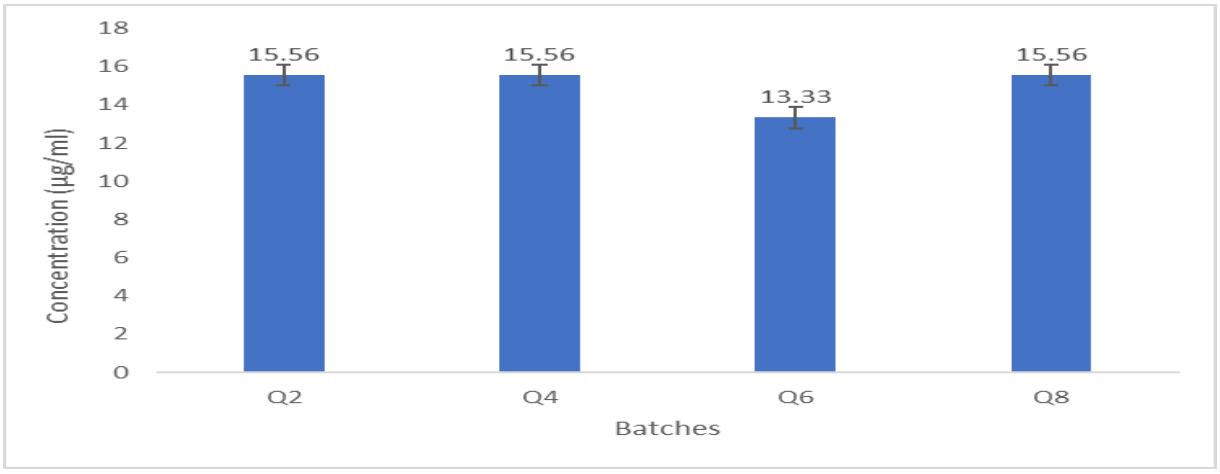
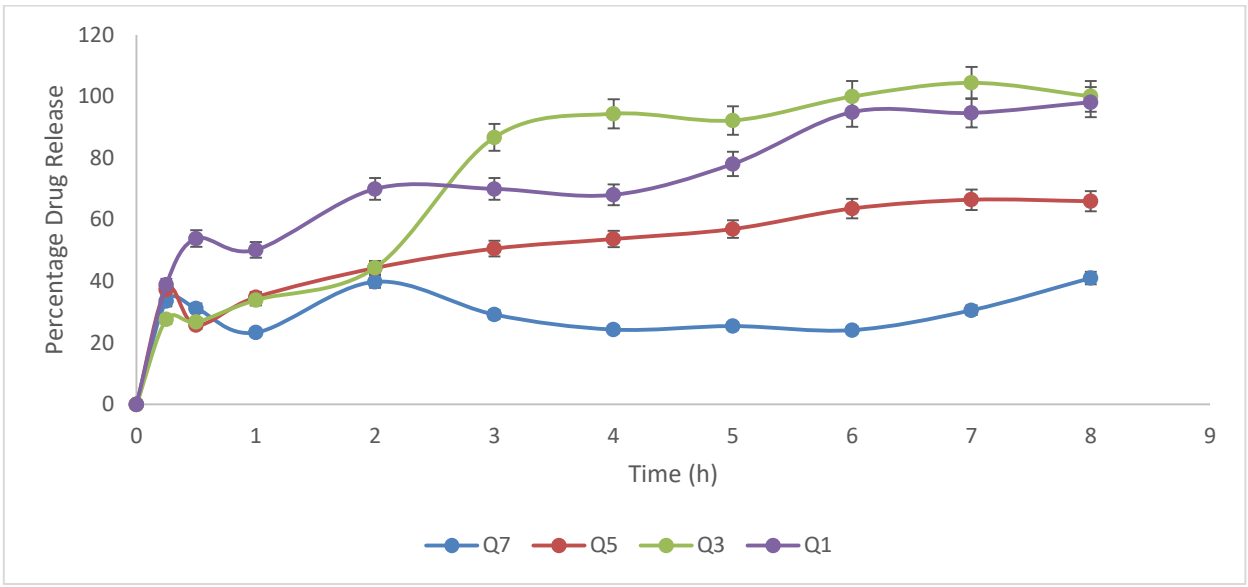


b)

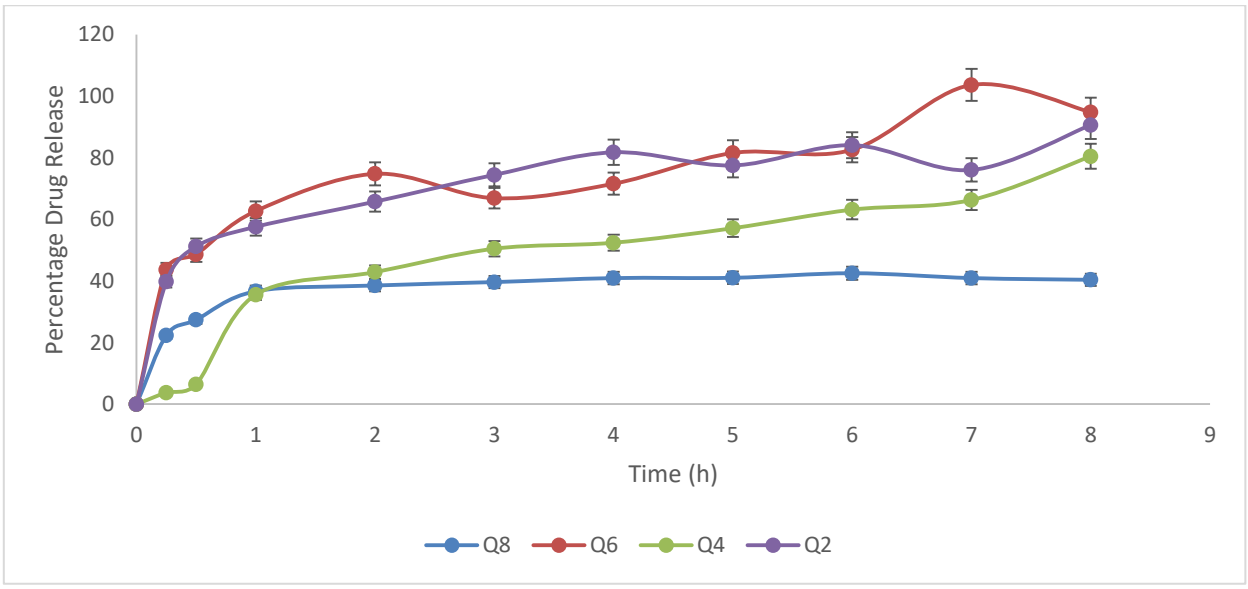
Figure 2: Drug content analysis in (a) SLNs containing Quinine and (b) NLCs containing Quinine

Table 4: Encapsulation efficiency and Drug-loading capacity of SLN and NLC drug formulations

Formulation	Batches	Percentage (%) unencapsulated	EE (%)	Loading capacity (%)
SLN	Q1	50.70	49.30	14.90
	Q3	-	-	-
	Q5	-	-	-
	Q7	54.79	45.21	11.91
NLC	Q2	54.00	46.00	16.20
	Q4	62.07	37.93	18.62
	Q6	47.18	52.82	14.15
	Q8	52.20	47.80	15.64



a)



b)

Figure 3: *In vitro* drug release analysis of (a) SLNs containing Quinine (b) NLCs containing Quinine

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formulation made with local beeswax had the smallest particle size (93.60±4.44 nm), while the largest particles (118.9±4.13 nm) were those formulated with Softisan® 154. The sizes for the NLCs were within 99.68±6.75 nm [formulations produced using foreign beeswax] and 117.3±4.60 nm [NLCs made with stearic acid], respectively. The larger particle sizes of NLCs compared to SLNs may be due to the presence of a liquid lipid. Previous studies have shown that the presence or high concentrations of liquid lipids increases the particle size of lipid-based formulations. Mehmood et al, 2017 [17] had earlier reported this trend while preparing alpha tocopherol nanoemulsion made with olive oil using ultrasonic energy. Increase in particle size was observed at higher oil content with increasing sonication time. Tang and co-authors reported a similar outcome in an aspirin-loaded nanoemulsion [18]. In both cases, enlargement of particle sizes was attributed to the fact that increase in oil content results in higher viscosity of the dispersed phase resulting in resistance to flow and restriction in the rate of droplet break-up. This increase is most pronounced when the concentration of surfactant is limited; hence, the smaller oil droplets produced at the start of sonication coalesce to form larger ones [17,19], due to the incomplete coverage at the oil-water interface by the emulsifier molecules [18]. However, breakdown of these large particles may occur when sonication is continued [17,20]. In other words, increased rates of collision frequency due to the high concentration of oil and reduced surfactant concentration, results in a higher probability of coalescence of droplets. On the other hand, the various solid lipids in the different formulations possess diverse molecular sizes and structures, which in turn can influence the size of particles. The particle size of all formulations had polydispersity indices ranging from 0.281±0.054 to 0.308±0.055. The polydispersity

index (PDI) is used to characterize the size distribution of nanoparticles. A PDI value between 0.1 and 0.3 suggests a compact size distribution, whereas a PDI larger than 0.5 shows a broad distribution [21]. From the results of the particle sizes and particle size distribution, it can be observed that local beeswax can be used to produce lipid formulations in nanosize range with acceptable PDI, just like other lipids used in this study.

The FT-IR spectra of the lipid matrices were similar to those of the individual components. Nevertheless, a number of negligible peaks were not observed in the lipid matrix spectra, possibly due to overlapping caused by hydrogen bonding. The pure quinine spectrum had characteristic peaks which were retained in the drug-loaded SLNs and NLCs. This implied the integrity of the drug was maintained after its incorporation into the matrices. The FT-IR spectra of the formulations showed that SLNs and NLCs produced using local beeswax and other lipids were successfully loaded with quinine and were compatible, since no new compound was formed [4].

Time-dependent pH testing serves to evaluate the long-term stability of pharmaceuticals. This is essential for optimizing drug release kinetics and guaranteeing product quality and safety for the duration of its shelf life. The pH of all the formulation batches remained relatively stable and were acidic, immediately after formulation and eleven weeks post formulation. This was due to the presence of organic acids in the lipid components. The pH of the NLCs were within the range of 4.6 to 5.3 (after 3 weeks) and 4.1 to 5.3 (after 6 weeks) and that of the SLNs were 4.7 to 5.3 (after 3 weeks) and 4.3 to 5.2 (after 6 weeks). However, after 11 weeks, the SLNs had higher pH range of 4.6 to 5.9, compared to that of the NLCs, 4.5 to 5.5. The result obtained eleven weeks after formulation showed that batches Q1, Q2, Q3, and Q7 exhibited a decrease in pH while batches Q4, Q5, Q6, and Q8 exhibited slight increase in pH after 11 weeks of storage. The unloaded nanoparticles also had the pH values within the acidic region, implying that the incorporation of the drug had no effect on the pH. The fluctuations observed over time may be attributed to the exposure to humid and oxygen-rich air, which can potentially impact the pH. In this study too, there was no noticable difference between the pH of the formulations made with local beeswax and those made with other foreign sourced lipids.

Encapsulation efficiency (EE) and drug-loading capacity (LC) are crucial factors in drug delivery systems, particularly when using nano-carriers such as Solid Lipid Nanoparticles (SLNs) and Nanostructured Lipid Carriers (NLCs). These properties characterize the efficacy and capability of these carriers in delivering pharmaceuticals. Generally, the encapsulation efficiency and loading capacity for all batches were relatively low, ranging from 37.93% to 52.82% and 11.91 to 18.62, respectively. Comparing EE of the SLNs to their corresponding NLCs, NLCs had better encapsulation than SLNs as shown in Table 4. The only exception was the EE of the SLN of local beeswax (49.30 %), which was slightly higher than that of the NLC (46.00). In the other lipids the NLCs (Q4, Q6 and Q8) were more efficient than their corresponding SLN formulations (Q3, Q5 and Q7) in encapsulating quinine. SLN made with local beeswax (Q1) was the most efficient SLN formulation for encapsulating quinine with EE of 49.30% and LC of 14.90, while NLC made with Softisan® 154 (Q6) was the most efficient NLC formulation with EE of 52.85%.

Possible parameters influencing the encapsulation efficiency of the formulations include the chemical nature of the solid lipids used and the active ingredient [22], as well as the type of formulation made (SLN or NLC). The different lipids used have different carbon-chain length and this in turn influences the formation and arrangement of crystals during formulations, and hence the amount of drug entrapped within the lipid crystals [23]. NLCs have been known to be more efficient than SLN in entrapping drugs due to their amorphous form, since the presence of a liquid lipid creates more imperfection within the solid lipid, providing more space for drug entrapment, more than SLN formulations which do not have liquid lipid. Compared to SLNs, NLC formulations contain lower concentration of water and [24] improve on drug payload and formulation stability by averting drug loss from the lipid matrix during storage . Moreover, the addition of liquid lipids leads to dissolution of more of the active ingredients [4,5].The interaction between the lipid matrix components and the active compounds can also have a significant impact on the polymorphism/crystallinity of the formulation [25] and also the encapsulation efficiency. Quinine has partial hydrophilicity [26,27] and, as a result, is not entirely encapsulated by lipid. This may be the reason for the lack of encapsulation of quinine in some of the SLN formulations as seen in Table 4. From the result of the EE of all the formulations, local beeswax showed better encapsulation of quinine better than foreign beeswax, and other lipids. More analysis to determine the quality of local beeswax that made it able to encapsulate a hydrophilic drug in an SLN formulation better than foreign beeswax and Softisan® 154 is recommended.

The *in vitro* drug release in the phosphate buffer exhibited a progressive and uniform pattern across all batches. All the formulations exhibited immediate and continuous drug release in eight hours, except stearic acid. Both SLN and NLC made with stearic acid showed a gradual and sustained drug release, achieving only 40 % drug release after 8 hours. In contrast, Q1, Q2 and Q6 (Local beeswax SLN, local beeswax NLC and Softisan® 154 NLC, respectively) released ≥50 % of quinine in an hour. Comparing the SLNs to their corresponding NLCs, SLN formulations made with all lipids except Softisan® 154, showed a faster drug release than their NLC counterparts. For instance the release profile for Q1 and Q3 [SLN formulations] were faster release overtime than Q2 and Q4 [NLC formulations]. Q8 had a consistent drug release, however, Q7 had the highest percentage release after eight hours. The release pattern and percentage drug release of local beeswax SLN and NLC was similar to that of the foreign

sourced lipids used in this study. Hence, locally sourced beeswax can serve as a more affordable and available alternative to these imported solid lipids in lipid-based formulations.

CONCLUSION

This study reveals that beeswax from honeycomb wax sourced locally, can serve as a suitable alternative to other imported solid lipids for formulating different lipid drug delivery systems. This can reduce cost, delays to delivery of imported solid lipids as wells remove restrictions experienced by researchers due to the limited available sample quantities provided by their manufacturers. This study has also demonstrated that the hot homogenization technique can be successfully utilized to develop solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLCs) using local beeswax as solid lipid. More studies investigating the use of locally sourced beeswax for other lipid-based drug delivery systems to assess its suitability is recommended.

AUTHORS' CONTRIBUTION

Conceptualization, C.P.A., A.G. N, U.C.; Data curation, U.C., A.G.N.; Formal analysis, C.P.A., A.U., U.C., ; Funding, C.P.A., N.M.I., A.P.A., U.C., A.G.N., A.A.A.; Methodology, C.P.A., U.C., A.G.N.; Project administration, C.P.A., A.A.A., N.P.O., Writing—original draft, C.P.A., U.C., A.G.N., A.U.; Writing—review and editing, C.P.A., O.K.C., A.U., A.G.N., U.C. All authors have read and agreed to the published version of the manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

ACKNOWLEDGMENT

We wish to thank the management of the University of Nigeria, Nsukka, and the where this research was conducted. Our appreciation also goes to Lipoid GmbH and IOI Oleo GmbH for the gift of Phospholipon® 90H, Softisan® 154, and Medium chain triglyceride.

FUNDING

The conducting of the research work was funded by 2021 African–German Network of Excellence in Science-Programme Advocating Women in Science (AGNES-PAWS) Grant for Junior Researchers, supported by the Federal Ministry of Education and Research (BMBF) and the Alexander von Humboldt Foundation (AvH). The funders provided financial support but did not influence the study design, analysis, and interpretation of data generated.

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