Formulation, characterization, and ex-vivo permeation studies on gentamicin-loaded transdermal patches based on PURASORB® polymers

Petra Obioma Nnamani1, Franklin Chimaobi Kenechukwu1, Esther Uju Dibua2, Celestine Chidi Ogbonna2,3, Mumuni Abdul Momoh1, Augusta Uche Olisemeka1, Agubata Chukwuma Obumneme4 and Anthony Amaechi Attama1

1Drug Delivery Research Unit, Department of Pharmaceutics, University of Nigeria, Nsukka 410001, Enugu State, Nigeria.
2Department of Microbiology, University of Nigeria, Nsukka 410001, Enugu State, Nigeria.
3Microbiology Unit, School of Bioscience and Biotechnology, University of Camerino, 62032 Camerino, Italy.
4Department of Pharmaceutical Technology and Industrial Pharmacy, University of Nigeria, Nsukka 410001, Enugu State, Nigeria.

Accepted 12 April, 2013

Topical administration of gentamicin, an aminoglycoside antibiotic commonly used for treatment of bacterial infections, is limited by toxicity and membrane impermeability. The purpose of this study was to develop an alternative non-invasive, convenient and cost-effective drug delivery system for enhanced skin delivery of gentamicin. The patches were formulated by solvent evaporation technique using PURASORB® polymers and evaluated for drug content, thermal properties, physicochemical performance, stability, skin irritability and ex-vivo drug permeation through rat skin using a modified Franz diffusion cell. The DSC results indicate absence of strong interaction between gentamicin and the polymers. The formulations showed good drug encapsulation, stability, physicochemical properties, tolerability on rat skin and ex-vivo drug permeation through rat skin. Compared with a commercially available gentamicin sulphate cream the transdermal patches gave higher ex-vivo skin permeation through rat skin with patches of PURASORB® PL 32 showing highest permeation flux (5.161 μg/cm².h) and permeation coefficient (1.032 × 10⁻³ cm²/h). The results of this study indicates that patches of PURASORB® PL 32 represent a new delivery system for enhanced skin delivery of gentamicin.

Key words: Transdermal patches, gentamicin, PURASORB® polymers, bioadhesive strength, ex-vivo drug release.

INTRODUCTION

The method by which a drug is delivered can have a significant effect on its efficacy. The setbacks associated with the various means of drug administration have led to the increasing interest in the development of bioadhesive controlled release dosage forms for the treatment of both topical and systemic diseases (Kaur and Tambwekar, 2003; Ofokansi et al., 2005). Bioadhesive dosage forms can bind to mucous or epithelial surface and can be...
mostly Gram-negative bacteria including against a wide range of human bacterial infections, and dermatosis (Chambers, 2004). Gentamicin is active against a wide range of human bacterial infections, mostly Gram-negative bacteria including *Pseudomonas, Proteus, Serratia*, and the Gram-positive bacteria such as *Staphylococcus aureus* (Sundin et al., 2001). Like other aminoglycosides, gentamicin is toxic to the sensory cells of the ear and also causes nephrotoxicity by inhibiting protein synthesis in renal cells. This mechanism specifically causes necrosis of cells in the proximal tubule, resulting in acute tubular necrosis which can lead to acute renal failure (Chambers, 2004). Although the clinical utility of gentamicin is limited to injection or topical dosage form, there are lots of problems associated with conventional routes of administration of gentamicin. In other words, despite the broad spectrum of activity of gentamicin against Gram negative organisms and resistant organisms such as *Pseudomonas aeruginosa* and *klebsiella*, it is expensive, with poor oral bioavailability and, when administered parenterally, is painful, has to be given by a professional and in case of toxicity, there is no way the drug can be removed from the system (Umeyor et al., 2012). More so, in case of intravenous (i.v) therapy, many problems are associated with the use of gentamicin in injection form — inconveniences of injection for long term administration, side effects such as nephrotoxicity and ototoxicity among others (Australian Prescriber, 2011). Though topical administration of gentamicin is not painful, the patient may not apply the ointment/cream as often as required. Therefore, there is a need for the preparation of a new dosage form of gentamicin. Owing to the advantages offered by transermal drug delivery systems (TDDS) over conventional routes of administration (Chandak and Verma, 2008; Mundargi et al., 2007), this study was designed to evaluate the transdermal delivery system of gentamicin so as to develop non-parenteral and needleless (non-invasive) gentamicin preparation that will not only ensure controlled delivery of gentamicin but also ensure patient’s compliance. A transdermal patch is not painful and is cost effective (Mutalik and Udupa, 2004). Technically, the patch is placed in a part of the body which releases the drug into the body for a long period of time (Brown et al., 2006; Valenta and Auner, 2004; Dnyanesh and Vavia, 2003). Polymeric matrices are usually employed as carriers for transdermal delivery of drugs/actives (Valenta and Auner, 2004; Dnyanesh and Vavia, 2003; Chandak and Verma, 2008; Mundargi et al., 2007; Singh et al., 2003; Verma and lyker, 2002; Gupta and Mukherjee, 2003). The novelty embodied in this study lies in the formulation of gentamicin transdermal patches using PURASORB® polymers, a well-established, safe, biocompatible and resorbable excipients commonly employed in the formulation of controlled release drug delivery systems. These biodegradable polyesters have wide applications, including in orthopedic implant devices, surgical sutures, cardiovascular products, tissue regeneration scaffolds, among others. PURASORB® materials allow for maximum flexibility in formulation technologies, ranging from extrusion and solvent processing to spray drying. Moreover, they are the material of choice for the...
production of implants, microspheres, and depot systems (Lyman, 2007; Yasukawa et al., 2001; Avitable et al., 2001).

The objective of this study, therefore, was to design and formulate transdermal patches incorporating gentamicin using biodegradable polyesters for the purpose of enhancing the delivery of the drug, by providing controlled delivery of the drug. The suitability of four different biodegradable polyesters (PURASORB® polymers: PLGA, PDL 05, PL 32 and PDL 04) for this purpose was assessed by evaluating some of the physicochemical properties of the patches formed, the efficiency of incorporation of the drug in the patches as well as the ex-vivo permeation of the incorporated drug across intact skin.

MATERIALS AND METHODS

The following materials and methods were used for further purification: Gentamicin (Schering, Rockville, MD, USA), Poly(D-L-lactide-co-glycolide) (PLGA)-PURASORB® PDLG 7502A, poly(L-lactide) - PURASORB® PL® 32, poly(DL-lactide)-PURASORB® PDL 04, and poly(DL-lactide) -PURASORB® 05, (PURAC biochem by Gorinchem, Holland), Ethyl acetate, Sodium borate, Ophthalidialdehyde and 2-mercaptoethanol (Sigma-Aldrich, USA), Polyvinyl alcohol, Propylene glycol (Merck, Germany), Isopropanol, Methanol and Formalin (Adwic El-Nasr, Chemical Co., Cairo, Egypt), Sodium hydroxide (BDH, England) and distilled water (Lion water, UNN, Nigeria). Gentamicin sulphate cream USP, 0.1% (Perrigo Bronx, New York, USA) was used as a commercially available topical gentamicin cream. All other laboratory materials were of analytical grade. 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 General Assembly, Seoul, October 2008.

Preparation of transdermal patches

The patches were prepared by solvent evaporation technique (Arora and Mukherjee, 2002), using gentamicin, plasticizers and other film forming polymers. Gentamicin (5 g) was dissolved with distilled water (5 ml) in a beaker followed by addition of 10 g of propylene glycol. The mixture was stirred continuously until a solution (drug reservoir) was formed. The backing membrane was cast by weighing approximately 10 g of the film forming polymer (PLGA) into a separate beaker, adding about 70 ml of ethyl acetate and vortexing (Vortex Genie Bouxemia, N.Y 11716, USA) the mixture for 5 min. Subsequently, the drug-containing solution (drug reservoir) was then poured into the solution containing the PLGA (backing membrane). This dispersion was properly stirred and poured into an aluminum foil-lined petri dish of defined area (10 cm²). A funnel of suitable size was inverted over the petri dish to minimize solvent evaporation. Casting solvent was then allowed to evaporate 48 h to obtain dry films. The above procedure was repeated using PDL 05, PL 32 and PDL 04 as the film forming polymers as well as appropriate quantities of either or both ethylacetate, chloroform and sodium hydroxide (0.1 N NaOH ), as depicted in Table 1. The patches were stored between sheets of wax paper in a desiccator until further analysis.

Differential scanning calorimetry (DSC)

The stability and compatibility of gentamicin and different polymers to be used for the development of gentamicin transdermal film formulations was studied using a differential scanning calorimeter (Netzsch DSC 204 F1, Germany). Sample (2.5 – 5 mg), placed in an aluminum crucible cell was firmly crimped with the lid to provide an adequate seal. The thermal properties such as melting temperature, enthalpy and glass transition of the drug and transdermal patches were determined in the range of 20 – 350 °C under a 20 ml/min nitrogen flux at a heating rate of 10 °C/min. The baselines were determined using an empty pan, and all the thermograms were baseline corrected.

Characterization of gentamicin transdermal films

Physical appearance and thickness

The patches were visually inspected for colour, clarity, flexibility and smoothness. The thickness of the films was assessed using a screwguage (Mitutoyo Co., Japan). Three randomly selected patches of each formulation were tested for thickness. The thickness was measured at three separate points of each patch in order to ensure uniform thickness. The mean value and standard deviation was calculated (Amunaikit et al., 2005).

Weight variation

The patches were tested for mass variation by individually weighing three randomly selected patches from each batch; the average weight and standard deviation were calculated (Verma and Iyker, 2002).

Moisture content

The film sample (2.0 × 2.0 cm) was weighed (W₁) and kept in a desiccator containing anhydrous calcium chloride at 37°C for 24 h. Films were removed from the desiccators and reweighed until a constant weight (W₂) was obtained. The percentage moisture loss (an index of moisture content) was calculated using the formula (Krishna and Pandit, 1994):

\[
\text{Moisture loss (\%)} = \frac{W_1 - W_2}{W_2} \times 100
\]  

Where W₁ is the initial weight of the film and W₂ is the final weight of the film.
**Moisture uptake**

The film sample (2.0 cm²) was weighed \(W_1\) and kept in a dessicator containing saturated solution of potassium chloride at 25 °C (90 ± RH) for 24 h. Films were removed from the dessicator and reweighed until a constant weight \(W_2\) was obtained. The percentage moisture uptake was calculated using the formula (Gupta and Mukherjee, 2003):

\[
\text{Moisture uptake (\%)} = \frac{W_1 - W_2}{W_2} \times 100
\]  

(2)

Where \(W_1\) is the initial weight of the film and \(W_2\) is the final weight of the film.

**Film folding endurance**

This was determined by repeatedly folding the patches (2 cm²) at the same place until a crack or break was observed. The number of times the film could be folded at the same place without breaking/cracking gave the value of folding endurance. Three randomly selected patches of each formulation were tested in triplicates and the mean and standard deviation calculated (Devi et al., 2003).

**Drug content**

Three randomly selected medicated patches were assayed. The drug content of the selected medicated patches was determined by dissolving each patch in 100 ml of the casting solvent distilled water. Gentamicin sulphate concentration was determined spectrophotometrically (Shimadzu UV-1601 UV/Vis double beam spectrophotometer, Japan) after derivatization with orthophthalaldehyde reagent by Zhang’s method (Zhang et al., 1994). Briefly, the o-phenaldialdehyde reagent was formulated by adding 2.5 g o-phenaldaldehyde, 62.5 ml methanol and 3 ml 2-mercaptoethanol to 580 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, ophenaldialdehyde 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-phenaldialdehyde 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 isindole whose absorbance was then measured at 332 nm (Chang et al., 2006).

**In vitro bioadhesive strength measurement**

The bioadhesive strength of gentamicin films was studied by evaluating the force required to detach the hydrated polymeric films from the surface of rat skin tissue using a Lecomte Du Nuoy tensiometer (Model Nr 3124, A. Kruss Hamburg, Germany) adapted for this purpose (Bazigha et al., 2011). Briefly, a portion of freshly sacrificed rat skin was obtained from an abattoir in Nsukka, Nigeria. The tissues were cut into small pieces (2 × 5 cm) and placed on a flat surface and irrigated with 0.5 ml of distilled water so as to enhance the interaction between the tissue and film. The film was attached to the surface of the ring of the tensiometer by glue. The film held by the wire ring was made to make contact with the tissue for 5 min, after which the film was pulled away from the tissue using the side knob of the instrument. The total force (in dynes) required to completely detach the film from the tissue was recorded. The experiment was repeated three times on different tissue surface for each batch of the films. The bioadhesive force \(F_b\) was calculated per unit area of the polymer film as follows (Ganesh et al., 2011):

\[
F_b = \frac{F}{A}
\]

(3)

Where \(F_b\) is the mucoadhesive force, \(F\) is the force applied, and \(A\) is the cross sectional area.

**Skin irritancy test of the prepared patches in rabbits**

Skin irritancy studies were performed on twenty healthy rabbits of either sex weighing 1.920 ± 0.087 kg (divided into five rabbits per group) according to an established method (Verma and Chandak, 2009). The hair of a skin area of around 5.0 cm² was shaved with an electric clipper, covering both sides of the vertebral column of each rabbit and care was taken to avoid skin damage during shaving. Each batch of the topical patches was applied onto the shaved surface 24 h after hair removal using a piece of cotton wool soaked in saturated drug solution on the back of the rabbits. The patch was placed over the skin with the help of surgical adhesive tape. An aqueous solution of 0.8% formalin was applied as a standard irritant. The animals were visually observed for 7 days and checked for any sign of edema or erythema.

**Preparation of rat abdominal skin**

Male Wistar rats were sacrificed with prolonged anesthesia and the abdominal skin of each rat excised. Abdominal hairs on the skin were removed with clipper, full thickness skin was surgically removed and dermis side was wiped with isopropyl alcohol to remove residual adhering fat. Heat separation technique (Otokanski et al., 2012) 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 using blunt forceps. The epidermis was washed with water and wrapped with aluminum 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 5.5) overnight before use.

**In-vitro skin permeation studies**

*In vitro* skin permeation studies were performed using a modified Franz diffusion cell with effective diffusion area of 2.27 cm². The excised rat abdominal skin (Wistar albino) was mounted between the donor and receptor compartments of the diffusion cell. The formulated patches were placed over the skin and covered with paraffin film in order to ensure that the patches do not dislodge from the skin during the test (Azarni et al., 2007; Repka et al., 2005). The receptor compartment of the diffusion cell was filled with phosphate buffer pH 5.5. 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 in order to simulate skin temperature. At predetermined time intervals for a total of 42 h, aliquots were withdrawn and the drug content was determined spectrophotometrically at 332 nm after derivatization with o-phenaldialdehyde reagent, as previously mentioned. The receptor phase was replenished with equal volume of phosphate buffer pH 5.5 at each sample withdrawal. The results were the mean values of three runs. The cumulative percentages of drug permeated per square centimeter of patches were plotted against time. Control experiment was performed using pure sample of gentamicin powder added directly into the donor compartment of
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Figure 1. DSC thermograms of transdermal patches and gentamicin. PL 32, PDL 04, PDL 05 and PLGA are gentamicin-loaded patches containing PURASORB® PL 32, PDL 04, PDL 05 and PLGA respectively (From to to bottom: gentamicin, PL 32, PLGA, PDL 05 and PDL 04, respectively).

the Franz diffusion apparatus.

Permeation data analysis

The flux \((\mu g \; cm^{-2} \cdot h^{-1})\) of gentamicin was calculated from the slope of the plot of the cumulative amount of gentamicin permeated per \(cm^2\) of skin at steady state against the time using linear regression analysis (Attama et al., 2009). The permeation coefficients were obtained from the steady-state flux values making use of the following equations.

\[
P = \frac{J}{C_0} \text{ (cm/h)}
\]

(4)

Where \(P\) is the permeation coefficient, \(C_0\) is the initial drug concentration in the drug compartment; \(J\) represents the steady-state flux obtained from Equation 5.

\[
J = \frac{dQ}{Adt} \quad (\mu g/cm^2 \cdot h)
\]

(5)

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.

Stability study of transdermal patches

Time resolved stability studies were carried out on the various batches of the formulations following the ICH guidelines (Ganesh et al., 2011). The transdermal patches were stored at 40 ± 0.5°C in a humidity chamber having a RH of 75 ± 5%. After four weeks, six months and one year of storage, the patches were withdrawn and evaluated for the drug content following the method stated above.

Statistical analysis

All experiments were performed in replicates for validity of statistical analysis. Results were expressed as mean ± SD. ANOVA and Student’s t-test were performed on the data sets generated using SPSS. Differences were considered significant for p-values < 0.05.

RESULTS

Figure 1 shows the thermograms of gentamicin and the patch formulations, while Table 2 presents the thermal properties of the transdermal patches. Gentamicin showed a melting peak of 249.1°C. When the gentamicin was loaded on the four different PURASORB® polymers, the DSC thermograms showed different melting peaks and thermal properties according to the nature of the PURASORB® polymer, as depicted in Figure 1.

The results of the physicochemical characterization of the patches are shown in Table 3. All the patches of the different polymers were transparent, colourless, smooth and uniform but the PDL 05 polymer had the greatest clarity. The patches made from PL 32 were firmer, more adhesive and more uniformly dispersed. After the addition of 5 ml of 0.1 N NaOH to PDL 04, the dispersion
Table 2. Thermal properties of gentamicin transdermal patches.

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Melting point (°C)</th>
<th>Enthalpy (Mw/mg)</th>
<th>Glass transition temperature (Tg) (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Onset</td>
<td>Middle</td>
<td>End</td>
</tr>
<tr>
<td>PLGA</td>
<td>...</td>
<td>...</td>
<td>31.3</td>
</tr>
<tr>
<td>PDL 05</td>
<td>...</td>
<td>...</td>
<td>38.7</td>
</tr>
<tr>
<td>PL 32</td>
<td>180.7</td>
<td>-1.981</td>
<td></td>
</tr>
<tr>
<td>PDL 04</td>
<td>126.5</td>
<td>-5.155</td>
<td></td>
</tr>
</tbody>
</table>

Table 3. Properties of gentamicin transdermal patches.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Formulation code</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PLGA&lt;sup&gt;a,b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Thickness (µm)</td>
<td>390.50 ± 9.08</td>
</tr>
<tr>
<td>Weight variation (mg)</td>
<td>20.90 ± 0.27</td>
</tr>
<tr>
<td>Drug content (%)</td>
<td>97.60 ± 2.43</td>
</tr>
<tr>
<td>Folding endurance</td>
<td>316.50 ± 4.17</td>
</tr>
<tr>
<td>Tensile strength (dyne cm&lt;sup&gt;-2&lt;/sup&gt;)</td>
<td>75.60 ± 1.07</td>
</tr>
<tr>
<td>Moisture absorption (%)</td>
<td>2.70 ± 0.28</td>
</tr>
<tr>
<td>Moisture content (%)</td>
<td>1.60 ± 0.29</td>
</tr>
</tbody>
</table>

<sup>a</sup>Mean±SD, <sup>b</sup>n=3, PL 32, PDL 04, PDL 05 and PLGA are gentamicin-loaded patches containing PURASORB<sup>®</sup> PL 32, PDL 04, PDL 05 and PLGA respectively.

of the drug became more even. The skin irritation study shows no erythema formation on the rabbit skin for PURASORB<sup>®</sup> PL 32 patches, but patches made from PLGA caused severe redness of the skin within 24 h while the reaction from patches containing PDL 04 and PDL 05 were mild.

The results of <i>in vitro</i> skin permeation studies of gentamicin from the transdermal patches are shown in Figure 2 whereas the permeation data (permeation coefficients and steady-state permeation fluxes) are presented in Table 4. The results indicate that PURASORB<sup>®</sup> PL 32-based patches gave the highest ultimate amount permeated after 42 h. The permeability assessment of the gentamicin from the formulations across the rat skin (Table 4) showed permeation fluxes of 4.761, 4.917, 5.161 and 4.839 µg/cm<sup>2</sup>·h for patches formulated with PURASORB<sup>®</sup> PLGA, PDL 05, PL 32 and PDL 04, respectively; while the permeation coefficients of the formulations in phosphate buffer were: 9.522 × 10<sup>-7</sup>, 9.834 × 10<sup>-7</sup>, 1.032 × 10<sup>-6</sup> and 9.678 × 10<sup>-7</sup> cm/h for patches formulated with PURASORB<sup>®</sup> PLGA, PDL 05, PL 32 and PDL 04, respectively. For the unformulated gentamicin, the permeation fluxes and the permeation coefficients were 4.126 µg/cm<sup>2</sup>·h and 9.048 × 10<sup>-7</sup> cm/h respectively.

Figure 3 shows the drug content of the formulations after storage for one year. There was an insignificant change in the content of gentamicin in the formulations based on the required storage conditions.

**DISCUSSION**

In this work, transdermal patches containing gentamicin sulphate in biodegradable polyesters-based matrices were developed and evaluated for physicochemical performance, stability and <i>in vitro</i> drug release properties. Differential scanning calorimetry (DSC) enables the quantitative detection of all processes in which energy is required or produced (that is, endothermic or exothermic phase transformations). The result of the DSC study shows that gentamicin melted at 249.1°C as shown in Figure 1. The enthalpy of the gentamicin was -1.966 mW/mg. Furthermore, the DSC results show that the drug-loaded polymeric matrices generally had low enthalpies compared to the drug. Reduction in enthalpy generally suggests less crystallinity of drug-loaded polymeric matrices (Nnamani et al., 2010). This shows that the formulations generated imperfect matrices, which may have created numerous spaces for drug localization (Umeyor et al., 2011; Nnamani et al., 2010). More so, the physicochemical compatibility of the drug and the polymers studied by differential scanning calorimetry suggested absence of any incompatibility. The results revealed the compatibility of gentamicin and the polymers as well as the stability of the drug in the polymeric matrices (formulations). This is because the formulations gave lower endotherms than gentamicin, implying that gentamicin exists in amorphous state in the formulations and also is properly solubilized in the matrix systems.
(Umeyor et al., 2012). Moreover, there was general disappearance of drug peak in all patches but PURASORB® PL 32 patch had the least enthalpy (-1.981 mW/mg) reflecting the degree of disorder in the matrix.

The formulated gentamicin-loaded patches exhibited good organoleptic and physicochemical properties. The values of the thickness of the patches indicated uniformity in thickness, while the weights of the different batches of the transdermal patches were relatively similar. All the transdermal patches had highly uniform drug content. The results showed that the process employed in the preparation of the gentamicin patches was capable of producing patches with uniform drug content and minimal patch variability. The little variation in the drug content may be as a result of API and matrix physicochemical and material characteristics (Dnyanesh...
The design and preparation of transdermal patches is
gaining pace as it seeks to exploit the attractive properties of polymeric and/or lipid carriers to improve the delivery of therapeutic molecules. In this study, gentamicin-loaded transdermal patches based on biodegradable polyesters were successfully prepared by the solvent evaporation technique, which is simple, cheap and reproducible. The patches were developed and evaluated for physicochemical performance, stability and ex-vivo drug permeation properties. All the formulations showed good stability, physicochemical and ex-vivo drug permeation properties, with PURASORB® PL 32 patches as the best formulation. It follows, therefore, that this delivery system (PURASORB® PL 32 patches) could offer a better and more promising approach for the delivery of gentamicin than the commercially available topical gentamicin sulphate cream. Further bioevaluation is advocated to establish the enhanced performance of the formulations for possible application in the treatment of topical infections caused by gentamicin-susceptible micro-organisms.

ACKNOWLEDGEMENTS

We wish to thank PURAC Biochem, Gorinchem, Holland for providing samples of the polyesters (PURASORB® PL 32, PDL 04, PDL 05 and PLGA) used in the study.

REFERENCES


