Original Research Article

ORAL GENTAMICIN SULPHATE NANOEMULSION FOR SYSTEMIC INDICATION: FORMULATION AND EVALUATION

ABSTRACT

Aim: To achieve systemic availability of an oral dosage form of gentamicin sulfate (GS), typically impermeable via the gastrointestinal tract.

Study Design: Formulation and physical characterization of GSnanoemulsion. Stability studies and *ex vivo* bioavailability evaluation of the emulsion.

Place and Duration of Study: Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University Awka, Anambra State, Nigeria; between January 2021 and May 2023.

Methodology: Pre-formulation experiments were conducted with adjuvants for use for the formulation. Using a homogenizer, an aquaphilicGS nanoemulsion was prepared with the adjuvants, including polysorbate 80 and span 80, as permeation enhancers.Theemulsionwasevaluated for itsglobulesize and polydispersityindex, and real-time physical stability parameters using pH and relative viscosity. Thebioavailabilityofgentamicinsulphatewasassessed as*exvivo*anti-*E.coli*activityafteroraladministrationoftheemulsiontoWisterrats and a confirmatory test was done using gas chromatograph-flame ionization detection (GC-FID) analysis.

Results: Theemulsion hadanaverageglobulesizeof66.55nm,andismonodispersedwith a polydispersityindexof0.45.The pH and viscosity values measuredfor60days ranged from 6.60±0.00 to 6.80±0.00 and .03±0.00 to .03±0.01 respectively (*P = .05*). The *ex vivo* antibacterial activity of the GS emulsion was significant at 3.00±0.00 mm 1 –hr post-administration to the Wistar rats,indicatingsystemicavailabilityof theantibiotic. The GC-FID analysis confirmed the presence of the drug in the serum.

Conclusion: The bioavailability of oral GS is achievable through simple specialized formulations containing adjuvants which contributes to an improved gastrointestinal permeability of the drug.

KeyWords:Gentamicin sulphate, Oral gentamicin bioavailability, Nanoemulsion, Oral administration, Permeation enhancer.

1. INTRODUCTION

Gentamicin sulphate (GS) is a broad-spectrum antibiotic for treating susceptible bacterial infections and has been presented in several dosage forms (1-5). It is very effective for systemic infections caused by microorganisms such as septicemia, meningitis, pneumonia, osteomyelitis, septic arthritis, pyelonephritis, urinary tract infections, sepsis, and surgical site infections (6, 7). GS is an important antibiotic for treating gram-negative bacilli and gram-positive cocci infections.There is also considerable interest in its use for treating hereditary diseases caused by nonsense mutations that create premature termination codons and lead to the production of truncated or non-functional proteins (8). It belongs to the aminoglycoside group of antibiotics (9, 10) and is reportedly administered as a mixture of five main C-subtypes partly because of the difficulty in chemically separating the individual components (4). Molecular gentamicin is a complex of species stated to have three major and several minor components with gentamicins C1, C1a, and C2 being the three major components of the drug complex (11). The C2 component consists of two stereoisomers (C2 and C2a). However, the component ratios in commercial GS preparations vary remarkably and are generally unknown (11, 12). GS is classified into class III of the Biopharmaceutics Classification Systems (BCS) due to its high water solubility and very poor intestinal permeability. Being hydrophilic and with its poor intestinal membrane permeability, the medical use (for systemic delivery) is confined to injection forms, which has several disadvantages (13). The oral route of GS administration for systemic effect is not common because of this low trans-intestinal absorption of the drug.

Oral administration of a non-systemic solid dosage form of GS for local activity in the gastrointestinal tract (GIT) has been achieved (14), and other complex formulations of the drug aimed at achieving permeation through the GIT mucosa into the systemic circulation and across the blood-brain-barrier (BBB) have also been reported (15, 16) but yet a simple oral formulation of the drug having systemic effect and commercial validity has been a challenge. One such example was its complex formulation as a surface-modified self-nano emulsifying formulation (SNEF) (16). In that study, the emulsions were structured with PEG4000 while employing a rational blend of surfactants with good homogenization, to obtain nano-sized particles. In the study by (13), a self-double emulsifying drug delivery system (SDEDDS) of GS was prepared by response surface methodology. Selected optimized formulations were evaluated for zeta potential, optical clarity, release at 420 minutes, emulsion stability index, and self-emulsification time and the *ex vivo* study on intestinal permeability revealed that SDEDDS improved membrane gentamicin permeability compared to drug solution. Although attempts have been made to formulate an oral GS drug in forms that would achieve a systemic effect and facilitate the crossing of the BBB; there is a need to seek cost-effective simple formulations that will overcome the permeability barrier posed by the intestinal mucosa. More so, the inclusion of adjuvants with inherent antibacterial effects in these simple formulations can provide an added advantage.
The goal of this research is to formulate and evaluate a simple oral aquaphilic GS nanoemulsion that will be systemically available after *ex vivo* characterization using laboratory animals. The adjuvants to be included during the formulation shall include a blend of permeation enhancers and a lab-extracted pumpkin seed oil (PSO).

2. MATERIALS AND METHODS

2.1 Materials

Fresh pumpkin seeds (purchased in Awka, Nigeria), Petroleum ether (Griffin and George, India), Gentamicin Sulphate (obtained from Juhel Pharmaceutical Nigeria Industry Ltd., Enugu Nigeria), Polysorbate-80 and Span-80 (Qualikems, India), Purified water (Nnamdi Azikiwe University Awka Nigeria), N-hexane and Sodium Sulphathein (Springfield Research Institute Lab, Awka, Nigeria), Mueller hinton agar and McCkonkey agar (Hi-media Chemical Ltd. India). All other microbiological reagents like the nutrient broth and nutrient agar were all of analytical grade.

2.2 Methods

2.2.1 Extraction of PSO

Fresh pumpkin seeds were carefully peeled and then dried under a tree shade(out of direct sunlight) for 3 days. The dried seeds were then ground with a household blender with a mill (AKAI BD038A-1031 Japan). The milled material was placed in a column within the collecting chamber of a Soxhlet extractor (B54R37RL4J Eisco Labs, U.S.A). Petroleum ether was added to the collection chamber and utilized to extract the PSO. After extraction, the oil was placed in a flat stainless steel container and stored in a dark location until the solvent scent was no longer detectable.

2.2.2Formulation of a Stock Surfactant Solution (SSS) and *In vitro* Antibacterial Activity Check of the PSO and SSS

SSS is a blend of permeation enhancers developed at the Department of Pharmaceutics and Pharmaceutical Technology drug research unit of Nnamdi Azikiwe University, Awka, Nigeria by a combination of polysorbate 80 and span 80 in a 9:1 ratio (the ratio realized through pre-formulation studies) and under homogenization of 10,000 rpm for 15 minutes at room temperature. The SSS so obtained had a potent emulsifying capacity.

Combinations of PSO and SSS, in different volumetric ratios, were assessed for *in vitro* antibacterial activity using a susceptible strain of *E. coli*. Antibacterial activity was tested by introducing exactly a total of 80 microliters (µL) of the PSO, SSS, or their combinations into holes bored in nutrient agar plates that were already seeded with *E. coli*. The plates were replicated into three and the readingswere taken. Incubation of the plates was for 24 hours at 37ºC. The average inhibition zone diameters (IZDs) were thereafter recorded.

2.2.3Formulation of Emulsion

Five batches of the emulsion were initially prepared by combining the PSO and SSS in different ratios **(Table 1)**. The 'SSS' served the dual purpose of an emulsifier and a hybrid permeation enhancer.

To produce the emulsions, the required portions of PSO and SSS for each batch were combined in a beaker and homogenized at 10,000 rpm for 15 minutes. Thereafter, 10 ml of purified water containing 500 mg of GS was introduced into each of the mixes. Homogenization was continued for the next 5 minutes and the products were packaged and properly stored. These gave a drug concentration of 5 mg/ml in the emulsion.

Table 1. The formula for the emulsion

Batch	PSO: SSS ratios	PSO-SSSmix(ml)	Water (ml)	GS Drug (mg)

1	1:9	90	10	500
2	2:8	90	10	500
3	3:7	90	10	500
4	4:6	90	10	500
5	5:5	90	10	500

2.2.4 Bio-guidedCalibrationof GSSolutions

A calibration curve of a pure sample of GSdrug was achieved by constituting different aqueous solutions containing 5, 10, 20, 40, and 80 mg/ml of the antibiotic. Equal volumes (80 µL) of the solutions were introduced into cups carved in nutrient agar gel that was already seeded with the susceptible strain of *E. coli.* The plates were incubated for 24 hours at 37ºC. Zones of inhibition were measured, and the mean values were plotted against the log10 of their corresponding GS concentration values.

2.2.5 *Ex vivo* Antibacterial Activity of the Emulsion (using Batch 1)

A total of twelve (12) Wistar rats comprising males and non-pregnant females weighing between 250 and 300 g were grouped into four - labeled A to D, with 3 rats in each group. The rats were acclimatized at laboratory room temperature (33 ± 2ºC) for two weeks with free access to chicken grower mash and drinking water.

Group A served as negative control and was administered with PSO-SSS oral mix at 13.33 ml/kg body weight (bw). Group B rats also served as negative control and were administered with an aqueous solution of GS orally at 71.4 mg/kg bw. Group C rats received the formulated emulsion orally at a dose of 71.4 mg/kg bw. Group D rats served as positive control and were administered with a commercially available GS injection intra-peritoneally at a dose of 10.66 mg/kg bw.

The blood samples of the animals were then collected via the retroorbital plexus using heparinized tubes at varying intervals of 0.5, 1, and 2 hourspost-treatment and immediately stored in ethylenediamine tetraacetic acid (EDTA) bottles. When needed for use, the blood samples were processed into sera with a centrifuge at a speed of 6500 rpm. The sera were then introduced unto holes bored on *E. coli*-seeded nutrient agar and incubated for 24 hours at 37ºC after diffusion. The zones of inhibition were recorded and the mean values were noted.

The dosingwas obtained considering only the maximum tolerable dose in an adult body weight for non-absorbable gentamicin sulphate for groups A – C as referenced in (14). Group D dosing is the exact dosing of the commercially available and standardized product.

2.2.6 Gas Chromatograph Flame Ionization Detection (GC-FID) Analysis of GS

Serum obtained from Group C animals1 hour post-administration of the emulsion, was used for the gas chromatography-flame ionization detector (GC-FID) analysis. For the analysis, extraction was carried out by dissolving 0.1 g of the serum sample in 1 ml of dichloromethane and extracted with 2 ml of phosphate buffer at pH 6.0. The dichloromethane layer was used for the analysis. Separation was carried out at 50ºC on a reversed-phase Lichrospher C8 60 RP column, of 250 mm. The chosen flow rate was 0.5

ml/min. Ionization was performed in positive selected ion monitoring (SIM) mode; the ions m/z values were 468.20 for GS. The temperature was 35ºC and the drying gas (12 L/min) and nebulizer gas (60 psi) were both nitrogen. For the mobile phase, a linear gradient of pentafluoropropionic anhydride (PFPA) (20 mM in ultrapure water) and hexane were used. For the chromatographic quantification of GS, extracted samples were diluted with equal volumes of a co-solvent system consisting of equal proportions of PFPA: methanol (1:1) and ether. 0.1 ml was injected into the column, maintaining the injected extract constant.
For comparison, this stated procedure was repeated with a prepared standard GS solution.

2.2.7 Globule Size Analysis and Polydispersity Index (PDI) of the GS Emulsion
The globule size analysis and PDI of theselected batch 1 emulsion was done using a zeta sizer (Zen Malvern 1600, Malvern instrument U.S.A) at a temperature of 25ºC.

2.2.8 Relative Viscosity (RV) Evaluation
The RV of the selected batch 1 emulsion was measured atregulated lab and cold temperatures of 33 ± 2ºC and 6 ± 2ºC respectively using the formula below and was evaluated using an Ostwald viscometer (SearchTech 1007 India) over sixty days (days 0, 7, 14, 28, 60). The mean values from triplicate recordings were calculated and used.

$$RV = \frac{(De \times Tfe \times Vw)}{(Dw \times Tfw)} \qquad (1)$$

Where 'De' = density of the emulsion.
'Tfe' = time of flow of emulsion through the equipment.
'Vw' = viscosity of water.
'Dw' = density of water.
'Tfw' = time of flow of water through the equipment.

2.2.9 pHEvaluation
The pH of the selected batch 1 emulsion was measured at regulated lab and cold temperatures of 33 ± 2ºC and 6 ± 2ºC respectively. The pH was measured post-formulation on days 0, 7, 14, 28, and 60; after the pH meter (SearchTechPHS-3C 1233) was calibrated with standard phosphate buffer solutions. The mean values from triplicate recordings were calculated and noted.

3. RESULTS AND DISCUSSION

3.1 PercentageYieldofPSO Extract
ThepercentageyieldofthePSO extractobtainedviaSoxhletextractionwas 75.84%.

3.2*Invitro*AntibacterialActivityResultofPSOandSSS
The inhibitory activities of PSO, SSS, or their combinations on a susceptible strain of *E. coli* were evaluated and recorded **(Table 2)**. The result shows a progressive reduction in anti-microbial activity with higher PSO quantity and also shows that the SSS has better antimicrobial activity than the PSO. The result obtained informed the combination ratio of

these adjuvants that would be used for the GS nanoemulsion formulation. The inhibition zone diameters (IZDs) obtained for PSO and SSS alone were converted to gentamicin equivalent concentration by bioassay method (17), as shown in Table 3. This was achieved by superimposing the IZDs produced by each of the adjuvants on the standard GS calibration curve **(Figure 1)** to obtain the GS equivalent concentrations. This translates into the GS equivalent of the adjuvant that will produce the stated zone of inhibition.
The PSO and SSS individually have an inherent anti-*E. coli* activity with GS equivalent of 3.7 mg/ml and 17.8 mg/ml respectively as seen from the results below**(Table 3)**. This confirms that their combination for use has a significant synergistic interaction, with a GS equivalent of 23.2 mg/ml. SSS served as a permeation enhancer as well as an emulsifier.

Five batches of emulsion were formed with the PSO and SSS, with compositions as shown in Table 1 above. These batches were evaluated initially based on physicochemical properties alone. Batch 1 emulsion containing more SSS (that has been proven in Table 2 to contribute significantly to a higher anti- *E. coli* activity) was also the most stable after a freeze-thaw cycle and was therefore selected for further analysis (*ex vivo* investigation, globule size determination, viscosity, and pH check).

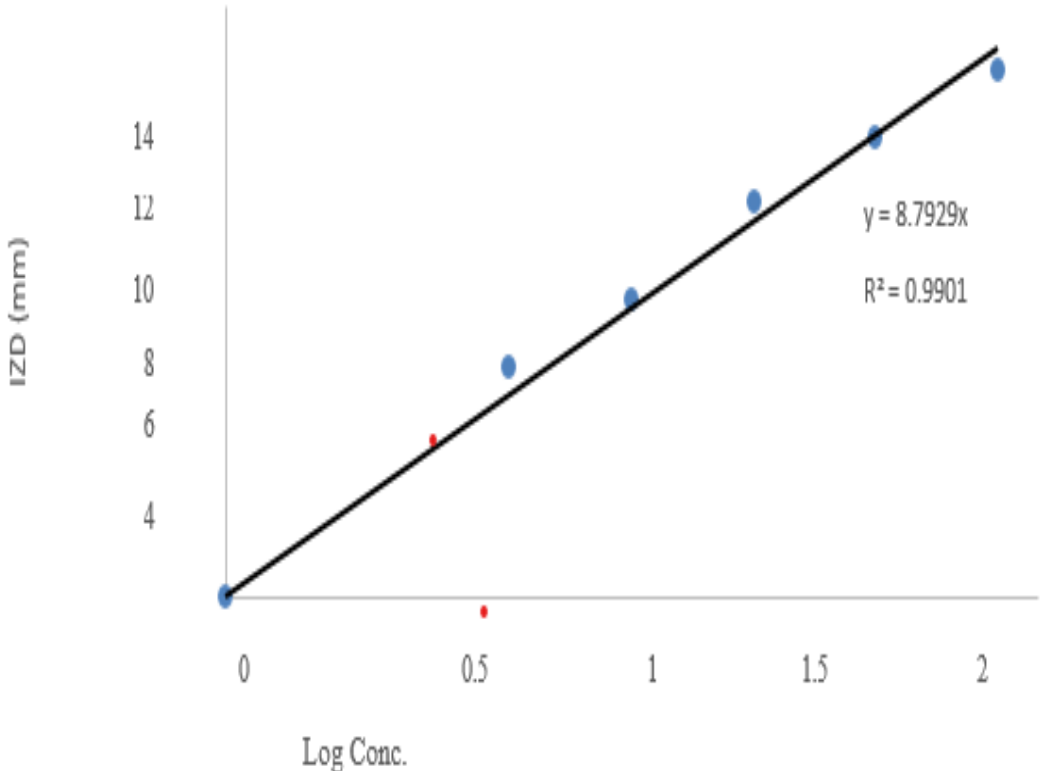

Figure1. Calibrationcurveofgentamicinsulphate (GS)using*E.coli*

PSO: SSS Ratios	Actual Values Used (in microliters)	Mean Inhibition Zone Diameters (mm)
0:10	0:80	11.00 ± 0.00
1:9	8:72	12.00 ± 0.00
2:8	16:64	10.67 ± 0.47
3:7	24:56	10.00 ± 0.00
4:6	32:48	9.67 ± 0.47
5:5	40:40	8.00 ± 0.80
6:4	48:32	7.67 ± 0.47
7:3	56:24	7.00 ± 0.00
8:2	64:16	6.00 ± 0.82
9:1	72:8	6.00 ± 0.00
10:0	80:0	5.00 ± 0.82

Table 2. *In vitro* activity of the PSO and SSS used for the formulation

Table 3. GS equivalent concentrations of the adjuvants (PSO and SSS)

Adjuvants	IZD(mm)	GS equivalent (mg/ml)
PSO	5.0 ± 0.82	3.7
SSS	11.0 ± 0.00	17.8
PSO/SSS mix	12.0 ± 0.00	23.2

3.3 Mean Particle Globule Size and Polydispersity Index (PDI) of the GS Nanoemulsion

The emulsion obtained was monodispersed with an average globule size of 66.55 nm, and PDI of 0.45. This also confirms the emulsion as a nanoemulsion.

3.4 *Ex Vivo* Antibacterial Activity (Systemic Bioavailability Result)

The formulations were administered by oral route to the rats to ascertain if the adjuvants and the homogenization technique employed during the formulation would aid the transport of GS across the GIT mucosa, thereby reaching the systemic circulation.

Following the administrations of PSO-SSS mix alone, the aqueous GS, the formulated GSnanoemulsion, and parenteral GS to groups A to D animals respectively; the result showed that the drug appeared in the serum **(Table 4)**. The group C animal serum exhibited anti- *E. coli* activity 30 minutes (0.5 hours) and 1 hour post-administration of the drug. The adjuvants alone (PSO and SSS) didn't appear in the serum after oral administration as seen with the group A animals but helped aidthe transport of GS into the bloodstream of the group C animals. This activity witnessed is most likely due to the effect of the SSS which has been shown from the preliminary findingsof this study, to possess anti- *E. coli* activity more than the PSO. The combination of the adjuvants was still helpful as it's already revealed in this study that they are synergistic in action.

It can be seen that the first placebo (PSO & SSS, without the drug) and the second placebo (pure GS solution) both administered orally did not produce activity. However, we have reported that the placebo which constitutes only PSO and SSS showed activity *in vitro* as indicated in Tables 2 and 3. A probability exists; in the sense that in line with normal metabolic processes, antibacterial principles present in the oil or SSS would have been inactivated during first-pass metabolism. The oral GS solution administered to the negative control didn't show any presence in the serum, as expected. However, the GSnanoemulsion administered orally and the parenteral dosage form administered intra-peritoneally appeared in the blood in enough concentrations to exert antibacterial activity.

The orally administered nanoemulsion (5mg/ml) given at a drug dose of 71.4 mg/kg exhibited declining time-dependent activity *ex vivo*. This is an indication of the very short

plasma life of GS in the formulated product.(14) have demonstrated that GS administered orally at doses below 500 mg/kg would not sustain therapeutic concentrations in the systemic circulation. The implication also is that a dose of 71.4 mg/kg (approximately 5 grams per 70 kg human bw) used in this study is not unduly high in an attempt to attain and sustain therapeutic plasma concentrations. The serum from the test group that received the oral emulsion exhibited the highest inhibition zone diameter (IZD) of 7mm after 30 minutes. Using the calibration curve equation for GS: Y=8.7929x in Figure 1, it thus means that:

$$IZD = 8.7929\,(\log \text{concentration}) \qquad (2)$$

We can deduce that assuming equilibrium volume of distribution, the serum concentration of the drug in a 0.25kg rat is about 6.25mg (and that is to say at most about 25mg/kg). Generally, toxicity can occur at higher doses usually above 7mg/kg/day with prolonged use or impaired kidney function when taken parenterally, and 7mg/kg is the recommended dose for a required pharmacokinetic and pharmacodynamics target (18). It is worth stating that pharmaceutical and clinicians researchers are always looking for novel drug carriers to maintain the drug level in the body in low and effective doses (9) but no reliable data exists presently on the maximum safe tolerable dose for oral GS administration, but it's expected to be much higher than that of the parenteral limit due to expected first-pass metabolism of the drug. However, this calls for more research to ascertain the exact safety limit of oral gentamicin formulations in humans and it can be very conservative to say that the dosage used in this study may be recommended for short-term use in patients with healthy kidney function, as there is an assured systemic circulation achieved with this study.

The produced nanoemulsion dosage form proposes two (2) desirable effects. Firstly, it can accommodate the large doses of GS required for trans-intestinal permeation and therapeutic plasma concentrations. And secondly, the chosen emulsifier 'the SSS', which also serves as a hybrid permeation enhancer facilitates GS permeation across the GIT mucosa to attain desired systemic concentrations.

Table 4.*Ex vivo* Antibacterial Activity Result

		Mean IZD Activity (in mm)		
Treatment mode	**Dose**	**0.5 hr**	**Ihr**	**2hrs**
PSO-SSS mix (oral)	13.33ml/kg	0.00±0.00	0.00±0.00	0.00±0.00
Aqueous GS (oral)	71.40mg/kg	0.00±0.00	0.00±0.00	0.00±0.00
GS nanoemulsion (oral)	71.40mg/kg	7.00±0.00	3.00±0.00	0.00±0.00
GS (parenteral)	10.66mg/kg	10.00±0.00	8.00±0.10	8.00±0.00

3.5 GC-FID Systemic Bioavailability Result

The GC-FID test was conducted to confirm that GS in the serum was responsible for the recorded antibacterial activity. This was done using the serum sample collected at 1 hour post-administration of the emulsion. The appearance of the peak in serum (A) and pure drug in solution (B) at nearly the same regions of the GC spectra (16.416 and 16.233 respectively) confirms that GS was present in the serum, and was responsible for the antibacterial action**(Figure 2)**.

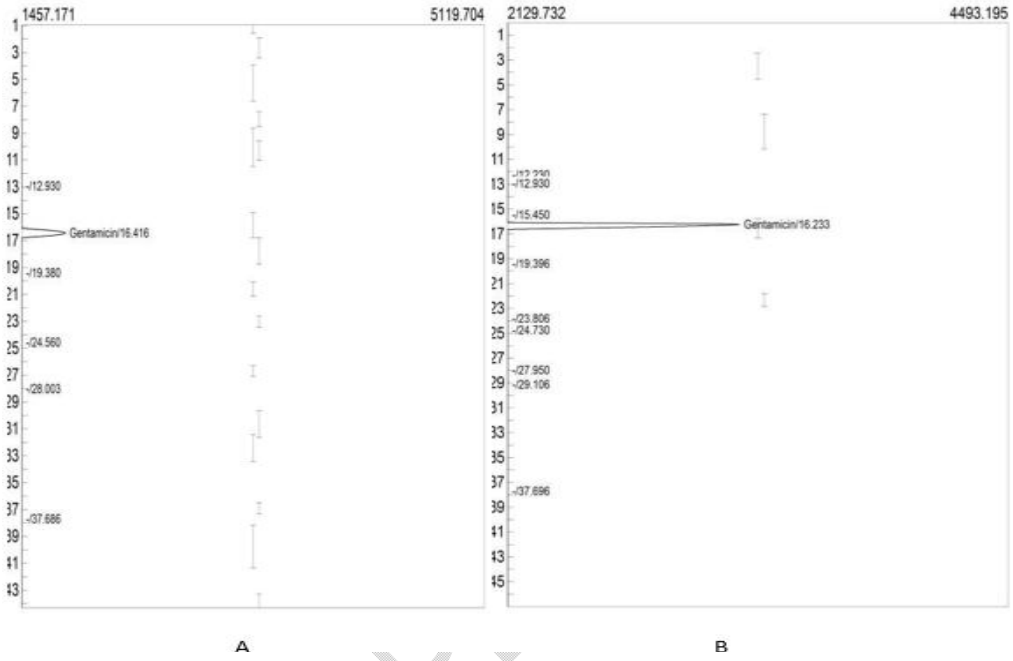

Figure 2: GC-FIDspectraofA(Nanomulsion)andB(PureGS)

3.6 PH and Relative Viscosity Results

Physicochemical properties exhibited by the nanoemulsion could be used to conclude the qualities of the product. It is evident from the result from **Table 5** that the pH and the relative viscosity of the emulsions were not significantly affected during the sixty days of storage at varying temperature conditions*(P =.05)*. The products were physico-chemically stable.

Table 5: PH and Viscosity Results

	RoomTemperature (33± 2°C)					ColdTemperature (6 ± 2°C)			
	Days								
	0	7	14	28	60	7	14	28	60
pH	6.80±0.10	6.70±	6.60±0.00	6.60±	6.60±0.	6.70±0.	6.70±0	6.70±0	6.70±

		0.10		0.00	00	00	.00	.00	0.00
Relative Viscosity (Pa)	.03±0.00	.03± 0.00	.03± 0.10	.03± 0.00	.03± 0.10	.03± 0.00	.03± 0.00	.03± 0.00	.03± 0.00

4. CONCLUSION

Aquaphilic GS nanoemulsions formulated by homogenization, with pumpkin seed oil (PSO) and permeation enhancers as adjuvants; exhibited significant *ex vivo*activity against *E. coli*.The bioavailability of oral GS is achievable through simple specialized formulations containing adjuvants, which contributes to an improved gastrointestinal permeability of the drug.

CONSENT

Not Applicable.

ETHICAL APPROVAL

Animal experiments were conducted after obtaining ethical approval from the Animal Research Ethics Committee (AREC) of Nnamdi Azikiwe University, Awka, Nigeria with approval number NAU/AREC/2022/00003. There wasn't any need for post-operative care and pain management for the animals (as the animal study was an *ex vivo* characterization with the animal serum).

All the authors also declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed.

REFERENCES

1. BārzdiņaA, Plotniece A, Sobolev A, Pajuste K, Bandere D, Brangule A. From Polymeric Nanoformulations to Polyphenols - Strategies for Enhancing the Efficacy and Drug Delivery of Gentamicin. Antibiotics. 2024;13(4):305.

2. Cao X, Dai L, Sun S, Ma R, Liu X. Preparation and Performance of Porous Hydroxyapatite/Poly (Lactic-Co-Glycolic Acid) Drug-loaded Microsphere Scaffolds for Gentamicin Sulfate Delivery. Journal of Materials Science. 2021;56(27):15278-98.

3. Nwankwo I, Edward K, Nwoba C, Uzuegbu N. Antibacterial Activity of Three Broad Spectrum Antibiotics against some Clinical Bacterial Isolates. Nigerian J Microbiol. 2021.

4. O'Sullivan ME, Song Y, Greenhouse R, Lin R, Perez A, Atkinson PJ, *et al*. Dissociating Antibacterial from Ototoxic Effects of Gentamicin C-Subtypes. Proceedings of the National Academy of Sciences. 2020;117(51):32423-32.

5. PurwantiT, Syamsuar M, Hariyadi DM, Erawati T. Characteristics and Release of Gentamicin Sulphate from Sodium Alginate Microspheres Entrapped in Emulgel. Int J Drug Deliv Technol. 2019;9:704-10.

6. Dhal C, Mishra R. *In Vitro* and *In Vivo* Evaluation of Gentamicin Sulphate-loaded PLGA Nanoparticle-based Film for the Treatment of Surgical Site Infection. Drug Delivery and Translational Research. 2020;10:1032-43.

7. EwuzieSR, Nwakile CD, Uronnachi EM, Umeyor CE, Dozie-Nwakile OC. A Review Of Gentamicin Sulphate Oral Preparation Containing Natural Oil (Pumpkin Seed Oil) for Systemic Indication. International Journal of Emerging Trends in Science and Technology. 2023:8032-42.

8. Wang S, Yang Z, Liu Y, Zhao M-T, Zhao J, Zhang H, *et al*. Application of Topical Gentamicin—A New Era in the Treatment of Genodermatosis. World Journal of Pediatrics. 2021;17:568-75.

9. Bayoumi A, Sarg MT, Fahmy TY, Mohamed NF, El-Zawawy WK. The Behavior of Natural Biomass Materials as Drug Carriers in Releasing Loaded Gentamicin Sulphate. Arabian Journal of Chemistry. 2020;13(12):8920-34.

10. NejatiL, Maram NS, Ahmady AZ. Preparation of Gentamicin Sulfate Nanoparticles Using Eudragit RS-100 and Evaluation of their Physicochemical Properties. International Journal of Nanoscience. 2021;20(05):2150049.

11. Bulman ZP, Cirz R, Hildebrandt D, Kane T, Rosario Z, Wlasichuk K, *et al.*Unraveling the Gentamicin Drug Product Complexity Reveals Variation in Microbiological Activities and Nephrotoxicity. Antimicrobial Agents and Chemotherapy. 2020;64(9):10.1128/Aac. 00533-20.

12. Maheshwari M, Memon N, Memon A, Khuhawer M, Memon A. Validated RP-HPLC Method Development for Gentamicin Microanalysis in Pharmaceutical Dosage Forms, Urine and Blood. Pharmaceutical Chemistry Journal. 2021;55(5):524-30.

13. Arka BN, Goutam M, Arpan C, Baishakhi M. Optimization of Self-Double Emulsifying Drug Delivery System Using Design of Experiments for Enhanced Oral Bioavailability of Gentamicin: *In Vitro, Ex Vivo And In Vivo* Studies. Journal of Pharmaceutical Sciences. 2024;113(3):659-68.

14. NwakileDC, Dozie-Nwakile O, Okoye E, UmeyorC, Uronnachi E, Uchendu I, *et al*. Non-Absorbable Oral Gentamicin Sulphate: Biopharmaceutical and Dosage Form Evaluation. European Pharmaceutical Journal. 2021;68(2):8-15.

15. Kenechukwu FC, Momoh MA, Nnamani PA, Attama AA. Solid Lipid Micro-Dispersions (SLMS) Based on PEGylated Solidified Reverse Micellar Solutions (SRMS): A Novel Carrier System for Gentamicin. Drug Delivery. 2015;22(6):710-22.

16. UmeyorC, Attama A, Uronnachi E, Kenechukwu F, Nwakile C, Nzekwe I, *et al*. Formulation Design and *In Vitro* Physicochemical Characterization of Surface Modified Self-Nanoemulsifying Formulations (SNEFS) of Gentamicin. International Journal of Pharmaceutics. 2016;497(1-2):161-98.

17. OkoreV, Umeh I, Nwakile C. Anti-Staphylococcal Activity of Jatropha Curcas Latex in the Presence of some Surfactants Or Human Serum: Implications for Treatment of Skin Infections. Bio-Research. 2007;5(2):285-9.

18. HodiamontCJ, Van Den BA, De Vroom Sl, Prins JM, Mathôt RA, Van Hest RM. Clinical Pharmacokinetics of Gentamicin in Various Patient Populations and Consequences for Optimal Dosing for Gram-Negative Infections: An Updated Review. Clinical Pharmacokinetics. 2022;61(8):1075-94.