

Non-Absorbable Oral Gentamicin Sulphate: Biopharmaceutical and Dosage Form Evaluation

Original Paper

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Received 20 July, 2020, accepted 7 February, 2021

Abstract Gentamicin sulphate is an antibiotic belonging to the aminoglycosides and to class III of the Biopharmaceutical Classification System (BCS). Gentamicin sulphate is highly water soluble, but has very low intestinal permeability. The wide use is because of its broad spectrum of activity. In the current study, the suitability of administering gentamicin sulphate orally for local action against susceptible gastrointestinal tract (GIT) bacteria was investigated. The possibility of the drug escaping into the systemic circulation even in the presence of some permeation enhancers was ascertained. Representatives of potential GIT bacteria pathogens were evaluated for their susceptibility to the drug at concentrations obtainable in the GIT using standard microbiological methods. Dose levels that will inhibit these potential bacteria pathogens were also established, as well as the frequency of their administration. Different batches of oral capsules of 250 mg gentamicin sulphate were formulated and their release profiles ascertained using standard methods. The results showed that the selected representatives of the GIT potential pathogenic bacteria were all susceptible to gentamicin sulphate. The drug at its plausible dosage levels of 14.28 mg/kg (1,000 mg/70 kg), 10.71 mg/kg (750 mg/70 kg) and 7.14 mg/kg (500 mg/70 kg) did not cross the mucosal barrier into the systemic circulation even in the presence of some permeation enhancers. The drug's frequency of administration were found to be on 8-hourly bases. Gentamicin sulphate (250 mg) granules formulated with polyethylene glycol (PEG 4000) as granulating aid were quick drying because the granules were not hygroscopic. The formulated gentamicin sulphate capsule batch released enough concentration of the drug that inhibits the test organism within 2 min of dissolution. The above stated doses are acceptable in the dosage form design; it is possible to formulate non-absorbable oral gentamicin sulphate dosage form for local activity in the GIT using existing conventional solid dosage formulating equipment.

Keywords Oral dosage form – gentamicin sulphate – enterobacteria – permeation enhancers – mucosal barrier – systemic circulation

INTRODUCTION

Gentamicin sulphate is an antibiotic belonging to the aminoglycosides and to class III of the Biopharmaceutical Classification System (BCS) (Pignatello *et al.*, 2016; Baasov *et al.*, 2015; Ito *et al.*, 2005). Gentamicin sulphate is highly water soluble, but has very low intestinal permeability. The drug has been used extensively through parenteral administration in the treatment of wide range of susceptible bacterial infections. The wide use is because of its broad spectrum of activity. Conventionally, gentamicin sulphate is administered parentally (Imamura and Adams, 2003; Labiris *et al.*, 1999). The drug has also been presented for dermal topical applications (Imamura and Adams, 2003; Ipsen *et al.*, 1991; Nnamani *et al.*, 2013; Pickett *et al.*, 1997; Ravis *et al.*, 2013) and as inhalations (Labiris *et al.*, 1999; Aquino *et al.*, 2012; Goldman *et al.*, 1990;

Lim *et al.*, 2002). It has been evaluated for rectal local activity (Fix *et al.*, 1983) as well as implants (Krasko *et al.*, 2007; Soriano *et al.*, 2000).

As noted earlier, the low intestinal permeability makes oral administration, hence formulation, very difficult. However, in veterinary practice, oral gentamicin sulphate has been recommended in weaning swine for the control and treatment of colibacillosis caused by strains of *E. coli* sensitive to gentamicin, and for the control and treatment of swine dysentery associated with *Treponema hyodysenteriae* (Legal Information Institute, 2016). It has also been evaluated in dogs in combination with labrasol to enhance its intestinal mucosa permeability (Rama Prasad *et al.*, 2003).

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In humans, oral administration of gentamicin sulphate has been included in the treatment trial of necrotizing enterocolitis, a condition of death of tissue in the intestine, often occurring mostly in premature or sick babies (Gephart *et al.*, 2012; Shah and Sinn, 2012). This is a form of localised drug action in which the gentamicin sulphate acts on the diseased tissues. The widespread use of gentamicin sulphate through the oral route in humans to achieve systemic therapeutic concentration has been limited by low intestinal permeability. Early researches have centred on the incorporation of permeation enhancers to achieve intestinal absorption and systemic circulation. Verma *et al.* (2014) in a review enumerated some of these permeation enhancers. Attempts have been made to formulate the drug for oral administration by incorporating some of these intestinal mucosa penetration/permeation enhancers to achieve systemic circulation (Anilkumar *et al.*, 2011; Rama Prasad *et al.*, 2003; Shaikh *et al.*, 2012). Umeyor *et al.* (2016) have achieved improved systemic circulation of orally administered gentamicin sulphate by surface modified self-nano-emulsifying formulations (SNEFs). However, the drug has not been formulated into dosage form(s) for local activity in the gastrointestinal tract (GIT). An unaided formulation of the drug administered orally will invariably accumulate in the gut, and the drugs' broad spectrum antibacterial activity could be used for local action against bacteria pathogens inhabiting the GIT. Representative enterobacteria, including, but not limited to, *E. coli*, *S. typhi*, *K. pneumonia*, *S. aureus* etc., have been known to cause diseases in the GIT (Walker *et al.*, 2014).

Gentamicin sulphate powder is well known for its hygroscopic nature. Presenting the drug powder into granules is hindered by atmospheric moisture absorption. Due to the drug powder's hygroscopic nature, wet granulation and direct compression into tablet/capsule dosage forms become impossible. Preliminary investigation had shown that it is possible to use polyethylene glycol 4000 (PEG 4000) as a granulating aid to hold the powder particles together.

In this research, therefore, gentamicin sulphate was evaluated for suitability in the treatment of GIT localised susceptible bacterial infections. The gentamicin sulphate powder was granulated using PEG 4000 as the granulating aid. The granules were formed into different batches of capsules of 250 mg and the release profile of the formed capsules ascertained.

MATERIALS AND METHODS

Selection of susceptible bacteria for the *in vitro* permeability study

The bacteria used were obtained from cultures maintained at the Medical Laboratory Department of University of Nigeria Teaching Hospital (UNTH) Ituku-Ozala, Enugu State, Nigeria. Twenty (20) ml of molten Mueller–Hinton agar was poured

Table 1. Formula for the production of different batches of gentamicin sulphate oral capsules.

Ingredients	Quantity (mg)		
	Batch A	Batch B	Batch C
Gentamicin sulphate	250	250	250
Carb-o-sil®	15	15	15
N-starch	30	30	30
Ac-di-sol®	5	5	5
PEG 4000	50	---	---
PVP	---	50	---
Gelatine powder	---	---	50

(--- no excipient)

into sterile Petri dishes, and standardised concentrations (McFarland 0.5) of overnight cultures of the test isolates were inoculated aseptically on the agar plates. The mixtures were gently rocked and allowed to solidify. Holes of diameter 5 mm were bored at the centre of the agar plates using a sterile metal cork-borer. A 20 µl of 4 µg/ml gentamicin sulphate reconstituted using rat sera and sterile water in the ratio of 1:1 was poured in each hole under aseptic condition. The plates were kept at tropical room temperature (27°C) for 1 h to allow for diffusion. The plates were then incubated at 37°C for 24 h, and the inhibition zones diameters (IZD) measured. The sample that showed the highest IZD (*S. aureus*), as shown in Table 1, was chosen for the *in vitro* permeability study.

Establishment of the baseline gentamicin sulphate dose level

Albino rats (Wister strain) weighing 150–250 g were randomly selected and used for the experiment. Doses of gentamicin sulphate as indicated in Table 2 were administered orally to the rats, and blood collected from the rats through the retro-orbital venous plexus at intervals of 0, 30, 60, 120 and 180 min. The blood was centrifuged, and sera collected from each time interval was introduced into holes bored on solidified 20 ml of molten Mueller–Hinton agar seeded with the *S. aureus*. The plates were allowed to stand for 1 h for sera diffusion and later incubated at 37°C for 24 h. The highest drug level that did not show activity was selected for combination with the permeation enhancers.

Table 2. Inhibition zone diameters (IZDs) of some bacteria for selection for the in vitro permeability study.

	Time (h)			
	0.5	1	2	3
Organism	IZD (mm)			
<i>B. subtilis</i>	13.3±0.6	7.0±0.0	10.0±0.0	3.6±0.6
<i>K. pneumonia</i>	8.0±0.0	5.0±0.0	2.0±0.0	0.0±0.0
<i>S. aureus</i> *	20.0±0.0	14.0±0.0	15.7±0.6	4.0±0.0
<i>E. coli</i>	6.0±0.0	4.0±0.0	3.3±0.6	0.0±0.0
<i>S. typhi</i>	8.0±0.0	3.0±0.0	3.0±0.0	0.0±0.0

*significant at $p < 0.05$

Table 3. Baseline gentamicin sulphate dose level using inhibition zone diameter (IZDs) (mm) produced by serum drug level of oral gentamicin sulphate against *Staphylococcus aureus*.

	Dose (mg/kg)		
Time (h)	400	500	1,000
0.0	0.0	0.0	0.0
0.5	0.0	7.3±0.6	10.3±0.6
1.0	0.0	8.7±1.2	11.3±0.6
2.0	0.0	8.2±1.2	12.3±0.6
3.0	0.0	7.3±0.6	13.0±0.0

Evaluation of permeability enhancement using the bioassay method

Albino rats (Wister strain) weighing 150–250 g were randomly selected and used for the experiment. Gentamicin sulphate alone was administered orally at 400 and 500 mg/kg. These are, respectively, the maximum concentration that cannot appear in the blood and the minimum concentration that can appear in the blood after oral administration of the drug (Table 2). Blood samples were collected from the rats through the retro-orbital venous plexus at 0, 30, 60, 120 and 180 min. The blood was allowed to coagulate, and sera collected from each sample were introduced into holes bored on solidified 20 ml of molten Mueller–Hinton agar seeded with the *S. aureus*. The plates were incubated at 37°C for 24 h after diffusion and the IZDs measured. More so, the solution of the drug was constituted using distilled water and thoroughly mixed with the respective quantities of the permeation enhancers as shown in the Table 3. Dose levels of gentamicin sulphate, 400 and 500 mg/kg, admixed with each permeation enhancer respectively were given orally to the rats. Blood sample was withdrawn and treated as above. The respective IZDs were measured planimetrically.

In vitro assessment of orally administered gentamicin sulphate against selected enterobacteria

Some pathogenic bacteria known to inhabit the GIT were obtained from cultures maintained at the Medical Laboratory Department of UNTH Ituku-Ozala, Enugu State, Nigeria, and used for this study. The selected bacteria include *E. coli*, *S. typhi*, *K. Pneumoniae* and *S. aureus*. White albino rats (Wister strains) weighing between 155 and 300 g were used for the study. Gentamicin sulphate dissolved in distilled water was administered at the following dose levels: 500, 14.28, 10.71, 7.14 and 3.57 mg/kg to the corresponding group of two rats per group. The number of rats was deliberately made small, to enable utilisation of the entire droppings produced by the group in a particular time interval. All the rat droppings were collected at 0-, 1-, 2-, 4- and 6-h interval. A 10% mixture of the dropping was made in distilled water and centrifuged after agitation for about 10 min. Centrifugation was done at 4,000 rpm for 10 min. A 20 µl of the decant was introduced into a 5 mm hole bored in a solidified Mueller–Hinton agar seeded with the respective enterobacterium and incubated for 24 h at 37°C. Zones of inhibition were measured planimetrically.

Assessment of duration of activity of orally administered gentamicin sulphate

The selected enterobacteria were used for this study. White albino rats (Wister strain) weighing 150–250 g were randomly selected for the study. Two rats were given gentamicin sulphate orally at 7.1 mg/kg (500 mg/70 kg). The droppings were collected at 0-, 4-, 6-, 8-, 10-, 12-, 14-, 16- and 18-h interval. A 10% mixture of the droppings was constituted with water and agitated mechanically for 10 min. The mixtures were later centrifuged for 10 min at 4,000 rpm. A 20 µl of the decant was introduced into a 5 mm hole bored in a solidified Mueller–Hinton agar seeded with respective enterobacterium and incubated for 24 h at 37°C. Zones of inhibition were measured as before.

Table 4. Inhibition zone diameter (IZDs) (mm) produced by serum drug level of orally co-administered gentamicin sulphate with permeation enhancers against *Staphylococcus aureus*.

Drug/P. enhancera	((mg/kg)/%)	0 h	0.5 h	1 h	2 h	3 h
Drug	400 500	0.0±0.0 0.0±0.0	0.0±0.0 0.0±0.0	0.0±0.0 8.0±0.0	0.0±0.0 8.7±0.6	0.0±0.0 8.0±0.0
Drug/PEG 4000	400/30 500/30	0.0±0.0 0.0±0.0	0.0±0.0 6.7±0.6	0.0±0.0 7.6±0.6	0.0±0.0 6.7±0.6	0.0±0.0 6.7±0.6
Drug/Tween® 80	400/15 500/15	0.0±0.0 0.0±0.0	0.0±0.0 6.7±0.6	0.0±0.0 6.7±0.6	0.0±0.0 6.7±0.6	0.0±0.0 7.0±1.0
Drug/Kolliphor® 188	400/1.0 500/1.0	0.0±0.0 0.0±0.0	0.0±0.0 8.7±0.6	0.0±0.0 8.7±0.6	0.0±0.0 9.0±0.0	0.0±0.0 9.0±0.0
Drug/Glycerine	400/1.5 500/1.5	0.0±0.0 0.0±0.0	0.0±0.0 7.7±0.6	0.0±0.0 8.3±0.6	0.0±0.0 8.0±0.0	0.0±0.0 7.0±1.0
Drug/SLS	400/2 500/2	0.0±0.0 0.0±0.0	0.0±0.0 0.0±0.0	0.0±0.0 9.0±0.0	0.0±0.0 10.0±0.0	0.0±0.0 9.3±0.6
Drug/Citric acid	400/2 500/2	0.0±0.0 0.0±0.0	0.0±0.0 9.0±0.0	0.0±0.0 8.7±0.6	0.0±0.0 8.3±0.6	0.0±0.0 8.3±0.6
Drug/Oleic acid	400/2 500/2	0.0±0.0 0.0±0.0	0.0±0.0 7.7±0.6	0.0±0.0 8.0±0.0	0.0±0.0 7.3±0.6	0.0±0.0 6.3±0.6
^a P. enhancer =	Permeation	enhancer,	SLS =	Sodium	lauryl	sulphate

Formulation of gentamicin sulphate capsules

Granules of gentamicin sulphate were formed using the formula shown in Table 4. PEG 4000, polyvinyl pyrrolidone (PVP) and gelatine were assessed as granulating aid. PEG 4000 was weighed out and poured into a beaker and melted on a hot plate. The diluent, (N-modified starch, a hydrophobic starch obtained from modifying maize starch with sodium hypochlorite (Nwakile, 2017), desiccant (Carb-o-sil®) and the disintegrant (Ac-di-sol®) were weighed out and mixed. The excipients were dispersed into the melted PEG 4000 and thoroughly mixed. The beaker was removed from the hot plate, and the gentamicin sulphate powder was introduced. The mixture was thoroughly mixed and allowed to cool. It was then granulated to the 0.8 mm sizes. A similar procedure was employed for the batch containing PVP. However, gelatine (3% w/w) was used to prepare batch C. The moisture absorbed by the gentamicin sulphate serves to moisten the mixture and enables lump formation for batch C. Batch B failed to give good granules, as the PVP failed to hold the particles together. Batch C, however, failed to give dry granules because of the presence of absorbed moisture continued to increase and weaken drying. Batch A, which gave quick drying granules, was packaged into gelatine capsule shells (size 00) to desired weight through manual filling.

Evaluation of gentamicin sulphate capsules

Dissolution of batch A-formulated capsules was done using BP basket method. Normal saline was used as the dissolution

medium. About 300 ml of the medium was introduced into a 1,000 ml beaker mounted on a hot plate magnetic stirrer and maintained at a temperature of 37±0.5°C and speed of 100 rpm. The capsule contained in a stainless basket was suspended into the medium using a retort stand. The dissolution was allowed to run for 60 min and 2 ml of the sink collected at a 5-min interval with replacement using fresh medium. The collected sink was used to inoculate the *S. aureus* seeded Mueller–Hinton agar whose IZDs were measured after 24 h.

A calibration curve was established using a solution of gentamicin sulphate with the following concentrations: 100, 50, 25, 12.5, 6.25 and 3.125 µg/ml. The organism used was *S. aureus*. Twenty (20) ml of molten Mueller–Hinton agar was poured into sterile Petri dishes, and standardised concentrations (McFarland 0.5) of overnight culture of the selected organism (*S. aureus*) introduced aseptically on the agar plates. The mixtures were gently rocked and allowed to solidify. Holes of 5 mm diameter were made in the agar plates using a sterile metal cork-borer. A 20 µl of the various concentrations of the drug were put in each hole under aseptic condition and kept at room temperature for 1 h to allow for diffusion. The plates were then incubated at 37°C for 24 h, and the IZDs measured and recorded. The size of the cork borer (5 mm) was deducted from the values recorded for the IZDs to get the actual diameter. This procedure was conducted in triplicate, and the mean IZDs calculated and recorded. Thereafter, the IZDs were plotted against the logarithm of the concentrations. Actual concentrations of drug in the serum were obtained by superimposing the IZD of the serum on

Table 5: Average inhibition zone diameter (IZD) (mm) of gentamicin sulphate after 6 h of oral administration at different concentrations.

			Dose (mg/kg)		
Microorganism	500	14.28	10.71	7.14	3.57
<i>E. coli</i>	12.0±0.8	6.3±0.5	6.0±0.0	4.3±0.5	0.0±0.0
<i>S. typhi</i>	13.3±0.5	6.0±0.0	6.3±0.5	4.0±0.0	0.0±0.0
<i>K. pneumonia</i>	15.0±0.8	8.0±0.8	10.0±0.0	8.3±0.5	0.0±0.0
<i>S. aureus</i>	14.3±0.5	10.0±0.8	7.0±0.8	6.3±0.5	0.0±0.0

the IZD axis and extrapolating to the log concentration. The concentration was then obtained by taking the antilogarithm of the log concentration.

Data collection and statistical analyses

The collected data were analysed statistically using mean, standard deviation and chi-square with $p < 0.05$ where appropriate.

RESULTS AND DISCUSSIONS

For a possible GIT local active dosage form, there is need to ensure that the drug is not absorbed appreciably from the GIT into the systemic circulation even when administered with potential permeation enhancers. To show that gentamicin sulphate does not get into the systemic circulation when administered PO, a sensitive bacterium was selected and used as a biomarker for the drug permeation study. Various bacteria species were incubated with 4.0 µg/ml of gentamicin sulphate. This represents the highest serum drug level after parenteral administration (CPACPS, 2002). The most sensitive bacterium being *Staphylococcus aureus*, as shown in Table 2, was therefore selected for the permeation studies.

It can be seen from Table 2 that all the bacteria were sensitive to gentamicin sulphate from 30 min after administration to 2 h after administration. Only *B. subtilis* and *S. aureus* were sensitive at the third hour. The *S. Aureus* had the highest sensitivity for all the period under review with IZD of 20, 14, 15.7 and 4 mm corresponded to the periods of 0.5, 1, 2 and 3 h, respectively.

It has been shown that gentamicin sulphate given to rats orally could appear in the blood of the rats if the administered dose is up to 10,000 mg/kg (EMEA, 2001). Hence, to ascertain the minimum concentration of gentamicin sulphate that can appear in the blood of rat after oral administration, gentamicin sulphate was administered orally to the rats at doses from 40 mg/kg and up to 1,000 mg/kg, as shown in Table 3. The oral administration was started at 40 mg/kg because this dose was preliminarily given parenterally (im) and the serum concentration after 1 h inhibited the test bacterium (*S aureus*). The 1,000 mg/kg corresponds to one-tenth of the orally administered dose elsewhere (EMEA,

2001). The serum collected at specified time interval after oral administration of various doses of the drug, as shown in Table 3, was used to inoculate *S. aureus*-seeded Mueller–Hinton agar plates. From Table 3, it can be seen that doses of 400 mg/kg gentamicin sulphate and below did not get into the blood at concentrations that inhibit bacteria growth *in vitro*. However, from gentamicin sulphate concentrations of 500 mg/kg and above, the drug was absorbed into the blood because the sera have concentrations that inhibited bacteria growth *in vitro* (Table 3).

To rule out the possibility of some permeation enhancers, at their pharmaceutically recommended usage levels aiding the drug at 400 mg/kg (and below) to cross the mucosal barriers, the drug was administered to the rats at the dose level of 400 mg/kg (using 500mg/kg as control) and in the presence of the permeation enhancers (Table 4) (Kibbe, 2000). It is quite evident from Table 4 that gentamicin sulphate concentrations of 400 mg/kg and below given orally could not permeate into the systemic circulation even in the presence of the permeation enhancers. However, at the dose of 500 mg/kg and above, the sera from rats inhibited the bacteria growth *in vitro*. This cannot be attributed to the permeation enhancers because similar values of IZDs were obtained in their absence (Table 4).

However formulating the drug at this dose level of 500 mg/kg, which permeates into the blood system, will correspond to giving a 70 kg body weight adult 35 g/dose as tablet of the drug; an outrageous dose. This of course indicates the suitability of the drug being evaluated as a potential drug for GIT local activity. Such evaluation was subsequently done by determining the dose of the drug. The doses of the drug were determined by administering gentamicin sulphate, as shown in Table 5. The filtrate collected from a 10% suspension of rat's droppings inhibited all the test bacteria at the following doses: 14.28 mg/kg (1,000 mg/70 kg), 10.71 mg/kg (750 mg/70 kg) and 7.14 mg/kg (500 mg/70 kg) body weights. There was no activity on any of the bacteria when gentamicin sulphate was given at 3.57mg/kg (250 mg/70 kg) body weight. These doses are very much acceptable and realistic in dosage form design.

The duration of activity of gentamicin sulphate administered orally was thereafter determined by monitoring the administered drug for 18 h, as shown in Table 6. The rats

Table 6. Duration of GIT local activity of orally administered gentamicin sulphate.

Time (h)	<i>E. coli</i>	<i>S. typhi</i>	<i>K. pneumonia</i>	<i>S. aureus</i>
0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
4	2.5±0.7	0.0±0.0	0.0±0.0	2.0±0.0
6	3.5±0.7	4.0±0.0	2.0±0.0	6.0±0.0
8 ^b	6.0±0.0	4.0±0.0	4.0±0.0	8.0±0.0
10	3.5±0.7	0.0±0.0	0.0±0.0	4.0±0.0
12	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
14	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
16	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
18	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0

^bAll organisms were inhibited at this hour

were given the drug at 7.14 mg/kg (500 mg/70 kg), the lowest dose that produces activity *in vitro*. The filtrate from a 10% suspension of rat droppings inhibited the growth of all the bacteria up to the 8th hour (Table 6). For all the bacteria tested, inhibition peaked at the 8th hour. This implies that the drug should be administered orally on 8-hourly intervals. This frequency of administration will maintain sufficient drug concentration level in the GIT to limit the proliferation of bacteria. However, doses of up to 14.28 mg/kg (1,000 mg/70 kg) and 10.71 mg/kg (750 mg/70 kg), as obtained in Table 5, could be administered. It is very important to note that gentamicin sulphate given orally does not cause lesion on the GIT mucosa at these acceptable doses of 14.28 mg/kg (1,000 mg/70 kg), 10.71 mg/kg (750 mg/70 kg) and 7.14 mg/kg (500 mg/70 kg). Such mucosal damage could not be possible because higher doses of 10,000 mg/kg body weight have been administered without such damage (EMEA, 2001). Hence, important fundamental biopharmaceutical and formulation issues that will enable ushering gentamicin sulphate into usage as oral tablets and solutions for the treatment of GIT localised infections that ravage the greater population of the world are possibly addressed.

Pure gentamicin sulphate powder is known for its hygroscopic character. The pure powder absorbs moisture from the environment and becomes sticky, making the powder difficult to granulate. This presents serious formulation challenges. To overcome this challenge requires the incorporation of excipients that will retard or discourage the absorption of moisture from the atmosphere (Table 1). To this end, N-modified maize starch possesses such attribute judging from its hydrophobic nature (Nwakile, 2017). This attribute of the N-modified maize starch is expected to help limit the atmospheric moisture-absorbing ability of gentamicin sulphate granules formulated with it. To limit the atmospheric moisture-absorption potential of the gentamicin sulphate powders, some other additive like silicone dioxide (Carb-o-sil®) was also included. Carb-o-sil® is a well-known desiccant approved for pharmaceutical use. Other ingredients included

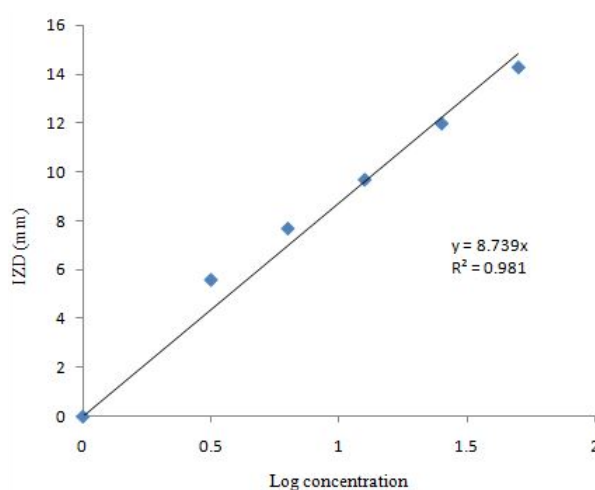


Figure 1. Calibration curve of Gentamicin Sulphate using *S. aureus*,

in the formulation were Ac-di-sol®. PEG 4000 was employed as the granulating aid because it performs better than PVP and gelatine in preliminary studies. Based on the administrable doses discussed earlier, 250 mg gentamicin sulphate granules were formed and manually packaged into capsules to a total weight of 350 mg of the gentamicin sulphate granules. Dissolution sink was tested for the presence of gentamicin sulphate by incubating the sink with *S. aureus*-seeded molten Mueller–Hinton agar. The inhibition zone diameter was used to deduce the concentration using the calibration curve established for the purpose. The calibration curve was established using *S. aureus* and pure gentamicin sulphate powder, as shown in the Figure 1.

As can be seen from Figure 2, the formulated gentamicin sulphate capsule released enough concentration of the drug that inhibits the test microorganism within 2 min of dissolution (Fig 2).

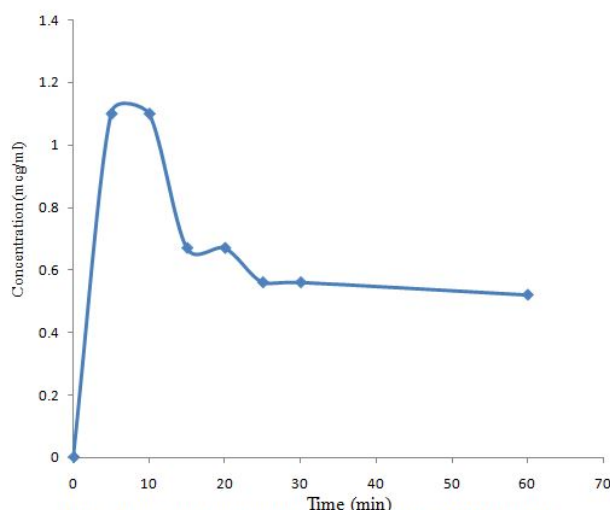


Figure 2. Concentration time curve of gentamicin sulphate capsule in normal saline.

CONCLUSION

Gentamicin sulphate at dose level of 400 mg/kg and below given orally could not get into the systemic circulation even in the presence of commonly available permeation enhancers that include PEG, Tween® 80, kolliphor® 188, glycerine, sodium lauryl sulphate, citric acid and oleic acid. The drug at 14.28 mg/kg (1,000 mg/70 kg), 10.71 mg/kg (750 mg/70kg) and 7.14 mg/kg (500 mg/70 kg) orally administered inhibited some frequently encountered pathogenic enterobacteria. Orally administered gentamicin sulphate was found to maintain

effective inhibition concentration in the GIT for 8 h pointing to an 8-hourly administration. The above-stated doses are acceptable in the dosage form design; it is possible to formulate a non-absorbable oral gentamicin sulphate dosage form for local activity in the GIT using existing conventional solid dosage formulating equipment. Such oral dosage(s) will be very important in controlling, in a very simple way, the ever debilitating GIT pathogens without recourse to drastic changes in conventional formulation pattern.

To that effect, gentamicin sulphate 250 mg granules was formulated. The gentamicin sulphate granules, which were quick drying, were packaged into capsules, which released enough concentration of the drug that inhibits the test organism within 2 min of dissolution. Oral dosage forms of gentamicin sulphate will expand the usage of gentamicin sulphate, thereby exploiting the drug's wide spectrum of activity.

FUNDING

This research was not funded by any grant agency or organisation.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

ACKNOWLEDGEMENT

Kind supply of gentamicin sulphate powder by Juhel Nigeria Limited is acknowledged.

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