Full Length Research Paper

Characterization and controlled release of gentamicin from novel hydrogels based on Poloxamer 407 and polyacrylic acids

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Parenteral administration of gentamicin, a hydrophilic aminoglycoside antibiotic commonly used in the control of Gram positive and Gram negative infections, is limited by adverse effects such as nephrotoxicity, ototoxicity and neurotoxicity. In this study, topical hydrogels of gentamicin were produced using three polymeric hydrogels of Poloxamer 407 and polyacrylic acids (Carbopols® 971P and 974P), and evaluated in terms of drug content, pH, swellability in different media, viscosity, spreadability, skin irritation on rats and time-resolved stability. The in vitro permeation of gentamicin from the hydrogel formulations was carried out in phosphate buffered saline using a modified Franz diffusion apparatus. Results obtained indicate that gentamicin-loaded hydrogels showed good encapsulation, stability, pH-dependent swelling, tolerability on rats, greater percentage drug release than the commercially available gentamicin ointment and pure sample of gentamicin. Overall, Poloxamer 407 hydrogels of gentamicin gave the most desirable properties in terms of drug permeation, spreadability, pH, swellability and viscosity, superior to polyacrylic acids hydrogels of gentamicin. This study has shown that Poloxamer 407 hydrogels of gentamicin could offer a promising approach for topical delivery of gentamicin for the treatment of skin infections caused by gentamicin-susceptible bacteria.

Key words: Poloxamer 407 (P407), topical hydrogels, gentamicin, polyacrylic acids (Carbopols® 971P and 974P), antimicrobial activities.

INTRODUCTION

The solubility characteristics of a substance greatly influence its ability to penetrate biological membranes (Eljarrat-Binstock et al., 2004). Both the aqueous solubility of a drug at the absorption site and the partition coefficient strongly influence the rate of transport across the biological membrane. The enhancement of drug dosage form formulation is connected with the application of auxiliary substances or with new technological possibilities, which may be intended for controlled drug delivery (Shazly et al., 2012). Controlled release of antibiotics at the site of infection is a new strategy being employed to treat chronic infections (El-Gendy et al., 2009). Localized delivery systems, based on biodegradable polymers are capable of slow and

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controlled release of drug for a required period of time, with initial burst effect to circumvent the infection (Kopecek, 1984; Graham and McNeill, 1984). Important factors, which influence drug release are polymer composition, drug diffusion, osmotic pressure, and bioerosion (Coppi et al., 1996; Khare and Peppas, 1995). The major advantage of these systems is steady and extended release of the antibiotics directly to infected tissue without systemic toxicity. Furthermore, no surgical intervention or procedures are required for their removal (Stephens et al., 2000).

Hydrogels, swollen three-dimensional networks of hydrophilic polymers held together by association bonds or cohesive forces, are suitable carriers for drug delivery (Eljarrat-Binstock et al., 2004). They are of special interest in controlled release applications, because of their soft tissue biocompatibility, the ease with which drugs are dispersed in matrix and the high degree of control achieved by selecting the physical and chemical properties of polymer network. Hydrogels have a high water content and rubbery nature similar to natural tissue, which make them desirable for biomedical applications. Hydrogels have been investigated extensively for application as carriers in diffusion-controlled release devices (Mayol et al., 2008; Raja et al., 2011; Changez et al., 2003; Ayhan and Özkan, 2007; Sokmen et al., 2008).

In this type of device, a drug or protein is incorporated into the system and then released in response to a change in the environment. The hydrogels are rendered insoluble due to the cross-linking which may be physical or chemical. These cross-links are responsible for providing the physical integrity and network structure of the hydrogels. The polymeric biomaterials are used to delay drug dissolution at a slower rate depending on exposure of drug molecules to aqueous environment surrounding the drug delivery system. Their usage is advantageous in safety, ease of manufacture, cost effectiveness, biocompatibility and biodegradability (Mayol et al., 2008). In general, the gelation of a polymeric solution can be triggered by a number of factors such as variations in temperature, as for Poloxamers (Desai and Blanchard, 1998), pH, as for Carbopols (Srividya et al., 2001; Cho et al., 2012), or the presence of cations, as for alginates (Cohen et al., 1997). Topical drug administration generally refers to topical application of agents to healthy intact skin either for localized treatment of tissues underlying the skin or for systemic therapy (Brown et al., 2006). It delivers drugs to the circulatory system and have been shown to possess controlled release rate, enhanced efficacy, stable plasma concentration, non-invasive administration, increased safety, reduced dosing frequency and simplicity of use, to mention few (Allen Jr., 2011). Yet, a common problem with it is permeation across stratum corneum (sc), which limits the size and property of drug molecules that pass through (Prausnitz and Langer, 2008). Flux across the skin is therefore dependent upon skin hydration, partitioning, transport as well as concentration gradient across the skin (Azarni et al., 2007). Hydrogels (including topicals) achieve sustained release by diffusion from a reservoir through microporous membrane into the skin (Mayol et al., 2008; Raja et al., 2011; Changez et al., 2003; Ayhan and Özkan, 2007; Sokmen et al., 2008).

Gentamicin sulphate is an aminoglycoside antibiotic commonly used topically in the control of severe Gram positive and Gram negative microbial infections especially in burns and wounds as well as for treating bone and soft tissue infections (Chang et al., 2006). Topical gentamicin is often used in the treatment of impetigo, infected bed sores, burns, nasal staphylococcal carrier state, pyoderma, infections of the external eye and adenexa (Nishijima and Kurokawa, 2002). Despite its benefits, bacterial barriers and adverse effects such as nephrotoxicity, ototoxicity and neurotoxicity upon prolonged use limit gentamicin daily dosage (Robert and Walters, 1998). In fact, many clinicians are reluctant to use it, even for a short term (Drusano et al., 2007). Efforts have been made to determine its optimal therapeutic regimens in order to increase its overall efficacy while minimizing drug toxicity. These include liposomes (Jia et al., 2008), solidified reverse micellar drug delivery systems (Umeyor et al., 2011, 2012a, b), hydrogels (Eljarrat-Binstock et al., 2004; Changez et al., 2003; Ayhan and Özkan, 2007; Sokmen et al., 2008) and more recently, gentamicin transdermal microgels (Nnamani et al., 2013), and gentamicin-gold nanospheres for antimicrobial drug delivery to Staphylococcal infected foci (Ahangari et al., 2013). Topical hydrogels could be employed as an alternative low dose regimen aimed not only at reducing toxicity associated with prolonged use of gentamicin but also assuring proper utilization of the benefits of gentamicin, especially its rapid bactericidal activity, particularly in blood stream infections. These hydrogels can retain large quantities of gentamicin solution and can be directly applied to the skin without need for sophisticated equipment. Thus, this work seeks to design a gentamicin-loaded topical hydrogel and evaluate its physicochemical characteristics in an attempt to achieve predictable and reproducible gentamicin delivery. Gentamicin-loaded hydrogels of poloxamer 407 and polyacrylic acids (Carbopol® 971 and 974) were prepared and evaluated with respect to physicochemical properties, swelling and in vitro drug permeation. The hydrogel takes the form of a topical dosage system, containing a defined drug loading available for release within a defined surface area. Such a system is expected to be self-adhesive, backed with a protective material and capable of delivering a drug dose comparable to that delivered by Geby the proprietary gentamicin creams and ointments.

MATERIALS AND METHODS

The following materials were used: gentamicin pure powder (a kind
Animal Care and Use Committee (Research Ethics Committee) of
animals were conducted in accordance with Ethical Guidelines of
obtained from an all-glass still. All experiments involving the use of
manufacturers without further purification. All other reagents were of
Mfg. Corp., California, USA) were used as procured from the
Chemical Co., Cairo, Egypt) and triethanolamine (Spectrum Chem.
Germany), propylene glycol and polyvinyl alcohol (Merck,
Chemical Co., USA), Poloxamer 407 (BASF Ludwigshafen,
Germany), gentamicin (0.03, 0.06 and 0.09 w/w) were dissolved in aliquots of
acetate, sodium borate, ophthaldialdehyde and 2-mercaptoethanol
(Sigma Aldrich, Germany), sodium chloride and sodium hydroxide
(BDH, England), monobasic potassium phosphate (Sigma-Aldrich
Chemical Co., USA), Carbopole® 971P and 974P (Lubrizol
Corporation, USA), Poloxamer 407 (BASF Ludwigshafen,
Germany), propylene glycol and polyvinyl alcohol (Merck,
Germany), isopropanol, methanol and formalin (Advic El-Nasr,
Chemical Co., Cairo, Egypt) and triethanolamine (Spectrum Chem.
Mfg. Corp., California, USA) were used as procured from the
manufacturers without further purification. All other reagents were of
analytical grade and were used as such. Distilled water was
obtained from an all-glass still. All experiments involving the use of
animals were conducted in accordance with Ethical Guidelines of
Animal Care and Use Committee (Research Ethics Committee) of
University of Nigeria, Nsukka, following the 18th WMA General
Assembly Helsinki, June 1964 and updated by the 59th WMA

Preparation of gentamicin hydrogels

Three gelling agents were employed: Poloxamer 407 (P407), and
two polyacrylic acids (Carbopole® 971P and 974P, that is, C971 and
C974). Poloxamer 407 was dissolved completely in purified water
pre-cooled to approximately 5°C. Graded concentrations of
gentamicin (0.03, 0.06 and 0.09 w/w) were dissolved in aliquots of
purified water and propylene glycol added and mixed until a
homogenous mass was formed. For the polyacrylic acid hydrogels, the same procedure was followed except that room temperature
purified water was used instead of pre-cooled water and that the
hydrogels were further neutralized by triethanolamine. All products
were adjusted to pH 5.5 and kept at room temperature for 24 h to
ensure absence of air-bubble before they were dispensed in
lacquered aluminum tube (60 g), securely closed and stored at
room temperature until used. The formulation compositions are
shown in Table 1.

Table 1. Optimized formula for Hydrogel preparations.

<table>
<thead>
<tr>
<th>Composition (g)</th>
<th>Plain hydrogels (w/w)</th>
<th>Drug-loaded hydrogels (w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P407 Carbopol®</td>
<td>0.03 0.06 0.09</td>
</tr>
<tr>
<td>Poloxamer 407</td>
<td>13.2 -</td>
<td>13.2 13.2 13.2</td>
</tr>
<tr>
<td>Carbopol 971P</td>
<td>- 5.0</td>
<td>5.0 5.0 5.0</td>
</tr>
<tr>
<td>Carbopol 974P</td>
<td>- 5.0</td>
<td>5.0 5.0 5.0</td>
</tr>
<tr>
<td>Propylene glycol(a)</td>
<td>12.0 -</td>
<td>12.0 12.0 12.0</td>
</tr>
<tr>
<td>Propylene glycol(b)</td>
<td>- 10.0</td>
<td>10 10 10</td>
</tr>
<tr>
<td>Triethanolamine(b)</td>
<td>- Drop</td>
<td>Drop Drop Drop</td>
</tr>
<tr>
<td>Purified water</td>
<td>40.0 44.0 q.s q.s q.s</td>
<td>q.s q.s q.s</td>
</tr>
</tbody>
</table>

(a) means quantity in Poloxamer 407 hydrogel; Amount of purified water in drug-loaded Poloxamer 407 hydrogels was 40 ml. Propylene glycol and Triethanolamine mean quantity in Carbopel hydrogels only; Amount of purified water in drug-loaded Carbopel hydrogels was 44 ml.

Rheological evaluation

Viscosity assessment of the hydrogels was done using a Brookfield
viscometer (GallenKamp, England). Due to the viscous nature of the formulations, 1, 3 and 5 g quantities of the semi-solid formulations were dissolved in 25 ml of purified water for 24 h and their viscosities determined. An average of three readings was carried out for validity of statistical analysis.

Spreadability determination

About 1 g of each formulation was sandwiched between two glass
slides; a lower calibrated slide marked into 5 cm spaces and an
unmarked upper slide. Different weights (50, 100, 200 and 300 g)
were placed over the upper slide at 1 min intervals. The diameter
occupied by the spreading gel was finally measured to determine
the area of spread (length × width). The sample weight was fixed in
order to have same assay for all the samples and to limit the glass from sliding.

Drug content analysis

About 0.5 g of hydrogel was dissolved in 10 ml of water, centrifuged
(TDL-4 B. Bran Scientific and Instru. Co., England) at 1000 rpm for
30 min, filtered through a Whatman No. 1 filter paper, adequately
diluted and the concentration of gentamicin determined
derpectrophotometrically (Shimadzu UV-1601 UV/Vis double beam
spectrophotometer, Japan) after derivatization with o-
phthalaldehyde reagent by Zhang’s method (Zhang et al., 1994).
Briefly, the o-phthalaldehyde reagent was formulated by adding 2.5 g ophthalaldehyde, 62.5 ml methanol and 3 ml of 2-
mercaptoethanol to 560 ml sodium borate in distilled water solution.
The reagent was stored in a brown bottle in a dark chamber for at
least 24 h before use. This reagent could be used only up to three
days. Gentamicin sulphate solution, o-phthalaldehyde reagent, and isopropanol (to avoid precipitation of the products formed) were
mixed in similar proportions and stored for 30 min at room
temperature. The homologous aromatic dialdehyde, o-
phthalaldehyde is essentially non-fluorescent until it reacts with a
primary amine of gentamicin in the presence of excess sulfhydryl
such as 2-mercaptoethanol to yield a fluorescent isoinodole whose
absorbance was then measured at 332 nm (Chang et al., 2006).

Evaluation of the formulations

Physical examination

The semi-solid formulations were physically examined for colour,
homogeneity, and consistency. The pH was also re-evaluated
(before each use) to make sure that it was stable within the skin pH
of 5.5 (El-Gendy et al., 2009).
Study of swelling behaviour of the hydrogels

The effect of pH on swelling of hydrogels were investigated as follows: 0.5 g quantity of hydrogel from each batch of hydrogels was weighed and placed in 10 ml of buffer solutions of different pH [0.1 N HCl (pH 1.2), double distilled water (pH 7.0) and phosphate buffered saline (PBS, pH 7.4)]. The hydrogels were removed from their respective swelling media at predetermined time intervals; blotted dry with tissue paper and their weights were observed on analytical balance. This process was continued until the sample appeared to be dissolved. The equilibrium weight swelling (ESW) of each hydrogel was calculated using the following equation (Raja et al., 2011):

\[ ESW = \frac{W_2 - W_1}{W_1} \times 100 \]  

(1)

where ESW is the equilibrium swelling, \( W_2 \) represents the weight of the swollen hydrogel at time ‘t’ and \( W_1 \) is the weight of the hydrogel before swelling.

Skin irritation test

After obtaining the study protocol approval from the University of Nigeria Committee on safe handling of experimental animals, experiments were performed on male albino Wistar rats weighing 150 to 180 g (Department of Pharmacology and Toxicology, UNN). The rats were maintained under controlled environmental conditions and 12-h light-dark cycle. All animals received standard laboratory diet and water ad libitum. The dorsal hair of the rats was carefully removed using electric clippers, while they acclimatized. Formulations (0.5 g) were applied to the shaved dorsal surface of the rats and spread until complete absorption. The rats (n = 18) were divided into four groups of test (5 rats per batch of 3 test formulations) and control experiment [commercial gentamicin ointment, drug solution (gentamicin ampoule), and the control group that did not receive any treatment], altogether involving two rats. All formulations had equal drug concentration of 1% with the commercial gentamicin ointment. After 1 h application of all treatments, the treated skin was each time examined visually for erythema and/or oedema while the treatment lasted for 5 days.

Preparation of the rat abdominal skin

Male Wistar rats were sacrificed with prolonged anesthesia and the abdominal skin of each rat was excised. Abdominal hairs on the skin of animal were removed with clipper and full thickness skin was surgically removed and dermis side was wiped with isopropyl alcohol to remove residual adhering fat. Heat separation technique (Nnamani et al., 2013) was used to separate the epidermis. The technique involved soaking the entire abdominal skin in water at 60°C for 1 min followed by careful removal of the epidermis from dermis with a blunt forceps. The epidermis was washed with water and wrapped with aluminium foil and stored at -20°C until used. The stored epidermis was allowed to thaw, cut into 4.5 × 4.5 cm² pieces and hydrated by placing in phosphate buffer saline (PBS, pH 7.4) overnight before use.

Permeation studies

The skin permeation studies were performed by using a modified Franz diffusion cell following an established method (Nnamani et al., 2013). The effective diffusion area was 2.27 cm² and the receiver chamber has a capacity of 32 ml. The excised rat abdominal skin (Wistar albino) was mounted between the donor and receptor compartment of the diffusion cell. After equilibration for 30 min, 500 mg of the gentamicin-loaded hydrogel was placed in the donor compartment containing 5 ml of PBS. The receptor compartment of the diffusion cell was filled with PBS. The whole assembly was fixed on a magnetic stirrer, and the solution in the receptor compartment was constantly and continuously stirred using magnetic beads at 50 rpm; the temperature was maintained at 32 ± 0.5°C. The samples were withdrawn at different time intervals, filtered through a 0.45 µm pore size cellulose membrane filter and analyzed for drug content spectrophotometrically at 341 nm, against the blank (PBS). The receptor phase was replenished with equal volume of PBS at each sample withdrawal. The experiment was carried out in replicates. The cumulative percentages of drug permeated per square centimeter of the rat skin were plotted against time. Control experiments were also performed in each case using pure sample of gentamicin and commercially available gentamicin ointment.

Permeation data analysis

The flux (µg cm⁻² h⁻¹) of gentamicin was calculated from the slope of the plot of the cumulative amount of gentamicin permeated per cm² of skin at steady state against time using linear regression analysis (El-Gendy et al., 2009; Nnamani et al., 2013). The permeation coefficients were obtained from the steady-state flux values making use of the following equations.

\[ P = \frac{J}{C_o} (\text{cm/h}) \]  

(2)

where \( P \) is the permeation coefficient; \( C_o \) is the initial drug concentration in the drug compartment. \( J \) represents the steady-state flux obtained from equation 5.

\[ J = \frac{dQ}{Adt} (\text{µg/cm}^2\cdot\text{h}) \]  

(3)

where \( Q \) indicates the quantity of substances crossing the rat skin, \( A \) is the area of the rat skin exposed and \( t \) is the time of exposure.

Kinetic modeling of drug release

To analyze the mechanism of drug release from the hydrogels, the release data were fitted to various release kinetic equations and models to determine the in vitro release kinetic models and mechanisms. Three kinetic models including the zero-order, first-order, and Higuchi square root models were applied to process the release data to find out the equation with the best fit (Stephens et al., 2000; Mayol et al., 2008; Raja et al., 2011; Nnamani et al., 2013).

\[ Q = K_t t \]  

(4)

\[ Q = 100\left(1-e^{-K_o t}\right) \]  

(5)

\[ Q = K_o (t) \frac{1}{2} \]  

(6)

where \( Q \) is the release percentage at time, \( t \). \( K_t \), \( K_o \), and \( K_S \) are the rate constants of zero-order, first-order and Higuchi models, respectively.

Stability study of hydrogel formulations

Stability study was carried out for the hydrogel formulations as per ICH guidelines (Nnamani et al., 2013) at 40°C in a humidity chamber having 75% relative humidity (RH) for three months. After
Table 2. pH of the gentamicin hydrogels.

<table>
<thead>
<tr>
<th>Batch</th>
<th>0 mg</th>
<th>30 mg</th>
<th>60 mg</th>
<th>90 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Poloxamer 407</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial pH</td>
<td>5.41</td>
<td>4.67</td>
<td>4.72</td>
<td>6.50</td>
</tr>
<tr>
<td>Adjusted pH</td>
<td>5.50</td>
<td>5.50</td>
<td>5.40</td>
<td>5.50</td>
</tr>
<tr>
<td><strong>Carbopol® 971P</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial pH</td>
<td>4.0</td>
<td>1.39</td>
<td>0.95</td>
<td>1.08</td>
</tr>
<tr>
<td>Adjusted pH</td>
<td>5.51</td>
<td>5.53</td>
<td>5.50</td>
<td>5.42</td>
</tr>
<tr>
<td><strong>Carbopol® 974P</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial pH</td>
<td>1.16</td>
<td>1.13</td>
<td>1.04</td>
<td>1.06</td>
</tr>
<tr>
<td>Adjusted pH</td>
<td>5.48</td>
<td>5.49</td>
<td>5.56</td>
<td>5.44</td>
</tr>
</tbody>
</table>


three months, samples were withdrawn and the drug content of the hydrogels re-evaluated.

RESULTS AND DISCUSSION

Characterization of gentamicin hydrogels

Physical evaluation and pH measurements

All the hydrogels were transparent, clear and colourless. Poloxamer 407 hydrogels were the most flexible and possessed the best physical assessment among all the hydrogel formulations. Hydrogels of Carbopol® 974P were more consistent than those of Carbopol® 971P. The pH values of the gentamicin hydrogels are presented in Table 2. The pH of the hydrogels of Carbopol® 971P and 974P before adjustment were more acidic than Poloxamer 407 (Table 2) that produced entirely basic hydrogels, hence the need to buffer the former with triethanolamine to pH of 5.5. This would enhance the efficiency of the formulations as topical drug delivery systems for gentamicin as well as make the formulations safe for use on the skin, consistent with previous report (El-Gendy et al., 2009).

Viscosity measurement

Figure 1a to c shows the results of viscosity determination of hydrogels based on P407, C971P and C974P, respectively. Results of the study indicate that hydrogels of C974P (Figure 1c) were the most viscous whereas hydrogels of P407 (batches A0–A3 of Figure 1a) were the least viscous among the polymeric hydrogels. This is justified since C974P which is a highly cross-linked polymer produced highly viscous gels with rheology similar to mayonnaise (Srividya et al., 2001; Cho et al., 2012; Wagner et al., 2000). On the other hand, P407, being the least cross-linked polymer among the polymeric hydrogels (Shazly et al., 2012; Graham and McNeill, 1984; Desai and Blanchard, 1998) employed in this study produced the least viscous hydrogels.

The results equally revealed concentration-dependent increase in the viscosities of all hydrogel formulations (that is, 5 g >3 g >1 g/25 ml of distilled water). It was also observed that the drug-loaded hydrogels (batches A1–A3, B1–B3 and C1–C3) were more viscous than the plain hydrogels (batches A0, B0 and C0), which may be related to the additional drug content in the formulation compositions of the former. Among the drug-loaded hydrogels, viscosities decreased with increase in drug loading irrespective of the polymeric hydrogel employed in the formulation, and as such hydrogel formulations loaded with 30 mg of gentamicin (batches A1–C1) were the most viscous whereas hydrogels loaded with 90 mg of gentamicin (batches A3–C3) were the least viscous. The reason for this is unknown but may be related to enhanced drug entrapment by the hydrophilic polymers. Viscosity of the gel matrix is an important factor to consider in evaluation of drug penetration from gels across the skin or artificial membrane (Azarmi et al., 2007) and is used to measure the extrudability of a gel (Nnamani et al., 2013). Decreased viscosity of the hydrogels at higher drug loadings (batches A2–C2 and A3–C3) not only implied that these hydrogel formulations would be easily extruded from their containers or packages, but also indicated potential improvement in the diffusivity of gentamicin within the hydrogel network which in turn would facilitate flux, consistent with previous reports (Azarmi et al., 2007; Nnamani et al., 2013).

Spreadability determination

The results of spreadability determination of the hydrogel formulations (Figure 2a to c) generally credited hydrogel formulations based on P407 as the best followed by hydrogel formulations of C971P and lastly C974P hydrogel formulations. This is in agreement with the results of viscosity determination, as the most viscous formulations were the least spreadable and vise versa. It was not surprising that hydrogel formulations based on C971P NF polymer were more spreadable than those of C974P NF polymer, because C971P polymer hydrogel is a lightly cross-linked polymer with long rheology which would result in flow-like honey (high spreadability) in a semi-solid formulation (Desai and Blanchard, 1998), as seen in batches B2–B3. The C974P polymeric hydrogel, which is a highly cross-linked polymer, produces highly viscous hydrogels (batches C0–C3) with rheology similar to mayonnaise (low spreadability) (Nnamani et al., 2013; Wagner et al., 2000). P407 is the least cross-linked polymer among the polymeric hydrogels (Shazly et al., 2012; Graham and McNeill, 1984; Desai and Blanchard,
Figure 1. Viscosity of P407 hydrogels (a), C971P hydrogels (b) and C974P hydrogels (c) at 1, 3 and 5 g/25 ml drug concentration in purified water (n=3). 
A1-C1, A2-C2 and A3-C3 contain 30, 60 and 90 mg of gentamicin sulphate respectively while A0-C0 are drug-free hydrogels.

1998), hence the most spreadable and the least viscous hydrogel formulations (batches A0-A3) were obtained from it. The results also revealed that drug-loaded hydrogel formulations (batches A1-A3, B1-B3 and C1-C3) were comparatively less spreadable than the plain hydrogels (A0-C0), since the latter were less viscous than the former, as earlier explained. Furthermore, it was observed from Figure 2a to c that there was load (weight)-dependent (300 g > 200 g >100 g > 50 g) as well as drug concentration-dependent (90 mg > 60 mg > 30 mg) increases in areas spread across all batches. Among the drug-loaded hydrogel formulations, batches A3-C3 containing 90 mg of gentamicin were the most spreadable, thus justifying the viscosity results (the least viscous drug-loaded batches); whereas those containing 30 mg of gentamicin (batches A1-C1) were the least spreadable, because they were the most viscous gentamicin-loaded hydrogel formulations. Overall, batch A3 hydrogel formulation containing P407 and the highest quantity of gentamicin (90 mg) (Figure 2a) was the least
Figure 2. Spreadability of P407 hydrogels (a), C971P hydrogels (b) and C974P hydrogels (c) based on 50, 100, 200 and 300 g weights (n=3). A1-C1, A2-C2 and A3-C3 contain 30, 60 and 90 mg of gentamicin sulphate respectively while A0-C0 are drug-free hydrogels.

Table 3. Drug content and kinetic models of drug release from gentamicin-loaded hydrogels.

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Zero order</th>
<th>First order</th>
<th>Higuchi</th>
<th>Drug content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>0.9269</td>
<td>0.8899</td>
<td>0.9935</td>
<td>68.52 ± 1.88</td>
</tr>
<tr>
<td>A2</td>
<td>0.8551</td>
<td>0.8793</td>
<td>0.9802</td>
<td>85.76 ± 4.00</td>
</tr>
<tr>
<td>A3</td>
<td>0.9427</td>
<td>0.9338</td>
<td>0.9955</td>
<td>77.05 ± 3.98</td>
</tr>
<tr>
<td>B1</td>
<td>0.8843</td>
<td>0.7275</td>
<td>0.9809</td>
<td>82.89 ± 2.83</td>
</tr>
<tr>
<td>B2</td>
<td>0.9660</td>
<td>0.8791</td>
<td>0.9904</td>
<td>93.00 ± 4.04</td>
</tr>
<tr>
<td>B3</td>
<td>0.9365</td>
<td>0.9516</td>
<td>0.9835</td>
<td>86.27 ± 3.75</td>
</tr>
<tr>
<td>C1</td>
<td>0.9891</td>
<td>0.7217</td>
<td>0.9981</td>
<td>79.10 ± 2.08</td>
</tr>
<tr>
<td>C2</td>
<td>0.8925</td>
<td>0.8942</td>
<td>0.8999</td>
<td>90.66 ± 3.90</td>
</tr>
<tr>
<td>C3</td>
<td>0.9955</td>
<td>0.9781</td>
<td>0.9966</td>
<td>84.91 ± 2.72</td>
</tr>
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*a*Mean±SD, *b* n=3 after 3 months, A1-A3, B1-B3, and C1-C3 are Poloxamer 407, Carbopol® 971P and Carbopol® 974P hydrogels containing 30, 60 and 90 mg of gentamicin sulphate respectively.
viscous and most spreadable, thus necessitating further development of the latter as topical dosage form.

Drug content analysis
The content of gentamicin in the hydrogel formulations is as shown in Table 3. The results indicate that drug encapsulation efficiency (EE) increased with increase in the concentration of gentamicin up to 0.06% w/w for all batches, yielding maximum EE (85.76, 93.00 and 90.66%) for hydrogels formulated with P407, C971 and C974, respectively. Thereafter, the drug encapsulation efficiency decreased with increased drug loading. So, the hydrogels loaded with 0.06% w/w gentamicin resulted in higher EE percent while those loaded with 0.09% w/w gentamicin gave the least. The implication is that gentamicin attained saturation solubility in the hydrogel matrices at 0.06% w/w loading for all batches, and so, more of the drug could not be solubilized and encapsulated, consistent with earlier reports (Umeyor et al., 2011; Umeyor et al., 2012a). However, all the batches had good EE percent. Overall, batch B2 formulated with C971P polymeric hydrogel and 0.06% w/w gentamicin gave the best drug content of 93.00%. Since gentamicin is freely soluble in water (hydrophilic), the result agrees with Küchler et al. (2009) that much of the drug was present in the aqueous phase of the formulations, loosely attached at or near the particle surface, because the more hydrophilic the substance, the weaker the interaction with particle surface, and eventually the compound could be localized in the surfactant layer. When more drug particles at the periphery of the particle surface eventually encounter the polymeric cross-linked gel-matrices of polyacrylic acid resins and P407, stabilization would occur (Souto et al., 2011). Thus, to improve the skin uptake and delivery of such a drug, a semi-solid vehicle such as topical hydrogels would be a better approach as it would exhibit high stability in addition to low toxicity.

Swelling behaviour of the hydrogel formulations
Swelling parameter is a vital factor for characterization of hydrogels, because there is fundamental relationship between swelling of the polymer and nature of the swelling medium (Raja et al., 2011). Swelling studies for the hydrogel were conducted in media of varying pH (0.1 N HCl, pH 1.2; double distilled water, pH 7.0; PBS, pH 7.4). The swelling profiles of hydrogel formulations based on P407 (batches A0-A3), C971P (batches B0-B3) and C974P (batches C0-C3) in these media are shown in Figure 3Aa to c, 3Ba to c and 3Ca to c, respectively. The results revealed that there was pH-dependent as well as time-dependent increases in swelling across all batches. From the study, it was observed that the rate of swelling was generally slow in acidic medium while it was high in neutral medium, but the highest in basic medium. The time to reach equilibrium swelling was less (approximately 8 h) in the hydrogel formulations based on P407 (batches A0-A3 of Figure 3Aa to c) compared to higher time required to reach equilibrium swelling (9 to 11 h) recorded for hydrogel formulations based on polyacrylic acids (batches B0-B3 and C0-C3 of Figure 3Ba to c and 3Ca to c). The longer time to reach equilibrium swelling exhibited by the latter might be attributed to higher viscosities and better pH-dependent gelation properties that were characterized by slow water sorption (Srividya et al., 2001; Cho et al., 2012). Generally, hydrogel formulations showed high swellings especially in PBS, which could be due to the presence of amorphous domains of the polymers or the amorphous nature of the P407 and the polyacrylic acids (C971P and C974P), which also served as solubilizing polymers. Moreover, Figure 3A to C indicates that drug-loaded hydrogels (batches A1-A3, B1-B3 and C1-C3) swelled better than plain hydrogels (batches A0, B0 and C0) in all media across all batches. There was also drug concentration-dependent increases in degree of swelling in all media across all batches (A0>A1>A2>A3, B0>B1>B2>B3 and C0>C1>C2>C3), although the swelling was best at higher pH. This could be attributed to the hydrophilic nature of gentamicin (Eljarrat-Binstock et al., 2004; Shazly et al., 2012; El-Gendy et al., 2009; Changez et al., 2003; Ayhan and Özkan, 2007; Sokmen et al., 2008; Chang et al., 2006; Drusano et al., 2007; Jia et al., 2008; Umeyor et al., 2011, 2012a,b; Nnamani et al., 2013; Ahangari et al., 2013). The faster swelling of drug-loaded hydrogels prepared from P407 (batches A1-A3) at 8 h especially in PBS (Figure 3Aa) suggests faster release of the incorporated drug and perhaps, faster antibacterial activity whereas lower degrees of swelling associated with gentamicin-loaded hydrogels of the polyacrylic acids batches (B1-B3 and C1-C3) suggest possibility of prolongation of release of the incorporated drug; since drug release is directly related to polymer swelling (Raja et al., 2011). Meanwhile, polymer swelling is controlled by many other factors (Geever et al., 2008).

Skin irritation and in vitro drug permeation
The skin irritability test was carried out to evaluate the tolerability of the gentamicin hydrogels after application. The results obtained agreed with literature knowledge of the useful polymers not adversely affecting the skin (Kopeczek, 1984; Desai and Blanchard, 1998; Srividya et al., 2001; Cho et al., 2012; Wagner et al., 2000; Küchler et al., 2009; Souto et al., 2011). There was no erythema or edema observed generally across all batches. Based on this, the gentamicin hydrogel formulations were well-tolerated by the rat.

The results of the permeation study, which is an important tool that predicts in advance how a drug would behave in vivo (El-Gendy et al., 2009; Nnamani et al.,
Figure 3. Swelling profiles of P407 hydrogels (A), C971P hydrogels (B) and C974P hydrogels (C) in (a) alkaline medium (PBS, pH 7.4), (b) neutral medium (double distilled water, pH 7.0), and (c) acidic medium (0.1N HCl, pH 1.2) (n=3). A₁-C₁, A₂-C₂ and A₃-C₃ contain 30, 60 and 90 mg of gentamicin sulphate respectively while A₀-C₀ are drug-free hydrogels.
Figure 4. Permeation profile of gentamicin sulphate from (a) P407 hydrogels, (b) C971P hydrogels, (c) C974P hydrogels in PBS, pH 7.4 (n=3). A₁-C₁, A₂-C₂ and A₃-C₃ contain 30, 60 and 90 mg of gentamicin sulphate respectively while S₁ and S₂ are commercial gentamicin sulphate ointment and pure sample of gentamicin, respectively.

2013) are shown in Figure 4a to c, whereas the permeation data (permeation coefficients and steady-state permeation fluxes) were presented in Table 4. It is evident from the figures that there was controlled permeation of gentamicin from the topical hydrogels without a burst effect in all the formulations.

In vitro permeation was performed in PBS which was the medium that recorded the highest swelling of the hydrogel formulations. The permeability assessment of gentamicin from the hydrogel formulations across the rat skin (Table 4) showed permeation fluxes of 4.917, 5.161 and 5.239 µg/cm².h for P407 hydrogel formulations containing 30, 60 and 90 mg of gentamicin, respectively (batches A₁-A₃); 4.126, 4.572, and 4.717 µg/cm².h for C971P-based hydrogel formulations containing 20, 60 and 90 mg of gentamicin sulphate, respectively (batches B₁-B₃); then 4.761, 4.839 and 4.961 µg/cm².h for C974P-based hydrogel formulations containing 30, 60 and 90 mg of gentamicin sulphate, respectively (batches C₁–C₃). The permeation fluxes for commercial gentamicin
The permeation coefficients of C971P-based hydrogel formulations were 9.048 × 10^{-7} cm/h for hydrogels containing 30, 60 and 90 mg of gentamicin sulphate, respectively (batches A1-A3); the permeation coefficients of C974P-based hydrogel formulations were 9.522 × 10^{-7} cm/h for hydrogels containing 30, 60 and 90 mg of gentamicin sulphate, respectively (batches B1-B3); the permeation coefficients of P407-based hydrogel formulations were 9.048 × 10^{-7}, 9.395 × 10^{-7} and 9.496 × 10^{-7} cm/h for hydrogels containing 30, 60 and 90 mg of gentamicin sulphate, respectively (batches B1-B3); the permeation coefficients of Carbopol® 971P-based hydrogel formulations were 9.496 × 10^{-7} and 9.834 × 10^{-7} cm/h for hydrogels containing 30, 60 and 90 mg of gentamicin sulphate, respectively (batches A1-A3); the permeation coefficients of Carbopol® 974P-based hydrogel formulations were 9.522 × 10^{-7}, 9.678 × 10^{-7} and 9.834 × 10^{-7} cm/h for hydrogels containing 30, 60 and 90 mg of gentamicin sulphate, respectively (batches C1-C3); while the permeation coefficients of gentamicin ointment BP and pure sample of gentamicin were: 8.329 × 10^{-7} and 7.106 × 10^{-7} cm/h, respectively. These values were within the range obtained for some lipophilic and lipophobic drugs (Nnamani et al., 2013; Küchler et al., 2007). By implication, polymeric hydrogels (P407, Carbopol® 974P and Carbopol® 974P) facilitated release of drug from the hydrogels and its subsequent transport across the rat skin, more than the commercially available gentamicin ointment and the unformulated drug (pure sample of gentamicin). This enhanced skin delivery of gentamicin would be of immense benefit in treatment of topical microbial infections caused by gentamicin-susceptible organisms since the topical formulations could be employed either for localized treatment of tissues underlying the skin (Brown et al., 2006).

### Kinetic modeling of drug release

Different mathematical models were used to describe the kinetics of gentamicin release from hydrogels. The criterion for selecting the most appropriate model was chosen on the basis of goodness-of-fit test (Stephens et al., 2000; Mayol et al., 2008; Raja et al., 2011; Nnamani et al., 2013; Melero et al., 2012). The result is presented in Table 3. A comparative evaluation of the coefficient of determination (r²) for hydrogel formulations shows that drug release from the formulations obeyed the Higuchi square root model better than other models. By implication, the release of gentamicin sulphate from hydrogels was governed predominantly by a diffusion mechanism.

### Stability study

Assessment of the stability of novel formulations is
always very important in drug product design and development. Stability could be viewed from the degradation of the active ingredients or physical property of the formulation (Nnamani et al., 2013). In order to determine the change in drug content on storage, stability study was carried out. Table 3 shows insignificant difference in drug content of gentamicin before and after storage for three months. The result indicates that the formulations were stable at the required storage condition.

Conclusions
The design and preparation of hydrogels is an exciting field of research that seeks to exploit the attractive properties of different polymeric carriers to improve the delivery of therapeutic molecules. In this research work, gentamicin-loaded topical hydrogels based on Poloxamer 407 and Carbopols® (971P and 974P) were developed and evaluated for improved skin delivery of gentamicin. All the formulations showed pH-dependent swelling, good drug encapsulation, tolerability on rats and stability, as well as good in vitro permeation properties, which were generally better than commercial gentamicin ointment and pure sample of gentamicin. The Poloxamer 407 based-hydrogels exhibited the best in vitro performance. It follows, therefore, that this delivery system could offer a promising approach for the treatment of topical infections caused by gentamicin-susceptible bacteria.

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Abbreviations: P407, Poloxamer 407; C971P, Carbopol® 971P; C974P, Carbopol® 974P.

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