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Phytolipids lipospheres formulation: Application in oral delivery of metformin hydrochloride to diabetic rats

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ABSTRACT

The antidiabetic activity of oral metformin is dependent on its bioavailability. The purpose of the research is to improve the solubility, bioavailability and therapeutic efficacy of metformin using structure lipospheres. Dika fat was extracted from *Irvingia gabonensis* var. excelsa (*Irvingia wombolu*), and matrix consist of Dika fat with soyabean oil 3:1 was prepared and thereafter use to formulated lipospheres loaded with increase concentration of metformin. The formulated lipospheres were characterized *in vitro. In vivo* antidiabetic activity of metformin-loaded lipospheres was evaluated in hyperglycemic rats. The result of our findings showed that Matrix from the lipid mixture generated a structural disoriented matrix with numerous spaces that accommodated metformin in a concentration-dependent manner with maximum DEE of 87 %. The particle sizes were in micro range (10.45-21.22 μm) and there were no significant changes in the pH (6.1-5.8) of the formulations, indicating stable preparation. Maximum blood glucose–lowering effect (76.22 %) of the lipospheres was comparably higher than the conventional formulation (54.89 %).

KEYWORDS: *lipospheres, lipid- matrix, metformin, glucose-lowering*

INTRODUCTION

For the last decade, plant and animal sources have widely gain favorable attention in the area of natural lipids research as an alternative to synthetics lipids. Results have proved that phytolipids when used as excipients for lipid-based formulation are very efficient in improving drug absorption after oral administration, especially for the absorption of hydrophobic and hydrophilic drugs [1-3]. Although chemical specificity and purity may not be optimal, these lipids may be superior to synthetic forms as regards toxicity and biocompatibility [2]. Natural fats may have better in vivo tolerability than synthetic fats [4]. However, a major concern with the use of animal fats as drug delivery basis for oral or parenteral administration is the possibility of increasing the blood serum cholesterol, hence plant lipids are generally preferred. The rapid growth in the use of lipid-based drug delivery systems is primarily due to the diversity and versatility of pharmaceutical grade lipid excipients amenable for formulation of liquid, semi-solid, and solid dosage forms.

Additionally, the widening availability of lipidic excipients with specific characteristics offers flexibility of application with respect to improving the bioavailabilty of both lipophilic and hydrophilic drugs and manipulating their release profile [5-6]. The proven safety and efficacy of lipid-based carriers make them potential alternative drug carrier materials to polymers as well as attractive candidates for preparing lipid-based formulations [7-8]. These

formulations allow for controlled/sustained drug delivery, among other advantages [9-11].

More so, lipid-based formulations have been shown to enhance the bioavailability of drugs administered orally [3, 12, 13]. Structured lipid matrices have been shown to have improved physical properties compared with the individual components and have the ability of combining the beneficial characteristics of component fatty acids. They may lead to increase in solubility of drug candidates, which is one of the prerequisites to obtain high drug loading [1].

Lipospheres are solid water-insoluble microparticles formed of a solid hydrophobic core having a layer of a phospholipid embedded on the surface of the core containing a biologically active agent in the core. The average particle diameter is between 0.3–250 μm [14].

Lipospheres have several advantages over other lipid delivery systems such as emulsions, vesicles, and liposomes, in terms of stability, low cost of reagents, ease of manufacture, high dispersibility in aqueous medium, and a release rate for the entrapped substance that is controlled by the phospholipid coating and the carrier. In a liposphere, there is no equilibrium of substances in and out of the vehicle as in an emulsion system. Lipospheres also have a lower risk of reaction of substance to be delivered with vehicle than in emulsion systems because the vehicle is a solid inert material. Moreover, the release rate of a substance from the lipospheres can be manipulated by altering either or both inner solid vehicle or the outer phospholipids [14]. The pharmaceutical uses include providing extended release of active agent including drugs such as vaccines and anesthetics; in oral formulations for release into the lower portions of the gastrointestinal tract.

Metformin is an oral anti-hyperglycemic agent, which shows incomplete absorption from the gastrointestinal tract and the absolute bioavailability of 50 – 60% with relatively short plasma half-life of 1.5 - 4.5 h. Oral absorption of metformin is confined to the upper part of the intestine. Therefore, the low bioavailability can be ascribed to a comparatively high pre-systemic clearance that takes place after administration.

More importantly, are the gastrointestinal side effects such as abdominal discomfort, nausea, and diarrhea associated with metformin intake and the needs for repeated administration to maintain an effective plasma concentration, that at times decrease patient compliance [15].

Dika fat is an edible vegetable fat derived from the kernel of *Irvingia gabonensis* var excelcia [16-18], with the melting point range of 38 ^{o}C to 41 ^{0}C. *Irvingia gabonensis* var. excelsa or *Irvingia wombolu* is a tropical tree, now generally recognized to belong to Irvingiaceae family. Irvingia species are commonly known as African mango, bush mango or wild mango and the nut commonly called dika nut. The seed or nut contains fat which can be used for food, pharmaceutical and cosmetic applications [19]. Fat derived from the nut is generally regarded as safe [19].

Soybean oil is edible oil derived from soya bean seed and is one of the most important bean sources in the world, providing vegetable protein and oil for millions of people and supplying important functional ingredients to the food, health care, pharmaceutical, and chemical industries [20]. Dika fat and soybean oil are completely biodegradable physiological lipids, and similar to other lipid excipients, they have generally regarded as safe status informing their use in this study.

Therefore, the danger of use of synthetic polymer matrix materials which often goes along with detrimental effects on incorporated drug during manufacturing of formulations or during the erosion of the polymers after application are completely avoided [5]. Lipids properties play an important role in oral drug delivery systems by prolonging the duration of residence of drug carriers and by increasing the closeness of contact between drugs and mucous membranes at absorption sites, thus enhancing permeability, reducing degradation, and eventually improving oral bioavailability [21]. Furthermore, many synthetics lipids and phytolipids have been extensively studied for its potential as an absorption enhancer across intestinal epithelium [22].

In this work, metformin-loaded liposphere was prepared using Dika fat as solid lipid and soya bean oil as liquid lipid; the two lipids were mixed together in a definite ratio to obtain a single lipid matrix as the final carrier. The performances and the pharmacological activities of this formulation were studied *in vitro* and *in vivo*.

MATERIALS AND METHODS

Materials

Metformin hydrochloride was a kind gift from Farmex Meyer Pharma Ltd. (Ikeja, Lagos State, Nigeria).. Thiomersal, sorbitol (BDH, England), and polysorbate 80 (Uniqema, Belgium), poly ethyleneglycol PEG-4000 (Cary Roth, Karlsruhe, Germany) were used as procured from their manufacturers without further purification. Soybean oil (Grand Cereal and Oils, Nigeria), n-hexane, ethyl acetate (Sigma-Aldrich, Germany), *Irvingia* fat was prepared in departmental laboratory and distilled water (Lion water, Nigeria) was used for lipospheres preparation. All other reagents and solvents except otherwise specified were analytical grade and were used as supplied.

Extraction dika fat from *I. gabonensis*

Irvingia gabonensis was purchased from a local market in Enugu State, Nigeria. Authenticate the seed of the plant was done by Mr. A.O. Ozioko, a consultant taxonomist with the International Center for Ethnomedicine and Drug Development (InterCEDD) Nsukka. The dika wax was extracted following the method earlier reported in our laboratory [18]. Briefly, dika fat was extracted by Soxhlet extraction using established procedure. *I. gabonensis* seed was milled in a hammer mill and extracted in a Soxhlet extractor using n-hexane at 100 °C. The n-hexane was allowed to evaporate at room temperature. Boiled distilled water which was twice the volume of the wax was poured into the molten wax in order to dissolve the hydrophilic gum contained in the wax. The hydrophilic gum was removed using a separating funnel. Ethyl acetate was equally poured into the molten wax in order to remove the hydrophobic gum from the wax. The principle of adsorption was employed in the purification of the extracted wax using admixtures of activated charcoal and bentonite (2:1) as the adsorbent blend. The molten wax (10 g) heated to 100 °C was allowed to run through the column (1 g). The purified wax was stored in a refrigerator until used.

Preparation of structure lipid matrices

The lipid matrices consisting of 3:1 mixture of dika fat and soya bean oil were prepared by fusion. Briefly, the lipids were weighed using Adventure analytical balance (Ohaus, China), melted together on a hot plate at 70 ºC and stirred using a magnetic stirrer (SR1 UM 52188, Remi Equip., India) until solidification to get lipid matrices

Preparation of metformin-loaded lipospheres

The preparation method for metformin-loaded lipospheres was adapted from the hot homogenization technique described previously [23]. In each case the appropriate quantities of lipid matrices as presented in **Table 1,** were melted together in a crucible at 60 ◦C. The drug was added and stirred thoroughly. Other material such as thiomersal and sorbitol were carefully weighed and dissolved in distilled water at 75°C and immediately transferred into the lipid phase containing the drug at the same temperature. The mixture was then homogenized with a mixer (Ultra Turrax T18, UK) at 1200 rpm for 4 min and allowed to recrystallize at room temperature. The above procedure was repeated using increasing quantities of metformin or active pharmaceutical ingredient (API) (100, 200, 300, 400 and 500 mg) to yield metformin-loaded lipospheres (coded as A1-A5), respectively. To have a reference sample, metformin free (unloaded batch) of the lipospheres was similarly formulated using the same procedure and was coded as A6.

Table 1. Quantities of materials used for Metformin-loaded lipospheres formulation

Batch	Lipid matrix (g)	Sorbic acid (mg)	PEG-4000 (mg)	Sorbitol (mg)	Drug Conc. (mg)	Distilled water, q.s (% w/w)
A1	5	30	300	400	100	100
A2	5	30	300	400	200	100
A3	5	30	300	400	300	100
A4	5	30	300	400	400	100
A5	5	30	300	400	500	100
A6	5	30	300	400	----	100

Characterization of metformin-loaded lipospheres

Percent recovery

Percentage recovery rate were calculated to evaluate the efficiency of method used in drug formulation, thus it helps in selection of appropriate method of production. The resulting lipospheres were collected and weighed to determine the practical yield or recovery of the formulation using equation (1).

$$\text{Recovery rate (\%)} = \frac{W_1}{W_2 + W_3} \times 100 \qquad (1)$$

where W_1 is the weight of lipospheres formulated (g), W_2 the weight of the drug (g) and W_3 the weight of lipids (g).

Particle size and morphological characterization of lipospheres

The particle size of the lipospheres was determined by computerized image analysis of at least 50 lipospheres on a photomicroscope (Lieca, Germany). Each of the batches was mounted on a slide and observed under a light microscope. With the aid of the software in the microscope, the projected perimeter diameters of the particles corresponding to the particle sizes of the lipospheres were determined and average calculated. The particle morphologies were also observed and photomicrographs taken. All these were done in a time-dependent manner (24 h, 1 and 3 months).

Periodic pH evaluation

The pH of the dispersions was evaluated using a validated pH meter (HANNA Instruments, Padova, Italy). The electrode component was immersed into 50 ml quantities of the dispersions and the reading recorded. Each measurement was performed in triplicate and the average calculated.

Encapsulation efficiency (EE%)

A 50 mg quantity of lipospheres was dispersed in 25 ml of phosphate buffer (pH 7.4). The dispersion was allowed to stand for 2 h after which it was mixed in a vortex mixer for 5 min and
then centrifuged at 4000 rpm for 30 min to obtain two phases (i.e. aqueous and lipid phases). A 1 ml volume of the aqueous phase was thereafter diluted 1000-fold using distilled water. The absorbance of these solutions was determined in a UV-spectrophotometer (Jenway, Germany) at a wavelength of 285 nm, and the amount of metformin contained in the various lipospheres formulation samples was calculated using equation (2)

$$EE\,\% = \frac{\text{Actual drug content}}{\text{Theoretical drug content}} \times 100 \qquad \text{Eq. 2}$$

In vitro release profile

To determine the amount of drug released from the lipospheres, a dynamic dialysis technique was used. A 50 mg, were placed in the dialysis bag (length 8 cm, diameter 2.5 cm, MWCO 10,000; Spectrum, Los Angeles, USA) containing 5 ml of phosphate buffer solution (pH 7.4). The dialysis bag was previously soaked in buffer before used as the release bag. It was then placed in dissolution apparatus which was maintained at 37 ^{0}C with constant orbital mixing (60 rpm). At specified time points 5 ml aliquot of the medium was removed and replaced with equal volume of fresh medium. The metformin concentrations of the aliquots were determined by using spectrophotometer, and the amount of metformin released from the lipospheres were

calculated. The percentage of metformin released was plotted versus time. Each data point is meaning (SD) calculated from three measurements.

In vivo studies

Animals

Male Wistar rats weighing about 250 g were used for pharmacodynims studies. Rats housed in cages were kept in a room with controlled temperature (20–22 °C) and a 12 h day–night cycle. SD male rats were rendered diabetic prior to the study by intravenous injection of 40 mg/kg alloxan in isotonic saline solution. They were considered to be diabetic when the baseline glucose levels were in the range of 13.89–16.67 mmol/L. All of the animal experiments adhered to the principles of care and use of laboratory animals and approved by the Institutional Animal Care and Use Committee of Faculty of Pharmaceutical Sciences, Enugu, Nigeria

Oral administration approach

Twenty-five diabetic rats were randomly divided into five groups (five animals for each group). All animals were fasted for 12 h before the experiment, though they had free access to water throughout the whole experiment. The rats in group I and II were given 2 ml of normal saline and unloaded liposphere (batch A6) orally, both serve as negative controls. The rats in group III were orally administered with equal dose of glucophage® 5 mg/kg as a positive control. The rats in groups IV through VI were given metformin-loaded lipospheres (batch A3-A5) respectively, orally in the same dose. The choice of these batches was based on the results of the *in vitro* evaluation. All above formulations were made in the medium of physiological saline. Blood samples were collected from the retro-orbital plexus of each rat at different times and centrifuged. Glycemia was determined in the supernatant solution using ACCU-CHECK machine (ROCH, USA) Results are shown as the mean values of plasma glucose levels of animals at each group.

Pharmacokinetics analysis

A group of 15 male rabbits weighing on an average of 1.6 kg were used for the pharmacokinetic studies. They were randomly divided into 3 groups with 5 animals in each group. Animal in the first group received formulated liposphere orally in a dose of (100 mg/ kg of body weight) in the form of suspended in 2 ml sterilized double-distilled water. The second and third groups, each received conventional sample and pure drug sample in the form of suspension at the same dose of the liposphere orally. At different times post dosage administration, blood samples were collected from tail vein and were subjected to analysis spectrophotmetrically. Parameters such as the area under the curve (AUC), *C*max, *T*max, rate of elimination (*K*el), and circulatory half-life (*t*1/2) for each test agents were evaluated according to standard pharmacokinetic computer formulae. The experimental protocols concerning the handling of rats were in accordance with the requirements of the Departmental Animal Care and Use Committee at the University.

Statistical analysis

Data were analyzed using SPSS Version 16.0 (SPSS Inc. Chicago, IL, USA). Data were analysed by one-way ANOVA. Differences between means were assessed using student's *t*-test. $P<0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

The purpose of this work is the development of lipospheres based on dika fat and soya bean oil blends, to be used as sustained release devices for hydrophilic antidiabetic compound, metformin. The ultimate goal is to generate a delivery system that, upon formulation oral administration will ensure the high drug loading efficiency, improve oral absorption and prolonged availability of the drug in the biological system for enhance therapeutic efficacy.

Percentage recovery of the lipospheres

The result presented in **Table 2** was obtained for the lipospheres formulated. The percentage recovery values were less than 100% due to loss accruing form preparation processes. Results indicate that all the metformin-loaded lipospheres (batches A1–A5) had higher recovery percentages than the unloaded lipospheres (batches A6). However, they had overall high percentage recoveries. This may be as a result of adoption of a reliable production process technology.

Particle size and morphology of lipospheres

The particles size and morphological appearance of the lipospheres was examined by optical microscopic method. The particle size of these lipospheres was

within the range of 13.12 ± 6.42 to 23.49 ± 20.92 µm **(Fig 2).** Elevations of the particle size of these lipospheres were found with the increasing incorporation of drug into formulations. This could be attributed due to the increase in viscosity of lipid blend. In other words, the particle sizes within one week of formulation were small and increased dose-dependently according to the concentration of entrapped drug as shown in **Table 1.** Previous work on lipid carrier suggested that, particle size may be a function of either one or more of the following: formulation excipients, degree of homogenization, homogenization pressure, rate of particle size growth, crystalline habit of the particle [23]. There was an increased in the particle size of the formulation within 90 day period. This increased were observed in the loaded drug and were dependent on the drug concentration with highest in batch loaded with 500 mg of the drug. In the unloaded batch (A6), the reverse is the case, there was a slight decreased in the particle size throughout the investigative periods. The result here shows that drug loading did significantly ($P<0.05$) affect the size of the lipospheres. Increase in particle size is usually as a result of aggregation and subsequent growth by Ostwald ripening or sintering. The stability of the formulation and the slow in the particle growth may be attributed to the presence of soluble polymer ie PEG-400 in the formulation. Previous researchers have identified that the rate of particle growth in a suspension can be significantly slowed down by the addition of a water-soluble polymer such as PEGs [24]. PEGs polymers exhibit inhibitory effect on precipitation of drug in a supersaturated solution [25]. The stabilization effect of the polymer is attributed to the increase in viscosity of the aqueous medium and the formation of a protective polymer layer on the surface of the solid particles in the suspension. An increase in viscosity of the medium can result in a slower rate of solute diffusion and the surface polymer layer can function as a barrier to the deposition of solute onto the solid particles. Both mechanisms play an important role in suppressing particle growth in a suspension.

The morphological images shown in **Fig. 2(A1-A6)** indicate that the lipospheres have a spherical shape and a rough surface irrespective of the concentration of drug in the formulation. There was no sign of aggregation within the 24 h of the formation indicating the initial stability of the preparation. These results agree with those previously described for other lipid based compositions [18, 26].

Time-resolved pH analysis

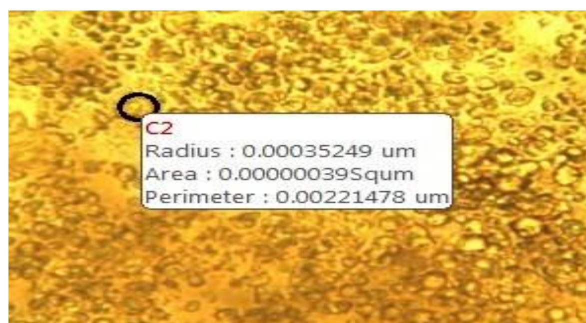
This test was carried out to determine the pH stability of the different batches of the lipospheres when stored at room temperature and at different time intervals. It was observed from the results **(Table 2)** that, after 9 months, there was no significant changes in the pH in the batches. The pH ranges of batch A1 to A5 were 4.9 ± 0.0 to 5.7± 0.1, while that of unloaded batch (A6) had a pH range of 5.4± 0.0 to 5.8 ± 0.2. The slight variation of pH with time could be a function of changes in the drug or excipients. Researchers have shown that stability of lipid formulation depends largely on the degradation of excipients with storage through generation of unfavorable pH or reactive species for the incorporated drug [1, 2, 23]. It is necessary to know the pH of maximum stability of an API or its stability profile and utilize that in designing a suitable formulation for the API. This will inform the formulation scientist whether to include a stabilizer or not in the new formulation. Consequently, time-resolved stability studies were carried out to determine the changes in the pH of the formulation as a function of the stability profile of the lipospheres when stored at room temperature. The results of the stability studies as a function of pH change of the lipospheres **(Table 2)** show that there was an insignificant change in the pH of the formulations over a period of 9 months, indicating that there was little or no degradation of the drug and/or the excipients used in the formulations within this period of time. Additionally, with respect to the metformin-loaded lipospheres, the change in pH from its day one of the formulation is very minimal to alter the expected activity of the formulation, as the degradation was only on the lipid matrix. This result was in agreement of an earlier work [27], who found out that the use of solid lipids is an attractive innovation that is advantageous because the solid lipid provides more flexibility in controlling the drug release and protects the encapsulated ingredients from chemical degradation. It’s worth to mention that, the change in pH of the unloaded formulation (Batch 6) was more than the loaded batches. This further confirms that the little change in pH may have resulted from the excipients and not from the drug. Thus, the formulations, within the investigated period were considered to be very stable.

Table 2: Properties of metformin-lipospheres formulations

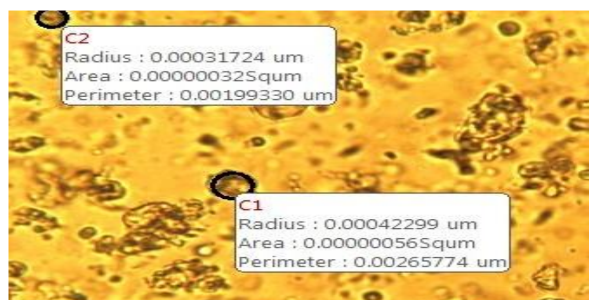
Batches		Particle size (µm)				Time-resolved pH analysis		
	RR (%)	24 h	1 month	3 months	DEF	24 h old	After 6 month	After 12 months
A1	89.0 ± 0.2	18.6 ± 0.2	22.0 ± 0.7	28.2 ± 0.0	91.30 ± 0.2	5.5± 0.3	5.2± 0.5	5.6±0.5
A2	93.1± 0.3	19.1 ± 0.4	17.0 ± 0.1	25.2 ± 0.3	93.11 ± 0.1	5.2± 0.1	5.1± 0.5	5.5±0.2
A3	98.1± 1.3	21.1 ± 0.1	22.0 ± 0.2	24.1 ± 0.4	92.10 ± 0.2	4.9 ± 0.0	5.7± 0.1	5.4±0.0
A4	94.0 ± 0.2	18.2 ± 0.0	18.7 ± 0.5	21.0 ± 0.2	91.30 ± 0.2	5.1± 0.0	5.4 ± 0.2	5.4±0.1
A5	96.1± 0.1	19.5 ± 0.6	18.5 ± 0.5	21.3 ± 0.3	96.11 ± 0.1	5.8± 0.0	5.4 ± 0.0	5.3±0.1
A6	82.1± 0.1	12.6± 0.1	12.9 ± 0.2	26.0 ± 0.7	* * *	5.4± 0.0	5.8 ± 0.2	5.8±0.0

Note: RR= recovery rate, DEE= drug entrapment efficiency

A1



A2



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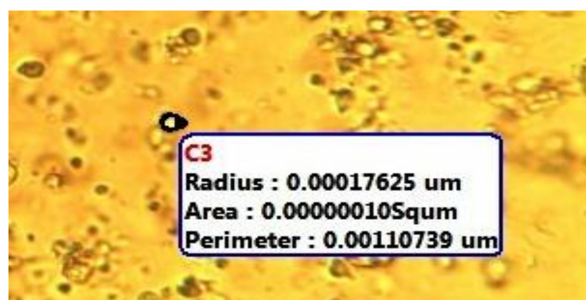

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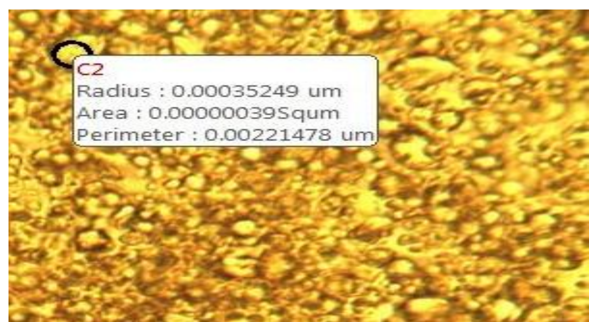
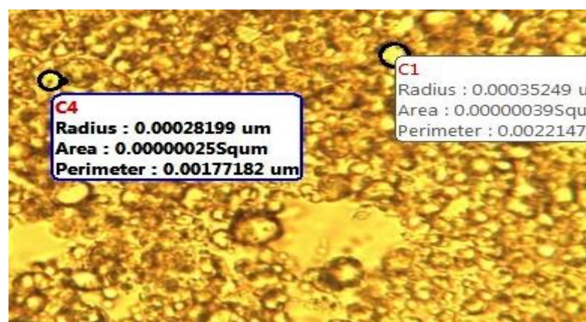


A5



A6

Figs. 1 (A1-A6). Photomicrograph of the lipospheres; (A1-A6) contain increase concentration of metformin; 100, 200, 300, 400 and 500 mg respectively, while A6 contain no drug (unloaded or drug free lipospheres) within one week of preparation. Magnification × 100

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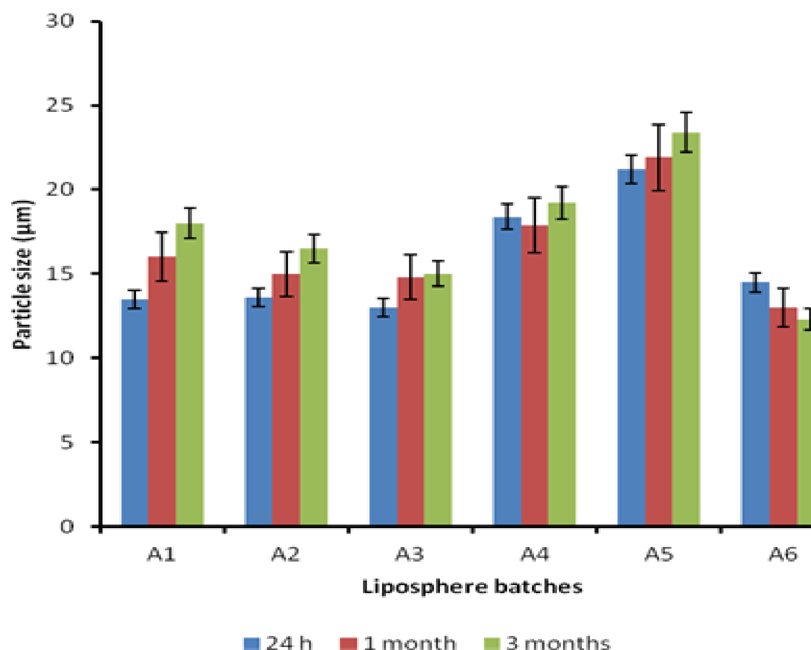

Fig. 2. Particle size analysis of the lipospheres containing 100, 200, 300, 400 and 500 of metformin for A1–A5, respectively. Batch A6 contain 0 mg of metformin (unloaded)

Drug entrapment efficiency (DEE%)

The role of the formulated lipospheres is to deliver the API to the target tissues intact. Thus, the ability of the lipospheres to accommodate active molecules is an important property. It can be expressed by the entrapment efficiency (DEE %). DEE % defines the ratio between the weight of entrapped API and the total weight of API added to the dispersion as shown in equation 3. The drug entrapment efficiencies in metformin-loaded lipospheres were within the range of 91.30 ± 0.2 (A1) and 96.11 ± 0.1 (A5) **(Table 2).** The highest drug entrapment efficiency was observed in batch A5 (96.11 ± 0.1), that was loaded with 500 mg of metformin. It was observed from previous worked [28, 29], that DEE % is dependent on several parameters, such as the lipophilic properties of the drug, the screening of the most appropriate lipid composition/ratio and surfactant combination, as well as the production procedure used. From the results, it was observed that there were variations in DEE % which dependent mostly on the drug loaded in the formulation. Report from previous researched shows that structured solid lipid with liquid lipid caused a structural reorientation in the lipid matrix thereby creating space for accommodating more drug at higher drug loadings [15]. Additionally, Dika fat was

said to have low crystalline nature which serve as an added advantage in the area DEE % [30]. Since, low crystallinity of lipids is desirable in order to create more spaces for drug localization and enhance the encapsulation or entrapment efficiency (DEE%) of drugs and increase the loading capacity of the lipids [1,9, 11, 31]. All the same, higher DEE% values were obtained in all the batches of the lipospheres. There was no significant difference ($p < 0.05$) in the DEE% obtained for batches of the formulations. However, the DEE% of the formulation increased as the amount of the drug in the lipid matrix increases.

In vitro release

Fig. 3 shows the release profiles of metformin from lipospheres in phosphate buffer (pH 7.2). It is here apparent that drug release *in vitro* underwent a very slow initial burst followed by slow dissolution. The initial release of metformin observed in all the formulations may be due to the un-encapsulated

drugs that were adhered at the periphery or the outer shell of the lipospheres particles, leading to a relatively short distance of diffusion and hence fast release of the drug [32]. From clinical view point, a fast release profile could be considered as an advantage as a sufficient amount of a drug which is refer to as loading dose and thus, the increase in the plasma concentration of the drug [33]. It was observed that following the fast release, was a sustained release in all the batches of the lipospheres. Then a prolonged, relatively steady and slow release was observed. Maximum release (83.51 ± 2.23% by 14 h) was observed in lipospheres prepared with 500 mg. The observed high release in this batch (Fig. 3) could be attributed to the quantity of the incorporated drug and the moderate particle size. This result is in agreement with an earlier works on the lipid drug delivery [1, 30]. Earlier works had highlighted that high quantity of drug in lipid carrier increased drug release via increased surface area and drug-lipid interaction thereby facilitate the increase in the drug release [12].

Blood glucose lowering effect

Fig. 4 shows the behavior of different formulations administered orally to diabetic rats. The efficacy of the formulations was assessed by measuring the blood glucose concentration and calculating the relative pharmacological availability. As expected, (Fig. 4), the oral distilled water (DW) and unloaded liposphere (batch A6) showed no hypoglycaemic effect or a reduction effect on the blood glucose level compared to the control group ($p > 0.05$}. The result of the conventional formulation (glucophage) that serve as the positive control also showed good hypoglycemic effect as there was an obvious reduction in blood glucose level, but not equal to that of loaded liposphere. In contrast, the blood glucose levels of the rats decreased remarkably after the oral administration of metformin-loaded liposphere, achieving a significant decrease at 3-4 h when compared with the control group ($P< 0.05$). More interestingly, the hypoglycaemic effect was maintained without recovery at the baseline for over 16 h of the study after dosing the diabetic rats at a dosage of 50 mg/ kg. There was a significant decrease in blood glucose observed in all the loaded lipospheres as compared to the positive control sample. Maximum blood glucose lowering (57 %) was encountered in the liposphere (A5) containing 500 mg of drug in the formulation, and this was quite comparable higher to the blood glucose reduction (31 %) observed in the conventional tablet sample (Glucophage®) . The duration of the glucose effect was more in the liposphere compared to that of the conventional formulation. In other words, the release rate of the lipospheres lasted over 16 h while that of conventional tablet released and lasted within 8 h. The result here indicates that liposphere exhibited prolonged drug release. In addition, when the batches (A1-A5) of metformin-loaded lipospheres were compared, it was observed that the effect were dependent on the concentration of drug loaded in the formulation. It can be argue or simply say that batch (A5) showed a stronger effect on blood glucose levels, indicating that dika fat structured with soyabean oil as matrix could enhance the intestinal absorption of metformin.

An explanation of this positive behavior of the liposphere could be put forward in terms of the demonstrated ability of dika fat and soy bean oil preparations to make the entrapped metformin more stable and protect it from early release as well as enhance its intestinal absorption. Additionally, the pharmacological action of this formulation or the mechanisms of bioavailability enhancement by liposphere is that, the structured lipid matrices possess adhesive properties that make them adhere to the gut wall and release the drug exactly where it should be absorbed [34]. In addition, lipids are known to have properties that promote the oral absorption of lipophilic and hydrophilic drugs in general [35]. For example, the fatty acid contents of dika fat have been described as absorption enhancers. Oleic acid has been shown to decrease the phase transition temperatures of membrane lipids with resultant increase in rotational freedom or fluidity of these lipids [36]. Lauric, palmitic and myristic acids have also been described to have penetration enhancing effects. Lauric acid, as its salt sodium laurate, has been shown to improve the oral absorption of drug in rats [37]. Since myristic acid is a component of membrane phospholipids, a more effective interaction might have occurred between dika fat (containing myristic acid) as the major component of the lipid matrix and the mucosa wall of the rats; thus enhances absorption. In general, better absorption and bioavailability enhancement enables dosage reduction and minimizes side effects [38, 39].

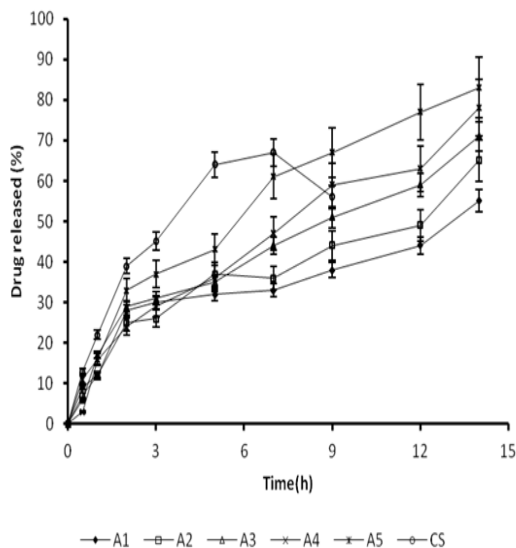

Fig. 3. Release profile of metformin from lipospheres formulated with Dika fat/soyabean oil matrix in phosphate buffer 7.2 (*n*= 3). A1–A5 contain 100, 200, 300, 400 and 400 mg drug, respectively. CS is the commercial sample.

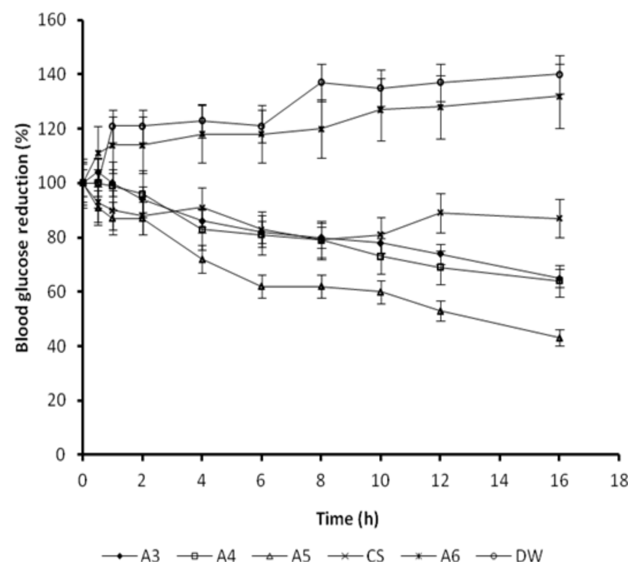

Fig. 4. Changes in blood glucose levels after oral administration of distilled water (DW) as a negative control, conventional sample (CS) as a positive control and metformin-loaded lipospheres (A3-A5) and the unloaded liposphere (A6) (mean ± SD, n = 5).

Bioavailability in rabbits

Results in Fig. 5 show that the mean plasma concentration of lipospheres following oral administration to rabbits was higher for the formulated products than for the conventional sample. Pharmacokinetic parameters for metformin, such as the maximum plasma concentration, peak time and the area under the concentration-time curve, are listed in Table 3. The T_{max} of metformin for the conventional sample was 2.0 h, which was faster than for the formulated liposphere. The *AUC* of the formulated liposphere, unlike C_{max}, was significantly ($p < 0.05$) higher than those of the commercial sample. The relative bioavailability of metformin in the formulated liposphere was about 3.5 times higher than the commercial sample, indicating the feasibility of further development of an efficient sustained release oral delivery system. The plasma metformin level peaked 5 h after oral administration of the formulation, and then slowly declined up to 14 h. The *AUC* (0–24 h) was 785μg mL–1 h–1, and was found to be significantly ($p < 0.05$) higher than the corresponding value (296.10 μg mL–1 h–1) for the commercial sample. The circulatory half-life of metformin was also prolonged when the formulated liposphere $T_{1/2}$ was administered compared to conventional sample ($T_{1/2}$=5.50 ± 0.32 h) or pure drug sample ($T_{1/2}$= 2.50 ± 0.11 h). These differences in pharmacokinetic pattern further affirmed the result obtained in the AUC as seen in Table 2. In all case, when the results of the liposphere were compared to the pure sample (unformulated powder), it was observed that by all indices the liposphere was significantly higher to that of pure sample

CONCLUSIONS

In designing a drug delivery device such as those based on lipids or combinations thereof, the choice as well as the combination ratio of lipids and other excipients should be considered based on the desired release pattern, route of administration, stability, and other physicochemical considerations. In this research work, the capability of dika fat and soyabean oil as a carrier was investigated. The effect of drug concentration on the release characteristics of the

formulation and the *in vivo* study were similarly evaluated. It was observed that the most concentrated formulation had the highest release and strong blood glucose lowering effect. One can suggest that batch (A5) containing 500 mg of API, offer the most promising formulation as regards capability to maintain prolongs release with improve in vivo effect on blood glucose reduction.

This indicates that sustain release of oral delivery of metformin for effective control of blood glucose is indeed possible using the right carrier system and formulation technique.

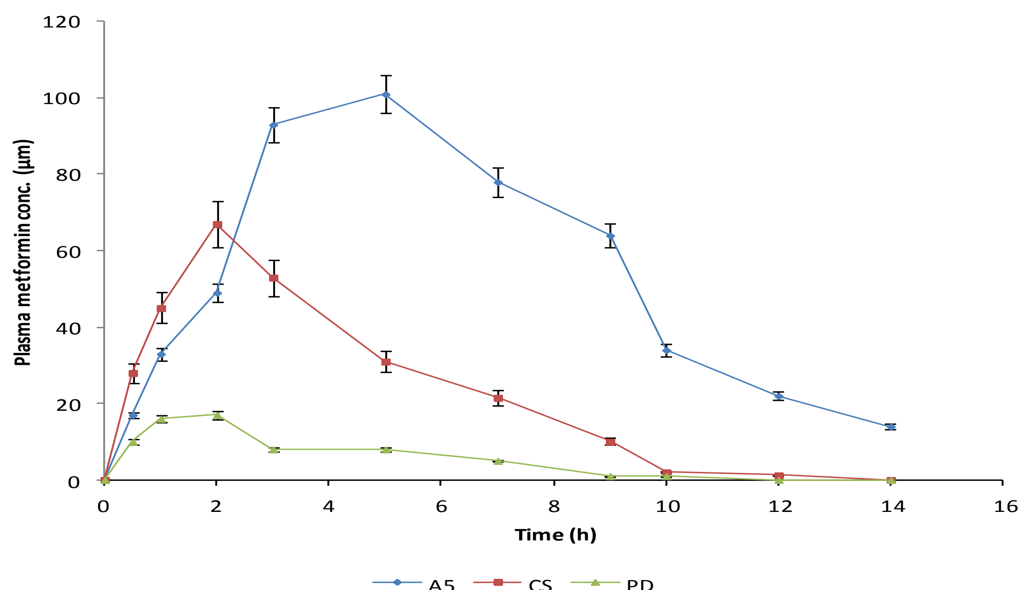

Fig. 5. Plasma concentration-time profiles of metformin after oral administration of the formulated metformin liposphere, the commercial sample and pure powder to rabbit (*n* = 5).

Table 3. Pharmacokinetic parameters of A5, CS and PD after oral administration to rats (mean ± SD, n=5).

Samples	AUC (µgh/ml)	C_{max} (µg/ml)	T_{max} (h)	$T_{½}$ (h)
A5	786.20 ± 1.21	101.00 ± 0.11	5.00± 0.39	9.00 ± 0.10
CS	296.27 ± 0.10	67.70 ± 1.00	2.00 ± 0.10	5.50 ± 0.32
PD	67.11 ± 0.10	17.22 ± 0.14	1.00± 0.30	2.50 ± 0.11

A5 = optimized batch of liposphere, CS= conventional sample and PD= pure drug

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