Application of Molecularly Structured Ben Oil in Gentamicin Entrapped Lipospheres

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Abstract

Objective: To formulate extended release gentamicin-entrapped lipospheres using natural lipids from Irvingia wombolu (IWF) and Moringa oleifera seed (MO) popularly known as Ben oil. Methods: Different lipid combinations including IWF and Phospholipon 90H (P90H) and IWF and MO were employed in the formulation of lipospheres. The formulations were analysed for particle size, encapsulation efficiency (EE), pH stability and antimicrobial studies amongst other tests. Also the in vitro release properties were studied in Phosphate buffer pH 7.2. Results: High EE of up to 90% were obtained for the various LM combinations. The pH was stable over 30 days and the formulations showed about 93% release of gentamicin at 12 h. Lipospheres formulated with MO matrices showed synergism in the microbial inhibition than other formulations. Conclusion: Natural lipids from Irvingia wombolu and Moringa oleifera seed could be used in formulating oral extended release gentamicin lipospheres.

Keywords: Moringa oil, Gentamicin, Lipospheres, Vegetable fats, Drug delivery

Introduction

Gentamicin is an antibiotics belonging to the aminoglycoside group and is used in the control of severe infections caused by Gram-positive and Gram-negative microorganisms [1]. It has rapid bactericidal activity and comparatively low levels of resistance in most community and hospital associated gram-negative pathogens and these properties made it a very useful empirical drug when rapid control of a serious infection is required [2]. However; it is limited by poor absorption; low bioavailability and toxicity [3-5]. Gentamicin is both nephrotoxic and ototoxic; but careful monitoring of plasma concentrations and short-term therapy (3 days or less) have been shown to have low incidence of nephrotoxicity; even though it will not prevent the rare occurrence of sudden idiosyncratic deafness [1]. Gentamicin toxicity remains a clinical problem; and many clinicians are reluctant in prescribing it even for a short term [6]. However; the use of structured lipid matrices in various novel drug delivery systems such as lipospheres could be employed in order to circumvent some of these limitations. Researchers have used this novel technology to increase the overall efficacy while minimizing toxicity of gentamicin [1,7-12].

The use of plant-derived lipids in drug delivery is generally preferred to lipids from animals due to the fact that lipids from animals have the possibility of increasing the blood serum cholesterol [12]. Moringa oleifera Linn. (Moringaceae) is native to the Indian subcontinent and naturalized in tropical and subtropical areas around
the world [13]. It is also known as the horseradish tree; drumstick tree and is now widely cultivated and has become naturalized in many locations in the tropics [14]. Almost every part of the plant (leaves; flowers; seeds; roots and bark) can be used as food or for medicinal and therapeutic purposes [15]; especially in developing countries [16]. The seeds can be used in a variety of ways including as medicinal and herbal remedies; nutritional supplements; and for industrial and agricultural purposes. The seeds contain 19 to 47 percent oil [17] known commercially as “Ben oil”; it is similar to olive oil and rich in palmetic; stearic; behenic and oleic acids [18,19]. The oil is clear; odorless and resists rancidity [19]. This oil contains about 75 % of oleic acid which makes it less vulnerable to oxidative stress than unsaturated fats [20,21].

Irvingia wombolu (Irvingiaceae) is a fruit tree with a wide distribution across West and Central Africa [22]. Irvingia kernels are used in soup making as they form an important part of the West and Central African diet. Fat extracted from the kernels can be used for food applications; such as in margarine or cooking oil; and is also suitable for soap; cosmetics and pharmaceuticals [23]. Lipid from Irvingia gabonensis have recently been studied as a carrier in solid lipid particulate delivery of drugs [24,25]. However; there are limited reports on the use of fat from Irvingia wombolu drug delivery system [21]; hence the interest in this novel lipid. Use of structured lipid matrices comprising two or more lipids in the preparation of lipid-based drug delivery have advantages of improved carrier capacity and stability and could be utilized in the preparation of lipospheres.

Lipospheres are particulate lipid dispersions of solid spherical particles of a particle size range 0.2 to 100 μm in diameter consisting of solid hydrophobic fat core such as triglycerides or fatty acids derivatives; stabilized by monolayer of phospholipid [26]. They have advantages over other delivery systems. Compared to emulsion based systems; including vesicles and liposomes; they have more stability and are more effectively dispersed than most suspension based systems. Further; the substance to be delivered does not have to be soluble in the vehicle since it can be dispersed in the solid carrier. Lipospheres also have a lower risk of reaction of substance to be delivered with the vehicle than in emulsion system because the vehicle is a solid material. Moreover; the release rate of substance from the lipospheres can be manipulated by altering either or both the inner solid vesicle and the outer phospholipid layer [27]. In this work we report formulation of gentamicin-loaded lipospheres using structured lipids from plants.

Materials and methods

Materials

Gentamicin (JUHEL Pharmaceutical Limited; Awka; Nigeria); ethanol; n-hexane; ethyl acetate (Sigma-Aldrich; Germany); hydrochloric acid; sodium hydroxide; monobasic potassium phosphate and Tween 80 (Merck; Darmstadt; Germany); activated charcoal (Bio-Lab. (UK) Limited; London); sorbitol (Across Organics; Germany); distilled water (UNN Water Resources; Nsukka; Nigeria). Moringa oil and Irvingia wombolu fats were obtained from a batch processed in our laboratory.

Extraction and purification of fats

The seeds of Moringa oleifera was purchased from Orba market in Enugu; Nigeria; in the month of February; 2013 and was authenticated by Mr. A.O. Ozioko; a consultant taxonomist with the International Center for Ethnomedicine and Drug Development (InterCEDD) Nsukka; Nigeria and the voucher specimen has been deposited in the herbarium of the Department of Pharmacognosy and Environmental Medicines; University of Nigeria; Nsukka. The shells of the seeds were removed manually and the seeds were milled with a blender (Moulinex Pika-lica) and extracted in a soxhlet extractor using 95 % ethanol [12,21]. The ethanol was allowed to completely evaporate at room temperature.

Irvingia wombolu kernels (popularly called “Ado” by the Igboes’ Nigeria) were also purchased from Nsukka market; Enugu State; Nigeria in the month of June: 2012 from Orba Market; Nsukka; Nigeria and authenticated. The seeds were milled with a hammer mill (500# grinder; China) and extracted in a soxhlet extractor using n-hexane [12,21]. The n-hexane was allowed to completely evaporate at room temperature. The extracted fat was successively depolymerised using boiling water and ethylacetate. Both fats extracts were further purified by passing them through a column of activated charcoal and bentonite (2:1) at 100 °C at a ratio of 10 g of fat and 1g of the column material [12]. The fats were stored in a refrigerator until used.

Formulation of structured lipid matrices

The structured lipid matrices were formulated using Irvingia wombolu fats and Moringa oil (2:1) and Phospholipon 90H and Irvingia wombolu fats (1:3). In
each case the lipids were weighed (Adventurer; Ohaus; China) and melted together in a beaker placed on a magnetic stirrer hot plate (SR1 UM 52188; Remi Equip.; India) and maintained at 70°C. The lipids were stirred until a homogenised transparent melt was obtained.

**Formulation of lipospheres**

The gentamicin-loaded and unloaded lipospheres were formulated by melt homogenization using the lipid matrices. The compositions of are shown in Table 1. A 5 g quantity of the lipid matrix was weighed and melted in a beaker placed on a magnetic stirrer hot plate at 70°C. Gentamicin was incorporated into the lipid melt. Distilled water containing the lyoprotectant (sorbitol); the preservative (sorbic acid) and the surfactant (Soluplus®) was maintained at 70°C. The hot aqueous phase was transferred into the lipid phase and homogenized at 5000 rpm for 10 min using an Ultra-turrax homogenizer (T25 Basic Digital; Ika Staufen; Germany). An emulsion (o/w) was obtained by phase inversion. The lipospheres were lyophilised using freeze-dryer (Amsco/Finn-Aqua Lyovac GTZ; Germany).

**Determination of yield**

The yield of lipospheres (%) was calculated using the formula:

\[
\text{Percentage Yield (\%) = } \left( \frac{W1}{W2+W3} \right) \times 100
\]

Where; W1 is weight of SLMs formulated (g); W2 is weight of rifampicin added (g) and W3 is weight of lipid; sorbitol; sorbic acid and Tween 80 (g) [12].

**Table 1: Composition of lipospheres.**

<table>
<thead>
<tr>
<th>Batch</th>
<th>LM combination</th>
<th>LM (%)</th>
<th>Sorbitol (%)</th>
<th>Sorbic acid (%)</th>
<th>Soluplus® (%)</th>
<th>Gentamicin (%)</th>
<th>Distilled water qS (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>IWF:MO</td>
<td>2:01</td>
<td>5</td>
<td>0.05</td>
<td>2</td>
<td>0.5</td>
<td>100</td>
</tr>
<tr>
<td>A2</td>
<td>IWF:MO</td>
<td>2:01</td>
<td>5</td>
<td>0.05</td>
<td>2</td>
<td>1</td>
<td>100</td>
</tr>
<tr>
<td>A3</td>
<td>IWF:MO</td>
<td>2:01</td>
<td>5</td>
<td>0.05</td>
<td>2</td>
<td>1.5</td>
<td>100</td>
</tr>
<tr>
<td>A4</td>
<td>IWF:MO</td>
<td>2:01</td>
<td>5</td>
<td>0.05</td>
<td>2</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>B1</td>
<td>P90H:IWF</td>
<td>1:03</td>
<td>5</td>
<td>0.05</td>
<td>2</td>
<td>0.5</td>
<td>100</td>
</tr>
<tr>
<td>B2</td>
<td>P90H:IWF</td>
<td>1:03</td>
<td>5</td>
<td>0.05</td>
<td>2</td>
<td>1</td>
<td>100</td>
</tr>
<tr>
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<td>1:03</td>
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<tr>
<td>B4</td>
<td>P90H:IWF</td>
<td>1:03</td>
<td>5</td>
<td>0.05</td>
<td>2</td>
<td>0</td>
<td>100</td>
</tr>
</tbody>
</table>

*LM: lipid matrix; IWF: Irvingia wombolu fats; MO: Moringa oil; P90H: Phospholipon 90H. Batches A1, A2 and A3 contain 0.5, 1.0, and 1.5% gentamicin and IWF: MO; B1, B2 and B3 contain 0.5, 1.0, and 1.5% gentamicin and P90H: IWF respectively, while Batches A4 and B4 contain no drug.

**Differential scanning calorimetry**

The differential scanning calorimetry (DSC) of Moringa oil; P90H; Irvingia wombolu fats; lipid matrices; gentamicin pure sample and the gentamicin-loaded lipospheres were analysed using a calorimeter (Netzsch DSC 204 F1; Wittelsbacherstraße 42 95100 Selb; Germany). In each case; 1 mg of each material was weighed into an aluminum pan; hermetically sealed and the thermal behaviour determined in the range of 10-400°C at a heating rate of 10 k/min under a 20 ml/min nitrogen flux. Baselines were determined using an empty pan; and all the thermograms were baseline corrected.

**Particle size and morphology**

The particle size of the lipospheres was determined by computerized image analysis. Little quantity of the lipospheres was dispersed in water on a microscope slide; covered with a cover slip and imaged under a binocular microscope (Wetzler; Hesse; Germany) attached with a Motic image analyser (Moticam; Fujian; China (Mainland)); at a magnification of x400. The particle morphologies were also observed [21].

**Time-dependent pH stability studies**

The pH of dispersions of gentamicin-loaded and unloaded lipospheres were determined at 1 day; 1 week and 3 weeks using a pH meter (pH ep® Hanna instrument; Padova; Italy.

**EE and loading capacity**

A 0.5 g quantity of the lipospheres was extracted with 100
ml of water by gentle shaking for 60 min. The extract was diluted with water; filtered with a filter paper (Whatman no. 1) and the absorbance readings were determined using UV spectrophotometer (Spectrumlab 752S; Breda; The Netherlands) at 203 nm. The concentrations of the drug were determined with reference to Beer's plot.

The EE% was calculated using the equation:
\[
EE\% = \frac{(Actual \ drug \ content)}{(Theoretical \ drug \ content)} \times 100 
\]

LC was determined using the relationship:
\[
LC = \frac{(Amount \ of \ API \ encapsulated)}{(Weight \ of \ lipid)} \times 100 
\]

In vitro release studies

The release of gentamicin from the lipospheres was studied using 500 ml of freshly prepared phosphate buffer (pH 7.4) maintained at 37 ± 0.5 °C. A 500 mg quantity of lipospheres was placed in a polycarbonate dialysis membrane (MWCO 6000-8000; Spectrum Labs; Breda; The Netherlands) (previously soaked in the medium for 24 h); containing 2 ml of the medium and securely tied with a non reactive thermo-resistant thread. It was immersed and firmly suspended in the dissolution medium. The paddle was rotated at 100 rpm; 5 ml portions of the dissolution medium were withdrawn at time intervals; samples were filtered; appropriately diluted and spectrophotometrically analysed at 341 nm. The volume of the dissolution medium was kept constant by replacing it with 5 ml of fresh medium after each withdrawal. Concentrations were determined with reference to Beer's plot and the procedure was repeated for each batch.

In vitro release kinetics

Various kinetic models were used to describe the in vitro release kinetics and mechanisms of release of gentamicin from lipospheres. The zero-order kinetics describes the systems where the drug release rate is independent of its concentration (Eq. 4). The first order Equation (Eq. 5) describes the release from systems where release rate is concentration dependent. Higuchi [28]; described the release of drugs from insoluble matrix as a square root of time dependent process based on Fickian diffusion (Eq. 6).

\[
Q = K_0 t 
\]

\[
\log Q_0 \log Q = K_1 t/2.303 
\]

\[
Q = K_2 t^{1/2} 
\]

where Q is the amount of drug released or dissolved at time t; Q0 is the initial concentration of drug; \( K_0 \); \( K_1 \); \( K_2 \) and \( K_3 \) are zero-order; first-order and Higuchi kinetic constant. The following plots were made: cumulative drug release versus time (zero-order); log cumulative of % drug remaining vs. time (first order kinetic model) and cumulative % drug release vs. square root of time (Higuchi model) [29].

Inhibition zone diameter

The plate agar diffusion method was used for this study. Molten nutrient agar (20 ml) was inoculated with 0.1 ml of Bacillus subtilis broth culture. It was mixed thoroughly; poured into sterile Petri dishes and rotated for even distribution of the organisms. The agar plates were allowed to set and a sterile cork borer (8 mm diameter) was used to bore six cups in the seeded agar medium. Different concentrations (100; 50; 25 and 12.5; 6.25 and 3.125 μg/ml) of the different batches of gentamicin-loaded lipospheres and the reference sample (pure gentamicin); were prepared. A 1 ml volume of each of the samples was added; respectively; into the different holes in each of the plates using Pasteur pipettes. The plates were allowed to stand at room temperature for 15 min to enable the samples to diffuse into the medium before incubating at 37 °C for 24 h. The diameter of each inhibition zone was measured. The procedure above was repeated for Salmonella typhi and E. coli (500; 250; 125; 62.5; 31.25 and 15.625 μg/ml was adopted). The diameter of each inhibition zone was measured and the average determined [1].The experiment in each case was carried out in three replicates.

Data and statistical analysis

Data were analyzed using SPSS Version 16.0 (SPSS Inc. Chicago; IL.USA) and one-way ANOVA. All values were expressed as mean ± SD. Differences between means were assessed using student's t-test and p < 0.05 was considered statistically significant.

Results

Differential scanning calorimetry

The results of the DSC thermograms of gentamicin and the lipids are shown in figure 1. The thermograms of gentamicin showed that the drug exhibited endothermic melting peak at 286 °C. The DSC thermograms of Irvingia wombolu fat showed a sharp endothermic melting peak at 42.3 °C; the thermograms of Moringa oil showed endothermic melting peak at 41°C; the thermograms of
P90H showed endothermic melting peak at 124 °C. The DSC results of the lipid matrices formulated with P90H and IWF showed melting peaks at 42.5 °C; while the LM produced with IWF and MO had endothermic melting peak at 42 °C. The thermograms of gentamicin-loaded lipospheres (Batch A1) showed broad endothermic peak of gentamicin between 250 to 270 °C.

**Figure 1**: DSC thermograms of some materials used. IWF: *Irvingia wombolu* fats; MO: Moringa oleifera seed oil; P90H: Phospholipon 90H.

**Particle size and morphology**

The results of the particle size and morphology of gentamicin-loaded and unloaded lipospheres are shown in table 2 and figure 2. Particle size ranged from 8 to 15 μm and were spherical in shape.

**Yield**

The results of the percentage yield of lipospheres are shown in table 2 and showed that the lipospheres generally exhibited high recovery that ranged from 91 to 96 %. The high percentage recovery may be attributed to the reliable and reproducible method adopted in formulating the lipospheres. There were also losses accruing from transference at various stages.

**EE and loading capacity**

The results of the PDC of gentamicin-loaded lipospheres are shown in table 2 and the results showed that the range were 69 to 84 % for lipospheres formulated with IWF:MO and 70 to 90 % for lipospheres formulated with P9OH:IWF.

**pH stability**

The results of the pH stability studies of gentamicin-loaded and unloaded lipospheres are shown in figure 3. pH ranged from 5.1 to 5.4 at day one and 4.6 to 5.2 at day 30. The results showed that the formulations exhibited an insignificant decline in pH over time ($p < 0.05$).

**Figure 2**: Photomicrographs of gentamicin-loaded lipospheres; A1 was formulated with IWF and Moringa oil; while B1 was formulated with P90H and IWF.

**Figure 3**: The pH of gentamicin-loaded and bland lipospheres formulations; batches A1; A2 and A3 contain 0.5; 1.0; and 1.5 % gentamicin and IWF: MO; B1; B2 and B3 contain 0.5; 1.0; and 1.5 % gentamicin and P90H: IWF respectively; while Batches A4 and B4 contain no drug.

**In vitro release**

The results of the in vitro release of gentamicin from the lipospheres are shown in figure 4 and showed that the formulations exhibited sustained release properties over the study period. At 0.25 h about 11 and 13 % of gentamicin were released from Batches A3 (containing
IWF:MO) and B3 (P90H:IWF) and 1.5 % gentamicin respectively. Also about 50 % of gentamicin was released from Batches A3 (containing IWF:MO) and B3 (P90H:IWF) and 1.5 % gentamicin respectively at 5 h. At 12 h about 93 % of gentamicin was released from Batch A3.

Also about 50 % of gentamicin was released from Batches A3 (containing IWF:MO) and B3 (P90H:IWF) and 1.5 % gentamicin respectively. Also about 50 % of gentamicin was released from Batches A3 (containing IWF:MO) and B3 (P90H:IWF) and 1.5 % gentamicin respectively at 5 h. At 12 h about 93 % of gentamicin was released from Batch A3.

Figure 4: In vitro release properties of gentamicin-loaded lipospheres in phosphate buffer; pH 6.8. Batches A1; A2 and A3 contain 0.5; 1.0; and 1.5 % gentamicin and IWF: MO; B1; B2 and B3 contain 0.5; 1.0; and 1.5 % gentamicin and P90H: IWF respectively.

In vitro release kinetics

The results of the in vitro release kinetics are shown in table 3. The regression analysis (r2) for the zero-order plots were linear (r2= 0.944 to 0.971). The first order plots also were linear; showing that drug release from the lipospheres were by mixed order. The Higuchi plots were linear; which showed that drug release followed diffusion controlled process.

Antimicrobial properties

The results of the IZD shown in figure 5 and showed that the formulations and the reference had higher activities against salmonella typhi. The order of antimicrobial activities was Salmonella typhi > E. fecalis > Staphylococcus aureus > Klebsiella pneumonia >E. coli.

Discussion

The DSC were studied in order to determine the thermotropic characteristics of the materials and to evaluate the crystallinity and the purity of the lipids and the drug. The results showed that the DCS thermograms of IWF; Moringa oil and P90H gave sharp endothermic peaks attesting to the fact that the lipids are of high purity. The thermograms of gentamicin pure sample also showed that the drug is pure and crystalline. The thermograms of the lipid matrices formulated with the lipid combinations showed that they all yielded a single peak; with reduced enthalpies. Ordinarily; one would expect higher transition temperature due to the melting transition of the P90H; but the phospholipid being amphiphilic; was actually solubilized in the molten Irvingia wombolu fats with lower melting point. Consequently; two transitions should not be expected as the phospholipid was molecularly dispersed in the IWF [21]. Reduction in enthalpy generally suggests less crystallinity of lipid matrices [12].

The DSC results of the structured lipid matrices showed that the structuring of P90H with Irvingia wombolu fats and P90G with Moringa oil respectively produced matrices with reduced enthalpies. There were modifications in the microstructure of the individual lipids respectively as a result of inclusion of a second lipid either Irvingia wombolu fats or Moringa oil. The lipid matrices generated imperfect matrices [12]. Lipids with less crystallinity or higher disorder are desirable because of the presence of more spaces for drug localization which would enhance the encapsulation of drugs and increase the loading capacity of the lipids.

The particles were within the limits for lipospheres [21]. Particle sizes of unloaded lipospheres were significantly lower than the gentamicin-loaded lipospheres (p < 0.05). The results also revealed that increase in drug loading significantly increased the particle size of the lipospheres. This could be due to an increase in the loading capacity of the inner fat core of the LM with increase drug loading.

The EE reduced with increase in the quantity of drug incorporated. Lipospheres having 0.5 % drug showed the highest EE values (Batches A1 and B1). This may be due to saturation solubility of the drug in the lipid matrix.
Table 2: Result of absolute drug content and EE%.

<table>
<thead>
<tr>
<th>Batch</th>
<th>Yield (%)</th>
<th>Particle size (μm)</th>
<th>TDC* (%)</th>
<th>ADC (%)*</th>
<th>EE (%)</th>
<th>LC (g API/ 100 g lipid)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>91.4</td>
<td>8.20 ± 0.96</td>
<td>0.25</td>
<td>0.21</td>
<td>84</td>
<td>4.2</td>
</tr>
<tr>
<td>A2</td>
<td>93.7</td>
<td>12.51 ± 0.86</td>
<td>0.5</td>
<td>0.38</td>
<td>76</td>
<td>7.6</td>
</tr>
<tr>
<td>A3</td>
<td>94.1</td>
<td>15.32 ± 0.72</td>
<td>0.75</td>
<td>0.523</td>
<td>69</td>
<td>10.46</td>
</tr>
<tr>
<td>A4</td>
<td>96</td>
<td>3.56 ± 0.45</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>B1</td>
<td>91.9</td>
<td>7.58 ± 0.52</td>
<td>0.25</td>
<td>0.227</td>
<td>90</td>
<td>4.54</td>
</tr>
<tr>
<td>B2</td>
<td>95.2</td>
<td>10.89 ± 0.45</td>
<td>0.5</td>
<td>0.425</td>
<td>85</td>
<td>8.5</td>
</tr>
<tr>
<td>B3</td>
<td>93.3</td>
<td>14.21 ± 0.62</td>
<td>0.75</td>
<td>0.521</td>
<td>70</td>
<td>10.42</td>
</tr>
<tr>
<td>B4</td>
<td>95.6</td>
<td>5.10 ± 0.38</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*TDC: Theoretical drug content; ADC: Actual drug content; EE: Encapsulation efficiency; LC: Loading capacity; Batches A1, A2 and A3 contain 0.5, 1.0, and 1.5 % gentamicin and IWF: MO; B1, B2 and B3 contain 0.5, 1.0, and 1.5 % gentamicin and P90H: IWF respectively, while Batches A4 and B4 contain no drug.

Table 3: In vitro release kinetics data of gentamicin-loaded lipospheres.

<table>
<thead>
<tr>
<th>Batch</th>
<th>Zero order</th>
<th>First order</th>
<th>Higuchi</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r2</td>
<td>r2</td>
<td>r2</td>
</tr>
<tr>
<td>A1</td>
<td>0.961</td>
<td>0.91</td>
<td>0.951</td>
</tr>
<tr>
<td>A2</td>
<td>0.971</td>
<td>0.92</td>
<td>0.931</td>
</tr>
<tr>
<td>A3</td>
<td>0.955</td>
<td>0.905</td>
<td>0.95</td>
</tr>
<tr>
<td>B1</td>
<td>0.976</td>
<td>0.939</td>
<td>0.938</td>
</tr>
<tr>
<td>B2</td>
<td>0.944</td>
<td>0.973</td>
<td>0.968</td>
</tr>
<tr>
<td>B3</td>
<td>0.967</td>
<td>0.976</td>
<td>0.978</td>
</tr>
</tbody>
</table>

Batches A1, A2 and A3 contain 0.5, 1.0, and 1.5 % gentamicin and IWF: MO; B1, B2 and B3 contain 0.5, 1.0, and 1.5 % gentamicin and P90H: IWF respectively.

The lipospheres generally exhibited overall high EE values. EE varied significantly across the sub-batches. However; EE was not significantly affected by the lipid matrix compositions; both lipid matrices used exhibited high encapsulation efficiency and could be used effectively in formulating gentamicin lipospheres.

The results of loading capacity of the lipid matrices also shown in table 2 showed that loading capacity increased with increase in drug loading in agreement with previous research [21].

The pH was studied in order to study the stability of the formulations in water over time. This is because of the possibility of presenting the dosage form as reconstitutable powder. Most of the formulations were stable. However; the decline seen in some of the batches may be due to release of free fatty acids from the lipids [12].

The in vitro release showed that the formulations exhibited good sustained release properties and could be recommended for once daily application. The results also revealed that there was no burst release in any of the formulations. The results of the release kinetics revealed that drug release were by dissolution and diffusion as depicted by values in table 3.

The results of the antimicrobial studies showed that gentamicin-loaded lipospheres showed insignificantly higher antibacterial properties against the test organism than the pure sample ($p < 0.05$). Moringa oil has been claimed to possess antibacterial properties [21]; which may be the reason for the synergism in antibacterial properties. These results showed that the formulation technique adopted were reliable as there was no reduction in the antibacterial properties of the formulations.

Conclusion

IWF and MO lipid matrices were successfully employed in gentamicin extended release oral lipospheres. Interestingly; the lipospheres had extended release properties and also exhibited good stability. MO impacted
an additional antimicrobial property on formulations containing it. This concludes that formulation of oral gentamicin with the use of these lipids has advantages in anti microbial chemotherapy in addition to the health benefits of phytolipids and plausibly economical concern for pharmaceutical industries.

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Declaration of interest

The authors state no conflicts of interest and have received no funding for the research or in the preparation of this manuscript.

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