Original Research Article

# Investigation of novel solid lipid microparticles based on homolipids from *Bos indicus* for the delivery of gentamicin

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## Abstract

**Background:** The aim of this study was to formulate solidified reverse micellar solution (SRMS)-based solid lipid microparticles (SLMs) using homolipids from tallow fat (*Bos indicus*) and evaluate its potential for enhanced delivery of gentamicin. **Materials and Methods:** SLMs were formulated by melt-emulsification using SRMS (15% w/w Phospholipon® 90G in 35% w/w *Bos indicus*), polyethylene glycol 4000 (PEG) and gentamicin (1.0, 2.0, 3.0% w/w), and characterized with respect to size, morphology, encapsulation efficiency % and pH-dependent stability. The *in vitro* release of gentamicin from the SLMs was performed in phosphate buffer (pH 7.4) while bioevaluation was carried out using clinical isolates of *Staphylococcus aureus* and *Escherichia coli*. **Results:** Results showed that the lipid matrix accommodated gentamicin in a concentration-dependent manner, and that stable and spherical SLMs with size range of 18.62 ± 1.24-20.59 ± 1.36 μm and 21.35 ± 1.57-50.62 ± 2.37 μm respectively for unloaded and drug-loaded formulations were obtained. The *in vitro* drug release studies revealed that SRMS-based SLMs could better be used to control the release of gentamicin than gentamicin injection. Results of sensitivity test revealed that the SLMs time-dependently and capacity-limitedly produced greater inhibition zone diameters (IZDs) than the standards, an indication of improved bioactivity against the test organisms, with greater IZDs against *S. aureus* than *E. coli*. Overall, SLMs containing 2% w/w SRMS, 3% w/w gentamicin and PEG 4000 entrapped the highest amount of drug, achieved complete drug release and gave highest IZD against the organisms within 420 min, while plain gentamicin gave the least. **Conclusion:** This research has shown that SLMs based on *Bos indicus* and P90G is a potential carrier system for dissolution and bioactivity enhancement of gentamicin.

**Key words:** *Escherichia coli*, solidified reverse micellar solutions, *Staphylococcus aureus*

## INTRODUCTION

There is increasing need to design and develop suitable drug carrier systems to control, localize and improve drug delivery.[1] However, designing a drug delivery system is challenging in terms of targeting the drug to specific sites and also to improve its therapeutic value with minimal side effects. Many different drug carriers can be used depending on the route of administration, among other factors. Oral drug administration is the most commonly employed route owing to its safety as well as convenience and ease of administration to the patient.[2] However, the development of oral forms of many drugs remains a challenge either on account of their stability or their absorption from the gastrointestinal tract (GIT) thus

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leading to sub-therapeutic bioavailability.[3] To overcome the poor absorption capacity of such drugs an array of lipid systems such as emulsions, micellar solutions, liposomes, lipid nanoparticles, structured lipid carriers, self-emulsifying lipid formulations, solid dispersions, dry emulsions, solid-liquid compacts, and drug-lipid conjugates is available to drug formulators.[4] Among the various lipid systems, solid lipid microparticles (SLMs) have been developed to address some issues such as stability and low payload capacity of some lipid systems.[5] SLMs have a lower risk of the reaction of the substance to be delivered to the vehicle than in emulsion system. In addition, the release rate of substance from the SLMs can be manipulated by altering either one or both the inner solid vesicle or the outer phospholipid layer. The combination of the solid inner core with phospholipid exterior ensures high dispersibility in an aqueous medium, and a release rate for the entrapped substance that is controlled by phospholipid coating and carrier, among other advantages.[1,6] SLMs offer a high solubilization rate for different types of drugs.[7-11] In addition, they have been widely investigated as potential drug delivery systems for hydrophilic drugs which encounter penetration and absorption problems.[12,13]

Gentamicin, a broad-spectrum hydrophilic bactericidal antibiotic of the aminoglycoside group, acts by inhibition of protein synthesis after binding to specific 30S-subunit ribosomal proteins.[14,15] It is very poorly absorbed from the GIT and is unstable in acidic pH of the stomach.[16] More so, its cationic nature affects its penetration into the mucosal walls of the GIT. Hence it is commonly administered topically, intramuscularly, intravenously, and subcutaneously.[17] Gentamicin is active against a wide range of human bacterial infections, mostly Gram-negative bacteria including *Pseudomonas*, *Proteus*, *Serratia*, *Escherichia coli* and the Gram-positive bacteria such as *Staphylococcus aureus*.[18] Like other aminoglycosides, gentamicin is toxic to the sensory cells of the ear and also causes nephrotoxicity by inhibiting protein synthesis in renal cells. This mechanism specifically causes necrosis of cells in the proximal tubule, resulting in acute tubular necrosis that can lead to acute renal failure.[19]

By tactical engineering of lipid-based drug delivery systems (LBDDS) such as solidified reverse micellar solution (SRMS)-based SLMs, the toxicity, penetration and absorption problems of gentamicin could be surmounted. Researchers have used this novel technology (SRMDS) to increase the overall efficacy while minimizing the toxicity of gentamicin.[2,12-14] Homolipids and heterolipids have gained renewed interests as excipients for LBDDS.[20-24] Homolipids are esters of fatty acids with various alcohols. Tallow fat is an edible animal fat derived from tallow fat (*Bos indicus*). SLMs based on *Bos indicus* has been evaluated as a basis for delivery of piroxicam.[25]

Thus, the aims of this study were to formulate SRMS (lipid matrix) consisting of P90G and *Bos indicus*, and SRMS-based SLMs containing gentamicin using melt-emulsification technique and evaluate the *in vitro* dissolution and bioactivity of gentamicin from such a delivery system.

## MATERIALS AND METHODS

### Materials

The following materials were used: Gentamicin pure sample (JUHEL Pharmaceutical Limited, Awka, Nigeria), tallow fat (a biodegradable homolipid was obtained from *Bos indicus* and purified in our laboratory), Phospholipon® 90G (Phospholipid GmbH, Köln, Nattermann, Germany), poloxamer 188 (Sigma Aldrich, Spain), polyethylene glycol 4000 (PEG) (Acros Organics, USA), monobasic potassium phosphate, sodium hydroxide and concentrated hydrochloric acid (BDH, England) and distilled water (Lion Water, UNN, Nigeria). All other reagents and solvents were analytical grade and were used as supplied.

### Extraction and purification of tallow fat from *Bos indicus*

The homolipid was extracted from the adipose tissue of *Bos indicus* by wet rendering following standard procedures.[23-26] Briefly, tallow fat was extracted from the adipose tissue of *Bos indicus* which was collected from freshly slaughtered cow, manually freed of extraneous materials, crushed and boiled in distilled water for 45 min, filtered through a muslin cloth and allowed to solidify at room temperature. The solid fat was manually removed and bleached/deodorized by passing it through a mixture of activated charcoal and bentonite (2:1) at 100°C at a ratio of 10 g of the fat and 1 g of the column material.

### Preparation of lipid matrix (solidified reverse micellar solution) and solid lipid microparticles

Lipid matrix consisting of a mixture of 35% w/w tallow fat (homolipid) and 15% w/w Phospholipon® 90G (P90G) was prepared by fusion method.[6-8] Briefly, the tallow fat and P90G were weighed using electronic balance (Mettler H8, Switzerland), placed into a crucible, melted together at 75°C on a thermo-regulated water bath shaker (Heto, Denmark) and stirred thoroughly. Thereafter, the mixture was allowed to cool and solidify at room temperature to obtain the lipid matrix (SRMS).

For the preparation of the SLMs, the melt-emulsification technique was adopted.[9-14] In each case, the SRMS was melted at 75°C, and the aqueous phase containing PEG 4000 and poloxamer 188 at the same temperature was added to the SRMS with gentle stirring with a magnetic stirrer (SR 1 UM 52188, Remi Equip., India), and the mixture was further dispersed with a mixer (T 25 digital Ultra-Turrax®; IKA, Staufen, Germany) at 8000 rpm for 5 min. The SLMs suspension obtained after cooling at room temperature was then lyophilized using a freeze-dryer (Amsco GT3, Germany). The above procedure was repeated using PEG 4000 and gentamicin (1.0, 2.0 and 3.0% w/w) and lipid matrix (4.0, 3.0 and 2.0% w/w), to obtained gentamicin-loaded SLMs (batches $A_1$-$A_3$, $B_1$-$B_3$ and $C_1$-$C_3$). The unloaded SLMs ($D_1$-$D_3$) were also prepared. The formulation compositions are shown in Table 1.

**Table 1: Formulation compositions of the SLMs**

| Batches | PEG 4000 (g) | Poloxamer 188 (g) | Gentamicin (% w/w) | Lipid base (15% w/w P90G in 35% w/w TF) (g) | Distilled water, q.s (% w/w) |
|---|---|---|---|---|---|
| $A_1$ | 1.0 | 2.0 | 1.00 | 4.0 | 100 |
| $A_2$ | 2.0 | 2.0 | 1.00 | 3.0 | 100 |
| $A_3$ | 3.0 | 2.0 | 1.00 | 2.0 | 100 |
| $B_1$ | 1.0 | 2.0 | 2.00 | 4.0 | 100 |
| $B_2$ | 2.0 | 2.0 | 2.00 | 3.0 | 100 |
| $B_3$ | 3.0 | 2.0 | 2.00 | 2.0 | 100 |
| $C_1$ | 1.0 | 2.0 | 3.00 | 4.0 | 100 |
| $C_2$ | 2.0 | 2.0 | 3.00 | 3.0 | 100 |
| $C_3$ | 3.0 | 2.0 | 3.00 | 2.0 | 100 |
| $D_1$ | 1.0 | 2.0 | — | 4.0 | 100 |
| $D_2$ | 2.0 | 2.0 | — | 3.0 | 100 |
| $D_3$ | 3.0 | 2.0 | — | 2.0 | 100 |

Batches $A_1$-$A_3$, $B_1$-$B_3$ and $C_1$-$C_3$ are gentamicin-loaded SLMs while batches $D_1$-$D_3$ are unloaded (zero-drug) SLMs, P90G: Phospholipon® 90G, TF: Tallow fat, SLMs: Solid lipid microparticles, PEG 4000: Polyethylene glycol 4000

### Particle size analysis and morphological characterization of solid lipid microparticles

The particle size and morphology of the SLMs were determined by computerized image analysis. Briefly, approximately 5.0 mg of the SLMs from each batch was dispersed in distilled water and smeared on a slide (Marinfield, Germany) using a glass rod. It was then covered with a cover slip and viewed with a photomicroscope (Hund®, Weltzlar, Germany) attached with a digital camera at a magnification of ×1000. With the aid of the software in the photomicroscope, the particle morphologies were observed and photomicrographs taken. The sizes of the particles were measured and average taken.

### Determination of encapsulation efficiency % and loading capacity

Approximately 0.5% w/v dispersion of the SLMs in distilled water was prepared, allowed to equilibrate for 48 h at room temperature, shaken, and filtered. The filtrate was adequately analyzed for gentamicin content spectrophotometrically (Unico 2102 PC UV/Vis4 Spectrophotometer, USA) at 203 nm. The amount of drug encapsulated in the SLMs was calculated with reference to a standard Beer's plot for gentamicin to obtain the EE % using the formula:[12-14]

$$EE\ \% = \frac{\text{Actual drug content}}{\text{Theoretical drug content}} \times 100 \quad (1)$$

LC expresses the ratio between the entrapped active pharmaceutical ingredient (API) and total weight of the lipids.[18] It is determined as follows:

$$LC = \frac{W_a}{W_l} \times 100 \quad (2)$$

Where $W_l$ is the weight of lipid added in the formulation and $W_a$ is the amount of API entrapped by the lipid.

### Time-resolved pH-dependent stability studies

The pH of dispersions of the SLMs from each batch was determined using a pH meter (Suntex TS-2, Taiwan) after 1-week, 1-month, and 3 months of storage.

### *In vitro* drug release studies

Phosphate buffered saline (PBS, pH 7.4) and the USP XXII rotating paddle apparatus (Erweka, Germany) were employed for this release study. The dissolution medium consisted of 500 mL of freshly prepared PBS maintained at 37 ± 1°C by means of a thermostatically controlled water bath. The polycarbonate dialysis membrane used was pretreated by soaking it in PBS for 24 h prior to the commencement of each release experiment. In each case, 300 mg of the formulated SLMs was placed in the dialysis membrane containing 5 mL of the PBS, securely tied with a thermo-resistant thread and then immersed in PBS under agitation provided by the paddle at 100 rpm. At 60 min intervals, 10 ml portions of PBS were withdrawn and replaced with equal volume of PBS to maintain a sink condition, filtered, and analyzed spectrophotometrically at 341 nm. The amount of drug released at each time interval was determined with reference to the standard Beer's plot for gentamicin in PBS. This test was replicated for all the batches, gentamicin pure sample, and commercial gentamicin injection.

### Antimicrobial studies

The antimicrobial activity of the SLMs was tested against clinical isolates of *S. aureus* and *E. coli* by agar diffusion technique using samples withdrawn during the *in vitro* drug release studies.[12] Molten nutrient agar was inoculated with 0.1 ml of *S. aureus* broth culture. It was mixed thoroughly, poured into sterile petri dishes and rotated for even distribution of the organism. The agar plates were allowed to set and a sterile cork borer was used to bore three cups in the seeded agar medium. Using a sterile syringe, a definite volume withdrawn from the receptor compartment of the diffusion apparatus at predetermined time intervals was used to fill the holes. The plates were allowed to stand at room temperature before incubating at 37 ± 1°C for 24 h. The diameter of each inhibition zone was measured and the average determined.[14] The procedure above was repeated for *E. coli*.

### Statistical analysis

All experiments were performed in replicates for validity of statistical analysis. Results were expressed as mean ± standard deviation. ANOVA and Student's *t*-test were performed on the

data sets generated using SPSS (Version 17, SPSS Inc., New York, USA). Differences were considered significant for $P < 0.05$.

## RESULTS AND DISCUSSION

Some physicochemical properties of the SLMs are presented in Table 2 while the photomicrographs of the formulations are depicted in Figure 1. The results indicate that the mean particle sizes of the gentamicin-loaded SLMs and unloaded SLMs were in the range of 21.35 ± 1.57-50.62 ± 2.37 μm, and 18.62 ± 1.24-20.59 ± 1.36 μm, respectively. More so, the results revealed that after 3 months of storage, drug-loaded SLMs, and unloaded SLMs had a mean pH range of 2.23 ± 0.19-4.18 ± 0.79 and 2.27 ± 0.17 to 2.29 ± 0.09, respectively. The photomicrographs [Figure 1] showed that the SLMs were discrete, spherical, and greenish brown. The EE% of the SLMs ranges from 39.20 ± 1.82% to 86.60 ± 3.17%. The EE % [Table 2] increased with increase in gentamicin concentration in the formulations. Thus, sub-batches $C_1$-$C_3$ gave highest EE % while sub-batches $A_1$-$A_3$ gave the least. Table 2 also shows that maximum LC of 52.50, 57.90, and 62.70 g of gentamicin per 100 g of lipid were obtained for sub-batches $C_1$-$C_3$ respectively containing 3% w/w gentamicin.

Results of the physicochemical tests on the SLMs showed that high drug loading resulted in large particle sizes, consistent with earlier reports.[2,12,13] The stability tests, which were carried out to determine the pH stability of the SLMs when stored at different time intervals, revealed an insignificant change ($P > 0.05$) in the pH of the SLMs over a period of 3 months, implying that there was little or no degradation of the drug and/or the excipients used in the formulations within this period. It was discernible from the EE % results that the lipid contents improved the EE % of gentamicin in the SLMs. The implication of the LC results is improved solubility of gentamicin in the lipid matrix. Further incorporation of P90G in the SLMs led to the formation of structured lipid matrix, which invariably enhanced gentamicin encapsulation in the core of the SLMs, consistent with previous reports.[2,12,13] In addition, PEG 4000 being a hydrophilic surfactant improved the solubilization of the drug within the core lipids.[22] Similarly, poloxamer 188 is a nonionic tri-block copolymer composed of a central hydrophobic chain of polyoxypropylene flanked by two hydrophilic chains of polyoxyethylene.[27] This amphiphilic nature and surfactant properties of the polymer, in addition to the lipid components of *Bos indicus* increased the solubility of the drug within the lipid core.[25]

The determination of drug loading (or drug incorporation) is an important tool to evaluate a potential drug carrier system.[9-11,21] It is obviously desirable to formulate SLMs with high drug content to decrease the amount of SLMs to be administered. The prerequisite to obtain a sufficient LC is a sufficiently high solubility of the drug in the lipid melt.[25] The highest drug encapsulation of 86.60 ± 3.17% was obtained from the SLMs which means that the PEGylated lipid matrix of P90G and tallow fat had enormous spaces which accommodated the gentamicin (enhanced dissolution).

Figures 2-4 depict the *in vitro* release profiles of gentamicin from the SLMs in PBS. Drug release from the SLMs followed the order: $C_1$-$C_3$ > $B_1$-$B_3$ > $A_1$-$A_3$. The *in vitro* release profiles

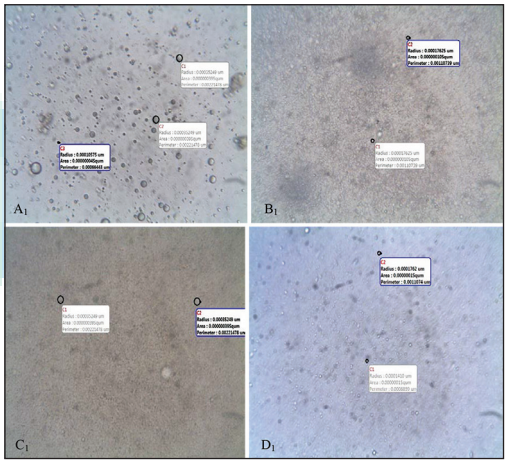


**Figure 1:** Photomicrographs of representative solid lipid microparticles batches. Key: Batches $A_1$, $B_1$, and $C_1$ are gentamicin-loaded solid lipid microparticles while batch $D_1$ is unloaded (zero-drug) solid lipid microparticles

**Table 2: Some physical parameters of the SLMs**

| Batches | Particle size (μm)[a,b] | pH[a,c] | | | EE (%)[a,c] | LC (g API/100 lipid)[c] |
|---|---|---|---|---|---|---|
| | | 1-week | 1-month | 3 months | | |
| $A_1$ | 21.35±1.57 | 2.20±0.51 | 2.25±0.45 | 2.24±0.19 | 39.20±1.82 | 24.20 |
| $A_2$ | 23.50±1.38 | 2.31±0.26 | 2.30±0.81 | 2.29±0.28 | 41.77±2.76 | 28.30 |
| $A_3$ | 25.53±1.72 | 2.21±0.09 | 2.27±0.33 | 2.23±0.93 | 55.10±2.65 | 31.50 |
| $B_1$ | 32.53±1.54 | 3.49±0.92 | 3.46±0.76 | 3.47±0.54 | 66.34±2.43 | 34.10 |
| $B_2$ | 36.70±1.46 | 3.50±0.28 | 3.49±0.98 | 3.48±0.71 | 70.96±2.10 | 41.80 |
| $B_3$ | 38.54±1.99 | 3.46±0.75 | 3.48±0.59 | 3.45±0.37 | 78.20±2.57 | 49.60 |
| $C_1$ | 43.55±2.90 | 4.10±0.81 | 4.13±0.84 | 4.11±0.56 | 82.55±3.82 | 52.50 |
| $C_2$ | 47.50±2.55 | 4.15±0.90 | 4.12±0.91 | 4.13±0.28 | 84.75±3.43 | 57.90 |
| $C_3$ | 50.62±2.37 | 4.17±0.76 | 4.20±0.99 | 4.18±0.79 | 86.60±3.17 | 62.70 |
| $D_1$ | 18.62±1.24 | 2.27±0.27 | 2.30±0.09 | 2.28±0.15 | — | — |
| $D_2$ | 19.51±1.79 | 2.28±0.99 | 2.27±0.18 | 2.29±0.09 | — | — |
| $D_3$ | 20.59±1.36 | 2.31±0.38 | 2.30±0.27 | 2.27±0.17 | — | — |

[a]Mean ± SD, [b]$n = 30$, [c]$n = 3$, Batches $A_1$-$A_3$, $B_1$-$B_3$ and $C_1$-$C_3$ are gentamicin-loaded SLMs while batches $D_1$-$D_3$ are unloaded (zero-drug) SLMs, EE: Encapsulation efficiency, LC: Loading capacity, SLMs: Solid lipid microparticles, SD: Standard deviation, API: Active pharmaceutical ingredient

indicate controlled release of gentamicin from the SLMs. In batch A formulations, sub-batch $A_3$ gave a maximum release of 92% while sub-batch $A_1$ gave the least (maximum release of 70%). Similarly, in batch B SLMs, sub-batch $B_3$ released the highest amount (i.e., 96%) of gentamicin while sub-batch $B_1$ released the least amount (77% of gentamicin). Furthermore, in batch C formulations, sub-batch $C_3$ recorded complete drug release while sub-batch $C_1$ released 83% of the encapsulated drug. Commercial gentamicin injection ($G_1$) and gentamicin pure sample ($G_2$) gave 65% and 62% drug release, respectively. Drug release is highly dependent on the compositions of the carrier system. The high and rapid release of gentamicin from the SLMs may be related to high rate of hydration and swelling of the SLMs in the medium, which might be attributed to the lipophilicity imparted on the drug by the excipients used in preparing the SLMs.[9-11] The controlled release observed in the study could be a consequence of the decreasing residual amount of drug in the SLMs and the build-up of drug concentration in the dissolution medium in the course of time.[25]

The anti-microbial results recorded as inhibition zone diameter (IZD) [Tables 3 and 4] indicate that gentamicin-loaded SLMs produced very significant IZD ($P < 0.05$) against Gram-positive organism (*S. aureus*) and Gram-negative organism (*E. coli*). The formulations recorded increasing IZDs against the organisms with time. Moreover, gentamicin-loaded SLMs gave greater IZDs than the plain gentamicin as well as commercial gentamicin injection against the organisms. Overall, sub-batch $C_3$ containing the highest PEG 4000 and gentamicin gave the greatest IZD against *S. aureus* (27.49 ± 2.38 μm) and *E. coli* (29.40 ± 3.07 μm)

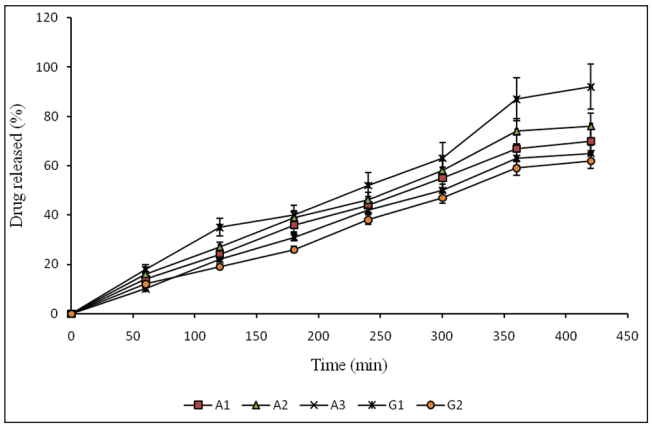


**Figure 2:** *In vitro* release profile of gentamicin from batch A solid lipid microparticles in phosphate bufferred saline, pH 7.4 ($n$ = 3). Key: $A_1$-$A_3$ contain 1.0%w/w of gentamicin while $G_1$ and $G_2$ are commercial gentamicin injection and plain gentamicin, respectively

**Table 3: Susceptibility of *Staphylococcus aureus* to gentamicin in the SLMs**

| Batches | IZD (mm)[a,b] | | | | | | |
|---|---|---|---|---|---|---|---|
| | Time (min) | | | | | | |
| | 60 | 120 | 180 | 240 | 300 | 360 | 420 |
| $A_1$ | 3.98±0.03 | 5.09±0.01 | 7.00±0.16 | 9.13±0.08 | 11.25±1.82 | 13.87±1.06 | 14.72±0.87 |
| $A_2$ | 4.00±0.27 | 6.32±0.54 | 8.19±0.09 | 10.08±0.12 | 12.28±1.75 | 14.64±1.17 | 16.00±1.96 |
| $A_3$ | 5.13±0.95 | 7.83±0.18 | 9.08±0.45 | 11.43±1.21 | 13.93±0.81 | 15.48±1.88 | 18.30±1.66 |
| $B_1$ | 4.63±0.84 | 6.82±0.50 | 9.53±0.26 | 12.95±0.98 | 15.27±1.09 | 18.14±1.19 | 19.63±1.57 |
| $B_2$ | 5.46±0.91 | 7.37±0.76 | 10.19±0.73 | 13.44±2.11 | 16.94±1.02 | 19.86±1.00 | 20.46±1.40 |
| $B_3$ | 6.03±0.78 | 9.16±0.53 | 11.62±0.78 | 14.95±0.36 | 17.32±0.53 | 24.18±1.13 | 26.53±1.39 |
| $C_1$ | 4.03±0.90 | 7.18±0.24 | 10.75±1.13 | 13.46±1.88 | 17.72±1.07 | 20.09±1.48 | 23.87±1.11 |
| $C_2$ | 5.59±0.22 | 8.73±0.49 | 11.10±1.87 | 14.89±1.01 | 18.64±1.55 | 22.47±1.89 | 25.59±1.25 |
| $C_3$ | 6.40±0.16 | 9.28±0.07 | 12.98±1.06 | 15.76±1.33 | 20.99±1.90 | 25.00±1.66 | 30.40±1.08 |
| $G_1$ | 3.18±0.09 | 4.64±0.18 | 5.99±0.15 | 7.05±0.22 | 9.19±0.14 | 11.19±1.75 | 13.47±1.87 |
| $G_2$ | 3.00±0.15 | 4.19±0.03 | 5.07±0.04 | 6.38±0.64 | 7.77±0.81 | 9.15±0.08 | 11.98±1.09 |

[a]Mean ± SD, [b]$n$ = 3, $A_1$-$A_3$, $B_1$-$B_3$ and $C_1$-$C_3$ are SLMs containing 1.0, 2.0 and 3.0% w/w of gentamicin respectively, $G_1$ and $G_2$ are commercial gentamicin injection and plain gentamicin, respectively, SD: Standard deviation, SLMs: Solid lipid microparticles, IZD: Inhibition zone diameter

**Table 4: Susceptibility of *Escherichia coli* to gentamicin in the SLMs**

| Batches | IZD (mm)[a,b] | | | | | | |
|---|---|---|---|---|---|---|---|
| | Time (min) | | | | | | |
| | 60 | 120 | 180 | 240 | 300 | 360 | 420 |
| $A_1$ | 3.04±0.15 | 4.89±0.09 | 6.47±0.17 | 7.78±0.85 | 9.15±0.16 | 11.37±1.50 | 12.72±1.94 |
| $A_2$ | 3.95±0.30 | 5.07±0.52 | 7.55±0.26 | 8.83±0.05 | 10.74±0.52 | 12.91±1.37 | 14.53±1.60 |
| $A_3$ | 4.68±0.19 | 6.97±0.07 | 8.66±0.49 | 10.34±0.74 | 12.52±1.09 | 14.81±1.55 | 17.56±1.35 |
| $B_1$ | 3.96±0.08 | 5.37±0.09 | 8.23±0.91 | 11.19±1.08 | 14.62±1.33 | 17.89±1.34 | 18.32±1.17 |
| $B_2$ | 5.02±0.41 | 6.98±0.13 | 9.67±0.27 | 12.87±1.32 | 15.63±1.07 | 18.95±1.10 | 19.64±1.46 |
| $B_3$ | 5.95±0.70 | 7.62±0.65 | 10.03±0.52 | 13.83±0.96 | 18.95±1.28 | 23.56±1.09 | 25.35±1.89 |
| $C_1$ | 3.99±0.55 | 5.59±1.71 | 8.43±1.10 | 11.96±1.71 | 14.82±1.54 | 18.19±1.14 | 21.22±1.30 |
| $C_2$ | 5.03±0.82 | 8.29±1.08 | 12.80±1.23 | 16.24±1.00 | 19.07±1.00 | 22.26±0.18 | 24.87±1.61 |
| $C_3$ | 6.00±0.27 | 9.57±1.64 | 13.23±1.10 | 17.04±1.55 | 20.68±1.51 | 24.80±1.56 | 28.49±1.82 |
| $G_1$ | 3.02±0.84 | 3.46±0.47 | 4.71±0.18 | 6.64±0.23 | 8.98±0.58 | 10.16±0.91 | 12.87±1.74 |
| $G_2$ | 2.98±0.60 | 3.28±0.30 | 4.59±0.81 | 5.77±0.17 | 6.14±0.92 | 8.81±0.50 | 10.77±0.90 |

[a]Mean ± SD, [b]$n$ = 3, $A_1$-$A_3$, $B_1$-$B_3$ and $C_1$-$C_3$ are SLMs containing 1.0, 2.0 and 3.0% w/w of gentamicin respectively, $G_1$ and $G_2$ are commercial gentamicin injection and plain gentamicin, respectively, SD: Standard deviation, SLMs: Solid lipid microparticles, IZD: Inhibition zone diameter

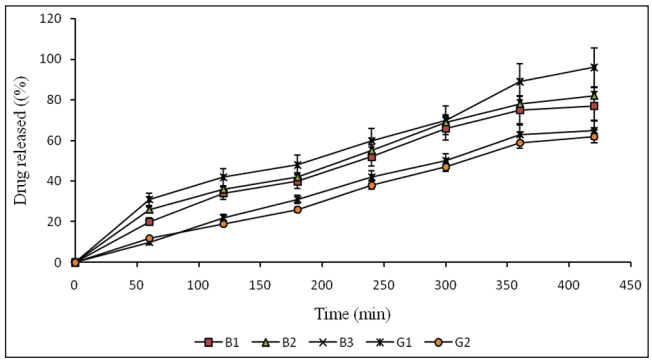


**Figure 3:** *In vitro* release profile of gentamicin from batch B solid lipid microparticles in phosphate bufferred saline, pH 7.4 ($n$ = 3). Key: $B_1$-$B_3$ contain 2.0%w/w of gentamicin while $G_1$ and $G_2$ are commercial gentamicin injection and plain gentamicin, respectively

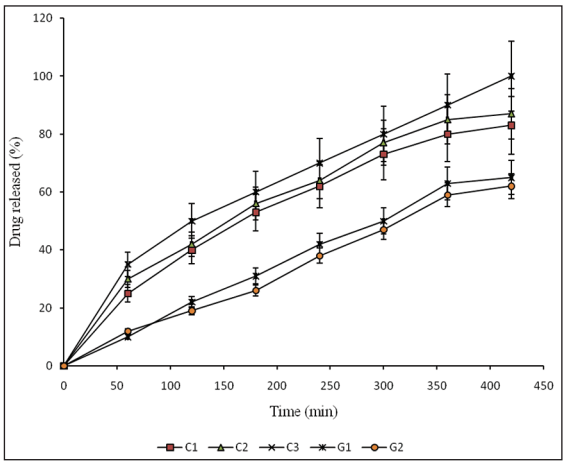


**Figure 4:** *In vitro* release profile of gentamicin from batch C solid lipid microparticles in phosphate bufferred saline, pH 7.4 ($n$ = 3). Key: $C_1$-$C_3$ contain 3.0%w/w of gentamicin while $G_1$ and $G_2$ are commercial gentamicin injection and plain gentamicin, respectively

after 420 min, whereas plain gentamicin and commercial gentamicin injection respectively gave IZDs of 13.47 ± 1.87 µm and 11.98 ± 1.09 µm against *S. aureus* and 12.87 ± 1.74 µm and 10.77 ± 0.90 µm against *E. coli* after 430 min. The bioevaluation was performed to establish that gentamicin did not lose activity during formulation as well as to show an increasing IZD over time during the *in vitro* dissolution study. Generally, the SLMs produced very significant ($P < 0.05$) IZDs against the organisms. Gentamicin is active against *S. aureus* and *E. coli*.[15-19] It was observed that the greater the amount of gentamicin loaded into the SLMs, the greater the IZD produced, in agreement with earlier reports.[12-14] By implication, the SLMs exhibited capacity limited bioactivity. Similarly, the antibacterial activity of the formulations was concentration and time-dependent, manifested by an increasing IZD against the organisms with time. High IZDs recorded against the organisms early in the study was an indication that these formulations would have exhibited the fastest release of the entrapped drug, hence the fast antibacterial activities; whereas time-dependent increases in IZDs within 420 min implies that the SLMs had potentials for sustained drug release. The improved lipid solubility conferred on the drug by the amphiphilic and surfactant properties of the solubilizers (active and passive) ensures sustained delivery of the drug through the gradual and sustained erosion of the lipid core. Furthermore, the SLMs generally gave greater IZDs than plain gentamicin and commercial gentamicin injection against the organisms. It is equally discernible from the results that the formulations were more active against *S. aureus* than *E. coli*. Overall, batch $C_3$ gave the greatest IZD against the organisms. This formulation would be a useful alternative to gentamicin injection for enhanced delivery of gentamicin in the treatment of infections caused by gentamicin-susceptible micro-organisms, thus encouraging further development of this formulation.

## CONCLUSION

This research has shown that PEGylated SLMs based on a homolipid from tallow fat (*Bos indicus*) and phospholipid (P90G) is a potential carrier system for dissolution and bioactivity enhancement of gentamicin. Compared with commercial gentamicin injection, the bioactivity and *in vitro* drug release studies undertaken with the formulations provided a basis to establish that SRMS-based SLMs could better be used to control the release of gentamicin. Further studies would seek to evaluate these formulations by employing pharmacokinetic models in experimental animals.

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### Conflicts of interest

There are no conflicts of interest.

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