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ORIGINAL ARTICLE

Development and characterization of a transdermal patch and an emulgel containing kanamycin intended to be used in the treatment of mycetoma caused by *Actinomadura madurae*

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Abstract

Background: Mycetoma is a chronic, degenerative, and incapacitating infection of the skin and subcutaneous tissue. *Aim*: This study focuses on developing a kanamycin-based auxiliary system intended to be used in the treatment of mycetoma caused by *Actinomadura madurae*. *Methods*: Transdermal patches (with two different formulations: one with free kanamycin [K] and the other one with kanamycin adsorbed in silica [K-SG]) and an emulgel were developed. Both patches were prepared by the casting-evaporation technique. To characterize them, differential scanning calorimetry, bioadhesion, post-moisture detachment, strength and rupture distance, gas exchange, water uptake, and dissolution studies were carried out. The emulgel (containing 0.57% of kanamycin) was prepared from an oil-in-water emulsion, which was then incorporated to a gel. *Results*: the patches with the best characteristics contained 22.9% of silica and 14.6% of kanamycin. Dissolution studies indicated that 8.8% of kanamycin released from K and 3.2% from K-SG at 24h. The emulgel containing 0.57% of kanamycin showed good technological characteristics for its application to the skin (viscosity, 44.9 ± 1.4 poises; pH, 6.9 ± 0.4; and penetrability, 52.7 ± 5.1). *Conclusions*: The optimal patches were those containing 15.9% of freely dispersed kanamycin (K) and 14.6% of kanamycin adsorbed in silica (K-SG), which corresponds to the batch 2-0.8. The assessments performed to both pharmaceutical forms (patches and emulgel) show that they have the adequate technological characteristics for being used as an auxiliary in the treatment of actinomycetoma caused by *A. madurae*.

Key words: Actinomadura madurae*; emulgel; kanamycin; mycetoma; transdermal patch*

Introduction

Mycetoma or madura foot is a chronic, degenerative, and incapacitating infection of the skin and subcutaneous tissue[1–3]. In Mexico, in 98% of cases, it is caused by bacteria from the group of aerobic actinomycetes (actinomycetoma), such as *Nocardia brasiliensis*[4], *Nocardia asteroides*, *Nocardia caviae*, *Actinomadura madurae*, *Actinomadura pelletieri*, and *Streptomyces comaliensis* or *Madurella mycetomatis*, and in 2% of cases by fungi (eumycetoma)[5,6], including *Madurella mycetomatis*, *Madurella grisea*, *Acremonium* sp, *Fusarium* sp, and *Pseudallescheria boydii*. The disease is predominant in India, Sudan, Brazil, Venezuela, and Mexico[5,7]. It has been found that 9.6% of cases are because of *A. madurae*[8]. Evolution time ranges from 1 to 10 years, although usually

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at 2 years complications such as fibrosclerosis and fistulas are already present[9].

Mycetoma treatment consists in the administration of one or several antibiotics[10,11]. In the particular case, when the disease is caused by *A. madurae*, either with bone or visceral involvement, the treatment used is that normally employed for actinomycetomas[12] and consists of diaminodiphenylsulfone 100–200 mg/day and trimethoprim–sulfamethoxazole 80/400–160/800 mg/day. When there is resistance to the usual treatment, amikacin sulfate (15 mg/kg) is used with satisfactory results[5,13]. Recently, treatment consisted of an in administering kanamycin (15 mg/kg-weight/day) plus trimethoprim–sulfamethoxazole for 14 days, with a resting period in which diaminodiphenylsulfone (100 mg/day) plus trimethoprim–sulfamethoxazole is administered for 2 weeks, thus completing a 4-week cycle. According to the severity of the case, one to four cycles are administered with satisfactory results[8].

The use of intramuscular kanamycin for the treatment of mycetoma caused by *A. madurae*, with bone involvement or resistant to conventional treatment, is completely novel. During treatment, it is advisable that the patient be hospitalized and monitored, as kanamycin adverse reactions include ototoxicity and nephrotoxicity. Once the patient has improved and is discharged home, there is the risk of a relapse; therefore, it is necessary to administer topical or transdermal auxiliary treatment for this disease. A kanamycin sulfate emulgel has been recently developed as auxiliary treatment for this infection with good results in patients previously hospitalized and discharged because of this disease[14,15].

This study had the aim of developing and optimizing two formulations, a patch and an emulgel, to be administered directly on the affected area, with the main objective of releasing antibiotic locally as a support to the systemic treatment, so that the patient can easily apply it, and if hospitalized, upon being discharged home, the risk of a relapse can be reduced. In this first stage of the investigation, it was sought that both pharmaceutical forms had adequate technological characteristics to be used as an auxiliary treatment for mycetoma due to *A. madurae*. In the case of the transdermal patch, kanamycin was placed free or adsorbed in silica gel, with the purpose of prolonging its release or adsorbing secretions in case of open lesions.

Materials and methods

Materials

Kanamycin A sulfate was kindly donated by Bristol-Myers Squibb (México city, DF, México); erythromycin ethylsuccinate and Eudragit E-100® (Röhm GMBH, Berlin, Germany) were provided by Helm (México city, DF, México). Silica gel 60 with a specific surface area of approximately 500 m^2/g (Merck, Naucalpan, México) and Ethocel Standard 10 Premium® (Ethylcellulose) were purchased from The Dow Chemical Company (Midland, MI, USA); triacetine and Carbopol 934P-NF® were donations of Industrias Monfel (Cuautitlán Izcalli, México) and Gattefossé (Noveon, México), respectively. Liquid Vaseline NFSS (Droguería Cosmopolitan, México city, DF, México), and Span 60® and Tween 60® (ICI Surfactants, Potsdam, NY, USA) were acquired from Aromgel S.A. de C.V. (Tlalnepantla, México); triethanolamine, glacial acetic acid, and 1-butanol were obtained from J.T. Baker (México city, DF, México); methanol and monobasic potassium phosphate were bought from Fermont (Monterrey, México) and ninhydrine from Sigma (St. Louis, MO, USA). Water was distilled with a Milli-Q quality system (Millipore Corp., Bedford, TX, USA).

Adsorption isotherms of kanamycin in silica and kanamycin–silica desorption profiles

To determine the optimal time for the adsorption of kanamycin in silica, 20 mg of silica gel 60 (SG), with a specific surface area of approximately 500 m^2/g, previously dried at 100°C for 2 hours, was added to 2 mL of aqueous solution saturated with kanamycin (~314 mg). The solution was shaken for 15, 30, 60, 90, and 180 minutes, by quintuplicate, and the drug concentration was determined using high-performance thin-layer chromatography (HPTLC), obtaining by difference the amount of adsorbed drug. On the other hand, different concentrations of kanamycin were assayed (20, 40, 60, 80, 100, 120, 140, 160, 180, and 200 mg/mL) in 800 mg of silica using only one shaking time (30 minutes) by sextuplicate.

For determining the desorption profiles, 800 mg of the drug-adsorbent system (equivalent to 848.7 mg of free kanamycin) was added to the dilution medium (distilled water) at 37°C, shaking at 100 rpm. Samples were taken every hour during 8 hours, and free kanamycin was quantified by using HPTLC.

Patch preparation

Patches with kanamycin and erythromycin contained in the adhesive (Eudragit E-100®) were developed. Kanamycin was used for fighting the actinomycetoma produced by *A. madurae* and erythromycin with the purpose of avoiding secondary infections[14,15]. The former because of the high prevalence of secondary bacterial infections in patients suffering from mycetoma, which as a consequence can develop other problems, from pain to bacteremia or septicemia. Erythromycin is a wide spectrum antibiotic often used for people having allergy to penicillins[16]. It results effective against Gram-positive[17]

as *Streptococcus pyogenes*, *Streptococcus pneumoniae*, *Streptococcus viridians*, *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Clostridium perfringens*, *Corynebacterium diphtheriae*, and *Listeria monocytogenes*, with intermediate action against Gram-negative as *Hemophilus influenzae* and *Neisseria meningitides*, very effective against *Neisseria gonorroeae*[17], *Pasteurella multocida*, *Borrelia* and *Bordetella pertusis*, *Campylobacter jejuni*, and rickettsiae as *Mycoplasma pneumoniae*, *Chlamydia lymphogranulomatis*, and *Chlamydia trachomatis*; there are also some mycobacterium as sensible as *M. scrofulaceum*, *M. kansassi*, and *M. intracellulare*[18].

The patches were covered with an ethylcellulose occlusive layer. The experiment was divided into two parts: In the first part, free-drug patches were developed, testing different proportions of ethylcellulose and silica (Table 1). In the second part, patches with both free kanamycin and kanamycin adsorbed in silica were produced.

During the first part of the experiment, a two-layer patch system was prepared. For the occlusive layer, a solution of ethylcellulose in methanol was poured on a teflon-coated container with an area of 132.7 cm^2, evaporating the solvent at room temperature for 24 hours (the ethylcellulose and SG proportions tested in the formulation are shown in Table 1). For the adherent layer, 4 g of Eudragit E-100® was dissolved in 13 mL of methanol, 1.2 g of triacetine was added as plasticizer, and this solution was placed on the ethylcellulose layer formed. Finally, different proportions of silica were dispersed (Table 1). In the second part of the development, the above-described steps were followed, dissolving 0.2 g of erythromycin in the adhesive layer and dispersing 1.4 g of kanamycin (free kanamycin or adsorbed in silica); these amounts were determined based on the technological properties of the different formulations used for preparing the patches during the first stage (Table 2). The thickness of the patches was measured using a Fowler & NSK digital vernier (Digitrix, Osaka, Japan).

Differential scanning calorimetry studies

The samples were carefully weighted (~5 mg) in closed aluminum pans and examined in a differential scanning calorimetry (DSC) (Q10 V6.16 TA Instruments Berlin, Germany) calibrating with indium. Thermograms were made in a heating ramp of 10°C/min in a temperature range from 0°C to 350°C, under a nitrogen flow of 20 mL/min. The examined substances were the following: (i) silica, (ii) kanamycin sulfate, (iii) kanamycin adsorbed on silica, (iv) erythromycin ethylsuccinate, (v) triacetine, (vi) Eudragit E-100®, and (vii) ethylcellulose. Regarding the patches, thermograms were obtained for the most significant batches with or without drug. The batches analyzed are listed following the nomenclature of Table 1. Patches without drug: (viii) 1.5–0.0, (ix) 1.5–0.4, (x) 2–0.8, (xi) 1.5–1.6, and (xii) 2–1.2; patches without drug, premoistened for 2 hours: (xiii) 1.5–0.4, (xiv) 1.5–1.6, and (xv) 2–1.2. Lastly, the patches analyzed with DSC that contained kanamycin and erythromycin were the following: (xvi) 1.5–0.0 with 0.7 g of free kanamycin and 0.1 g of erythromycin dissolved in the adhesive layer, (xvii) 1.5–0.4 with 0.7 of kanamycin adsorbed on silica plus 0.1 erythromycin dissolved in the adhesive layer and (xviii) 2–0.8 with 1.4 g of kanamycin adsorbed on silica plus 0.2 g of erythromycin dissolved in the adhesive layer.

The heating cycle method (heating, cooling, and reheating) was also used, under the same conditions as those in the heating ramp (10°C/min), with the aim of corroborating that kanamycin was adsorbed on silica. In this case, the studied substances were (i) kanamycin sulfate, (ii) kanamycin adsorbed on silica, and (iii) a physical mixture of kanamycin and silica.

Table 1. Ethylcellulose and silica proportions studied for patch formulation.

	Silica gel 60 (g)				
Ethylcellulose (g)	0.0	0.4	0.8	1.2	1.6
1.0	1–0.0	1–0.4	1–0.8	1–1.2	1–1.6
1.5	1.5–0.0	1.5–0.4	1.5–0.8	1.5–1.2	1.5–1.6
2.0	2–0.0	2–0.4	2–0.8	2–1.2	2–1.6

Table 2. Patch formulations, amounts expressed in % (p/p).

	Kanamycin formulation	Kanamycin adsorbed in silica formulation
Ethylcellulose	22.7	20.8
Eudragit E-100®	45.5	41.7
Triacetine	13.6	12.5
Erythromycin	2.3	2.1
Kanamycin	15.9	0
Kanamycin adsorbed in silica gel	0	22.9[a]

[a]Equivalent to 14.6% of free Kanamycin.

Bioadhesion studies

Bioadhesion studies were made in sextuplicate for all patch batches with different ethylcellulose to silica ratios shown on Table 1. A healthy 30-year-old female volunteer participated, with no cosmetic, pharmacological, or dermatological treatment for at least 12 hours before the study. The patch, with an area of 0.91 cm^2, was premoistened with 300 µL of distilled water for 3 seconds and immediately afterwards it was put into contact with the spherical probe of a texturometer TA.XT2 (Texture Technologies Corp., Scarsdale, NY, USA). The volunteer placed her forearm on the lower part of the equipment, then the probe was lowered at a speed of 2 mm/s until putting the patch in contact with

the volunteer's forearm, applying a compression force of 800 g/10 s. The patch was then withdrawn at a speed of 10 mm/s, considering a maximum detachment distance of 30 mm, measuring the necessary strength required to remove the patch.

Postmoisture detachment studies

The test was made under the same conditions as the bioadhesion studies, except that the patch was premoistened, exposing the adhesive face to 100 mL of distilled water for 1 hour.

Determination of patch rupture distance and strength

The assessment was made by using the same texturometer mentioned in the Section 'Postmoisture detachment studies' by triplicate for all batches. It consisted of placing a patch with an area of 132.7 cm^2 in a bottomless square support and using a spherical probe to measure the distance as well as the compression strength at which the patch was ruptured. The compression speed was 2 mm/s and was set at a maximum compression distance of 30 mm.

Gas exchange

This study was carried out by using a Tewameter™ (Courage+Khazaka, Cologne, Germany). Ten milliliters of phosphate buffer (pH 7.4) was placed in the receptor compartment of a Franz-type diffusion cell, while the patch was placed in the membrane zone (between the receptor and the donor) and was fixed to the cell by the use of pliers. Then, the water loss measurement was made by placing the sensor of the Tewameter TM 210 in the place of the donor. The measurement was made every 4 seconds for 1 hour, by triplicate for all batches.

Water uptake

The dry patch was weighted and moistened with 20 mL of distilled water for 15 minutes, removing excess of water carefully with cotton and weighting again. This experience was repeated for 1 hour, and afterwards every hour during 4 hours, by triplicate, for all batches. Water uptake was obtained by the difference in weight.

Dissolution studies

Dissolution studies were made in a DT 1 paddle melter (Optimal Control Inc., Cary, North Carolina, USA) that complies with the characteristics specified by The United States Pharmacopoeia[19]. In the lower part of the glass, a circular teflon device was placed to which the patch, with an area of 13.85 cm^2, was adhered, and 250 mL of phosphate buffer (pH 7.4) was added. Temperature was kept at 32.5°C and constant agitation at 100 rpm. One milliliter of the solution was taken, replacing the volume with fresh medium every hour during 8 hours and subsequently at 24 hours. The studied patches, following the nomenclature of Table 1, were 2.0–0.0 plus 1.4 g of free kanamycin (dissolution was followed for 195 hours) and 2.0–0.8 with 1.4 g of kanamycin adsorbed in silica (dissolution was followed for 24 hours). In both cases, 0.2 g of erythromycin was added to the adhesive layer. The quantification of kanamycin was made by HPTLC.

Analytical method

In those tests that required analytical method, kanamycin was quantified by a previously validated HPTLC method. The sample was applied to C-18 plates (UV_{254}; Merck, Frankfurt, Germany) with an automatic CAMAG TLC Sampler III (version 2.12, Switzerland). The plate was then placed in a chamber (CAMAG, Berlin, Germany) containing the mobile phase [10% phosphate solution: 98% glacial acetic acid (10:0.5)] and was left to displace up to 4 cm on the plate (R_f 0.8). The mobile phase was evaporated from the plate at 110°C for 10 minutes. A solution of 10% ninhydrine in 1-butanol was used for development, letting it dry again at 110°C for 10 minutes. The measurement of the areas was made with a Scanner 3 (CAMAG, Muttnez, Switzerland) and CATS software (version 4.06) with a mercury lamp as detector (λ = 553 nm).

Emulgel preparation

Emulgel was prepared according to a procedure modified from Palma-Ramos[14]; however, because of some instability found with the emulsion proposed by these authors, an adjustment with regard to the surfactants was performed, as explained in the following lines. The oil-in-water (o/w) emulsion was prepared by incorporating the oily phase (liquid vaseline, span 60, and erythromycin ethylsuccinate) to the aqueous phase (Tween 60, water, and kanamycin sulfate) at 70°C. It should be noted that some characteristics of the emulsion were varied (surfactant proportion, 5% and 10% and HLB, 9.01 and 10) to determine the most stable one by the centrifuge test (10 g of the emulsion at 5000 rpm/10 minutes). The emulsion with 10% of surfactant and HLB = 10 was chosen to prepare the emulgel. The gel was made by placing 1 g of Carbopol 934P-NF in 20 mL of water and neutralizing with 1.5 g of triethanolamine. Finally, the emulsion was incorporated to the gel with constant stirring until obtaining a semirigid consistency. Table 3 shows the final formulation of the emulgel. It is important to mention that Tween 80 originally included in the emulgel proposed by Palma-Ramos was replaced by Tween 60. pH was measured with a potentiometer 430 (Corning, Cambridge, UK).

Table 3. Emulgel formulation.

	% (p/p)
Emulsion o/w	
Kanamycin	0.570
Erythromycin	0.084
Liquid vaseline	26.640
Span 60	2.880
Tween 60	3.120
Water	26.670
Gel	
Carbopol 934P-NF	1.780
Water	35.590
Triethanolamine	2.670

Emulgel viscosity studies

Viscosity was determined by placing 50 µL of the emulgel on the plate of a digital viscosimeter CAP 2000 (Brookfiled, NY, USA) using a No. 3 circular needle: i) A temperature interval of 20°C to 50°C at a fixed speed of 50 rpm and ii) speeds of 50 to 150 rpm at fixed temperature of 25°C.

Emulgel penetrability test

To perform this test, the texturometer mentioned above (Section 'Bioadhesion studies') was used, applying a compression force on the emulgel with a conic probe. Testing conditions are mentioned in Table 4.

Results and discussion

Adsorption isotherms of kanamycin in silica and kanamycin–silica desorption profiles

Although apparently there is a less adsorption percentage the greater the contact time, the analysis of variance indicates that there are no significant differences in the percentage adsorbed with respect to contact time (F = 1.23 and $F_{0.05/4,20}$ = 2.87). The time selected to perform the adsorption isotherms was 30 minutes.

Table 4. Conditions in which the emulgel penetrability test was performed.

Velocity prior to the study	5 mm/s
Velocity during the study	2 mm/s
Velocity after the study	10 mm/s
Penetration distance	15 mm
Contact strength	1 g
Temperature	25°C
Narrow-mouth bottle filled to the top	
Upper diameter	3.5 cm
Lower diameter	2.5 cm
Lower diameter height	2.0 cm
Upper diameter height	5.0 cm
Total height	7.0 cm

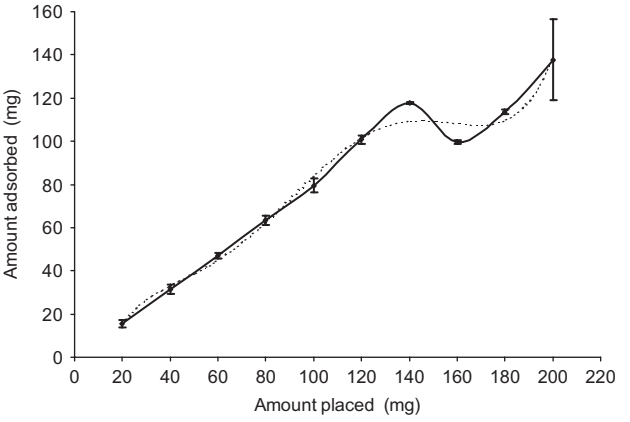

Figure 1. Kanamycin adsorption isotherm in 80 mg of silica (n = 6), wherever $y = -3E-11x^6 + 3E\text{-}08x^5 - 1E-05x^4 + 0.0019x^3 - 0.1346x^2 + 5.0253x - 44.704$ and $R^2 = 0.9871$.

Adsorbing the drug on silica has the purpose of prolonging the release time of the drug in the skin, while adsorbing sweat and fluids from the mycetoma-affected skin. Furthermore, while fluids are being adsorbed, the release of kanamycin into the skin is triggered. Figure 1 shows the adsorption isotherm of kanamycin on silica. As can be seen, a graph with a sigmoidal shape is obtained, which is described by a polynomial equation, indicating the formation of multiple layers. This occurs in surfaces without pores or with micropores that accept multiple layers of adsorbate bound to the surface. The inflection point (140 mg of kanamycin) indicates the formation of the first layer[20]. DSC studies confirmed kanamycin adsorption.

The kanamycin desorption follows a logarithmic profile. At 5 hours, desorption rate decreases. At this time, 496.16 mg of kanamycin has been desorbed (i.e., 58.45%) and at 8 hours, 505.93 mg (i.e., 59.61%).

Patch preparation

According to patch properties, two formulations were selected for being optimal. They are shown in Table 2 and correspond to the 2–0.8 batch of Table 1. The first one includes free kanamycin, and the second one kanamycin adsorbed in silica. The thickness of the different patch formulations ranged from 0.45 to 0.73 mm. Formulation 2–1.6 was the thickest one (0.73 mm) showing significant statistical differences with formulations 2–0.4 (0.45 mm) and 1–1.2 (0.47 mm) (F = 3.746 and $F_{0.05/14,30}$ = 2.037, SD = 0.228).

Differential scanning calorimetry studies

Figure 2 shows the thermograms obtained from the raw materials and kanamycin adsorbed on silica gel. As can be seen, kanamycin shows two endothermic peaks; the first one, very small, at 286.81°C, which corresponds to a weight

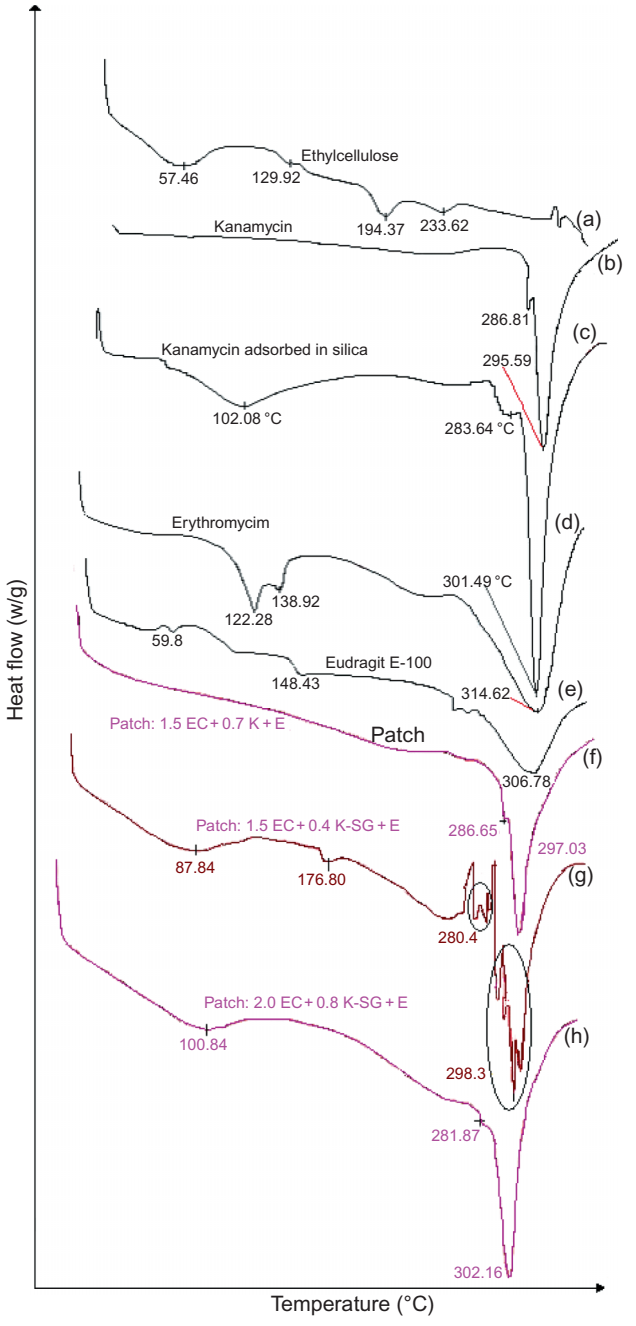

Figure 2. Thermograms of the excipients used in the elaboration of patches.

loss (it can be due to a fusion-decomposition phenomenon) and the second one at 295.59°C, which corresponds to the ignition of kanamycin. By adsorbing it in silica, three peaks are obtained: the first one at 102.08°C, corresponding to water loss; the second one (283.64°C) attributable to a process of desorption of the sample; and in the third peak because of the ignition of kanamycin (301.49°C).

On other hand, it is evident that whether moistened or not, the patches show a first peak because of water loss, although, as expected, this peak is much larger in the premoistened patches. It is also possible to observe that the DSCs have the same shape and that the peaks correspond to their excipients, without showing significant modifications. Therefore, it is possible to state that there are no chemical interactions between the different components. Finally, Figure 2f–h shows the thermograms of some patches. As seen, all of them present the peak due to water and kanamycin, whether free (f) or adsorbed on silica gel (g and h), which confirms that there is no chemical interaction between the excipients and that kanamycin is adsorbed on SG.

Bioadhesion studies

The bioadhesion force of all patch batches, covering the range from 28.2 to 176.1 $\vec{g}$ (Figure 3) was determined. Statistical differences were found between the patch formulations ($F = 9.203$ and $F_{0.05/14,79} = 1.829$). Figure 3 shows that the patches with less adhesion strength are those corresponding to formulations 2–0.4 and 1–1.6. Likewise, those with the greatest adhesion are 1.5–1.6 and 2–1.6. We can also see that in cases where there is no SG, as well as with 0.8 and 1.6 of SG, the greater the amount of ethylcellulose, the greater the bioadhesion. Formulations 2–0.8 and 1.5–1.2 have an intermediate adhesion strength (86.7 and 80.3 $\vec{g}$) compared to the other formulations.

Postmoisture detachment studies

This study has the purpose of verifying the strength with which the patch remains attached to the affected site once it has been moistened, as it is expected that in vivo they will be gradually moistened. Therefore, the patches were premoistened for 2 hours and retested for the strength with which they remain attached to the skin. It should be mentioned that statistically significant differences were found between the different formulations ($F = 14.334$ y $F_{0.05/14,75} = 1.826$). Figure 3 shows that patches without silica and 2–0.4 increase their adhesion strength, compared to nonmoistened patches; contrary to this, greater amounts of silica, for example, 2–0.8 and 1.2 and 1.6 of SG, reduce adhesion strength with increasing moisture; this is because of the fact that the patch loses integrity quickly with greater amounts of silica. The rest of the batches have similar adhesion strengths, whether or not they are moistened. On the other hand, no irritation was observed on the volunteer's arm. It can therefore be considered that the strengths obtained in these tests are adequate for being applied to the skin.

Determination of rupture distance and strength

Figure 4 shows the rupture distance, where it can be seen that by increasing the amount of SG, the rupture distance was smaller, even with formulation 1–1.6,

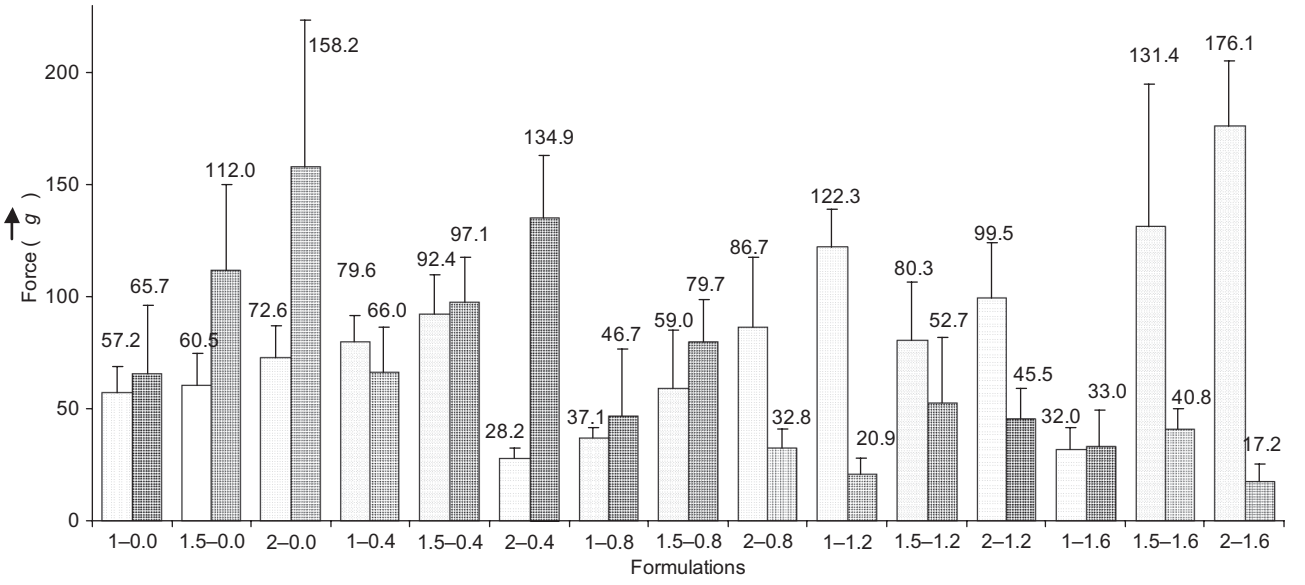

Figure 3. Bioadhesion ⬚ and postmoisture detachment ▦ of patches corresponding to the different formulations indicated in Table 1, $n = 6$.

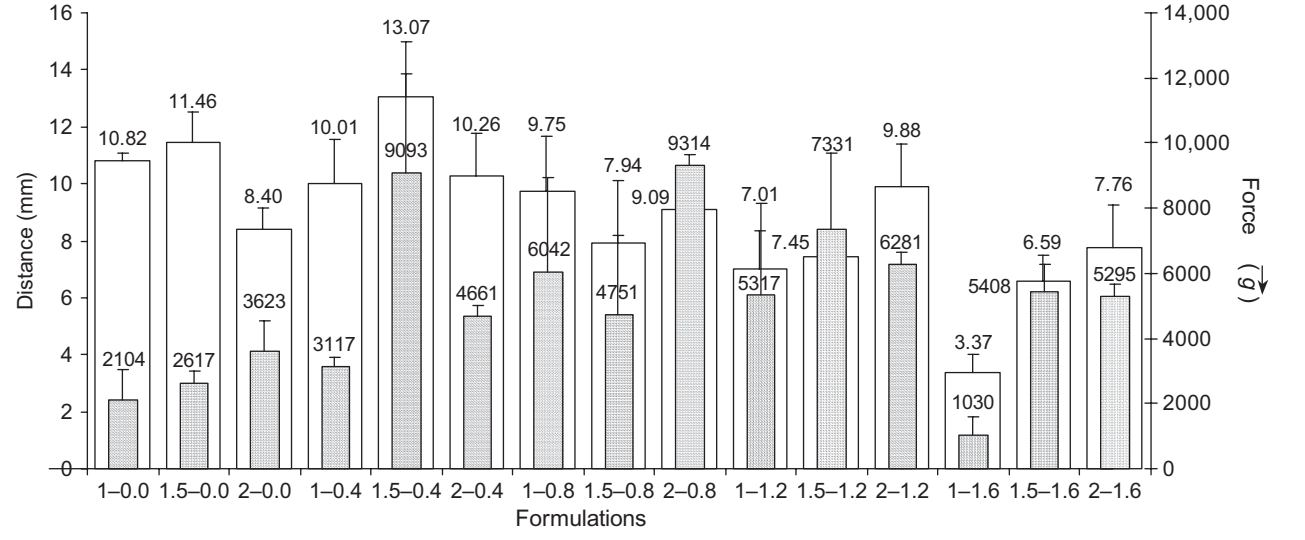

Figure 4. Distance ☐ and rupture strength ▩ of patches corresponding to the different formulations indicated in Table 1, $n = 3$.

which was the one that ruptured at a smaller distance and therefore sooner. We can see that with little (0.4 g) or no SG, the patch ruptured at a smaller distance if the amount of ethylcellulose was increased. However, with a greater amount of SG, the rupture distance increased if the amount of ethylcellulose was increased, as it rendered the patch resistant. It was observed that there are statistically significant differences between formulations ($F = 9.015$ and $F_{0.05/14,22} = 2.173$).

On the other hand, regarding rupture strength, the results were compared with those obtained by Padula et al.[21], who reported a tension strength between 1020 and 1836 $\bar{g}$ (10–18 N/mm^2) for bioadhesive films with lidocaine (whose main excipients were PVAL 72000, Glycerin, Water, and Plastoid E35M). In this work, it was found that all systems show greater rupture strength than that reported by these authors, suggesting that the systems are highly resistant. Those that show greater resistance to rupture are formulations 1.5–0.4 and 2–0.8 and that shows less resistance is formulation 1–1.6 because of little support of the ethylcellulose layer and the great amount of SG solids. Statistically significant differences were found between formulations ($F = 3.746$ and $F_{0.05/14,22} = 2.037$).

Gas exchange

Besides all the characteristics already described, it is necessary to have a semiocclusive patch, so that while the SG takes water from the skin and the adsorbed kanamycin

is released, the skin's breathing cycle in the site on which the patch is adhered may continue. The TEWL values were between 2.5 and 6.7 g/h m^2; therefore, with any of these systems the semiocclusive requirement was fulfilled, as normal TEWL values have been found between 8 and 13 g/h m^2.[22] There were statistically significant differences only with formulation 2–0.4, which had the lowest TEWL and 2–1.12 and 1.5–1.6, which showed the highest TEWL ($F = 11.141$; $F_{0.05/14,120} = 1.775$, SD = 4.126).

Water uptake

Results showed that by increasing the time of exposure to water, patches increased their weight and therefore the amount of water adsorbed. However, after 2 hours, a dramatic weight reduction was seen in almost all patches. This was attributable to the fact that the patch began to disintegrate, which caused some SG granules to detach from the patch and thus lose weight. This was more evident for patches with greater amounts of SG (1.6 g). By performing a two-factor variance analysis, we can see that there were statistically significant differences between the formulations tested ($F = 14.893$; $F_{0.05/14,180} = 1.747$) and exposure times ($F = 9.561$; $F_{0.05/5,180} = 2.264$), with evidence of interaction between the formulations and the exposure time ($F = 1.781$; $F_{0.05/70,180} = 1.371$).

Dissolution studies

To perform the dissolution studies, the formulation with the most adequate technological characteristics was chosen—this was formulation 2–0.8—both with kanamycin adsorbed in silica and with kanamycin. Regarding patches with free kanamycin, Figure 5a shows the percentage of dissolved kanamycin versus time. As mentioned above, the dissolution was followed for 195 hours; this graph shows that at 122 hours virtually all the kanamycin contained in the patch (99.9%) has been released.

Palma-Ramos et al.[14] reported in an antibiogram study that to produce an inhibition diameter of 2.3 cm with an area of 13.85 cm^2, 1.164 mg of kanamycin sulfate is needed. With the patch, it was found that after 1.3 hours, this amount (0.674%) has been released. The patch is intended to remain adhered for 24 hours, by this time 8.8% has been released, which ensures that during the time the patch remains on the skin it is releasing kanamycin.

Applying the Peppas semiempirical model, shown in Equations (1) and (2), where M_t is the amount of drug released at a given time t, M_0 is the total amount of drug in the system, and k is the constant related to the release mechanism, it was deduced that $k = 0.00276$ and $n = 1.10159$ ($R = 0.96742$). In this case, n is almost 1, which for some geometrical shapes of matrix systems or swelling-controlled release systems is related to a membrane-type diffusion-controlled system. This type of release kinetics is characterized by constant drug-release rates[23]. It is worth noting that between 1 and 99.5 hours, the possible application of the Higuchi model for matrix systems was also tested (Equation 3), but the data did not fit that model ($R^2 = 0.805$).

$$F = \frac{M_t}{M_0} = k \cdot t^n \tag{1}$$

$$\ln\left(\frac{M_t}{M_0}\right) = n \ln t + \ln k \tag{2}$$

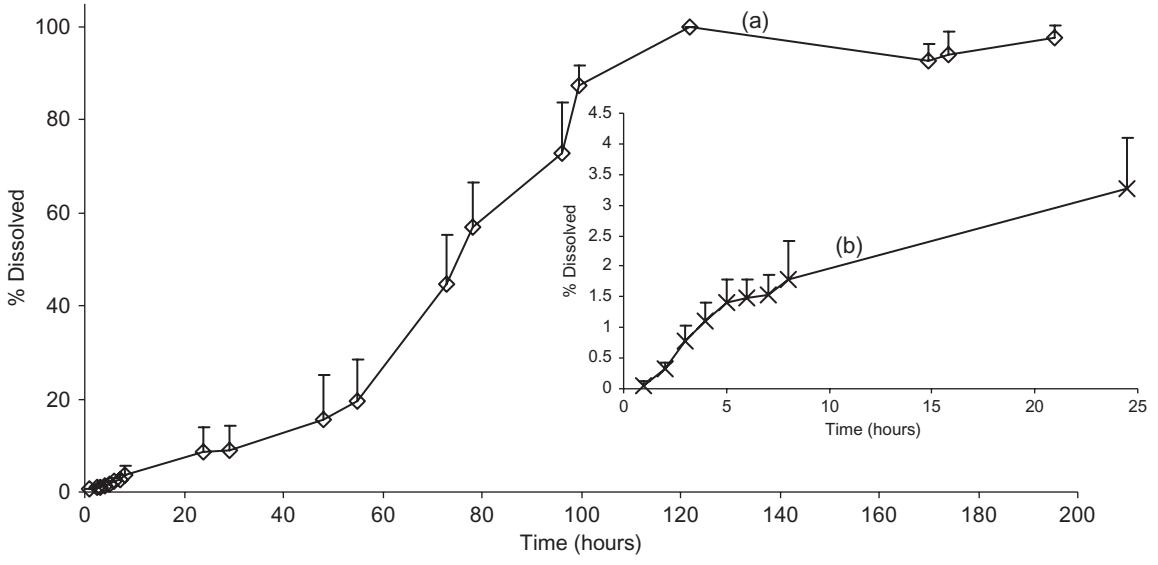

Figure 5. (a) Dissolution of kanamycin freely dispersed in the patches, $n = 5$. (b) Dissolution of kanamycin adsorbed on silica and dispersed in the patches, $n = 4$.

$$F = \frac{M_t}{M_0} = k \cdot t^{1/2}. \quad (3)$$

Figure 5b shows the percentage of kanamycin dissolved after 24 hours for patches with kanamycin adsorbed in silica. As it was expected, a slower release rate was achieved, reaching 3.2% (5.1 ± 1.3 mg) of kanamycin released after 24 hours (the patch with free kanamycin released 8.8% at 24 hours). It is important to point out that the 0.78% of kanamycin released at 3 hours is enough to produce a 2.3-cm inhibition diameter in an antibiogram according to what is indicated by Palma-Ramos et al.[14]

The release data were fitted to the Higuchi equation (Equation 3), where M_t is the amount of drug released at time t, M_0 is the total amount of drug in the system, k is a constant, and t is the time. A good correlation was found (R^2 = 0.9765). This indicates that the patch behaves as a granular matrix and Fickian release takes place. It is worth noting that the Peppas model was also applied, obtaining a R^2-value of 0.825, n value of 1.278, and a k-value of 0.001956. In this case, n indicates a Fickian diffusion in the matrix, but with boundary conditions, such as the presence of a stagnant layer or a net facing the release surface[24].

Emulsion and emulgel characteristics

The emulsion that remained stable after the centrifuge test was the one corresponding to 10% surfactant, HLB = 10, and pH 7.9. Emulgel had a pH value of 6.9 ± 0.4.

Regarding the emulgel, Figure 6 shows significant differences in the viscosity of the system (F = 57.431 and $F_{0.05/20,105}$ = 1.671) as the temperature changed. In the Figure 6a, as expected, viscosity decreased as the temperature increased, a phenomenon associated with the fact that the thermal movement is increased, and the hydrogen links are broken[25]. However, by decreasing the temperature again, the viscosity did not increase proportionally; this was because of the fact that the cooling velocity was very fast (3°C/min). Separately, the sample was heated up to 50°C and was cooled down slowly, and the characteristics of the emulgel were indeed restored.

While determining the viscosity, keeping the temperature constant at 25°C and modifying the rate, a pseudoplastic behavior was observed, typical of a polymeric matrix (Figure 6b).

Penetrability test provides information on the consistency or texture of the product being developed[26]. In this study, a penetration force of 52.7 ± 5.1 $\bar{g}$ was determined at a depth of 15 mm and at 25°C, indicating a very compact product.

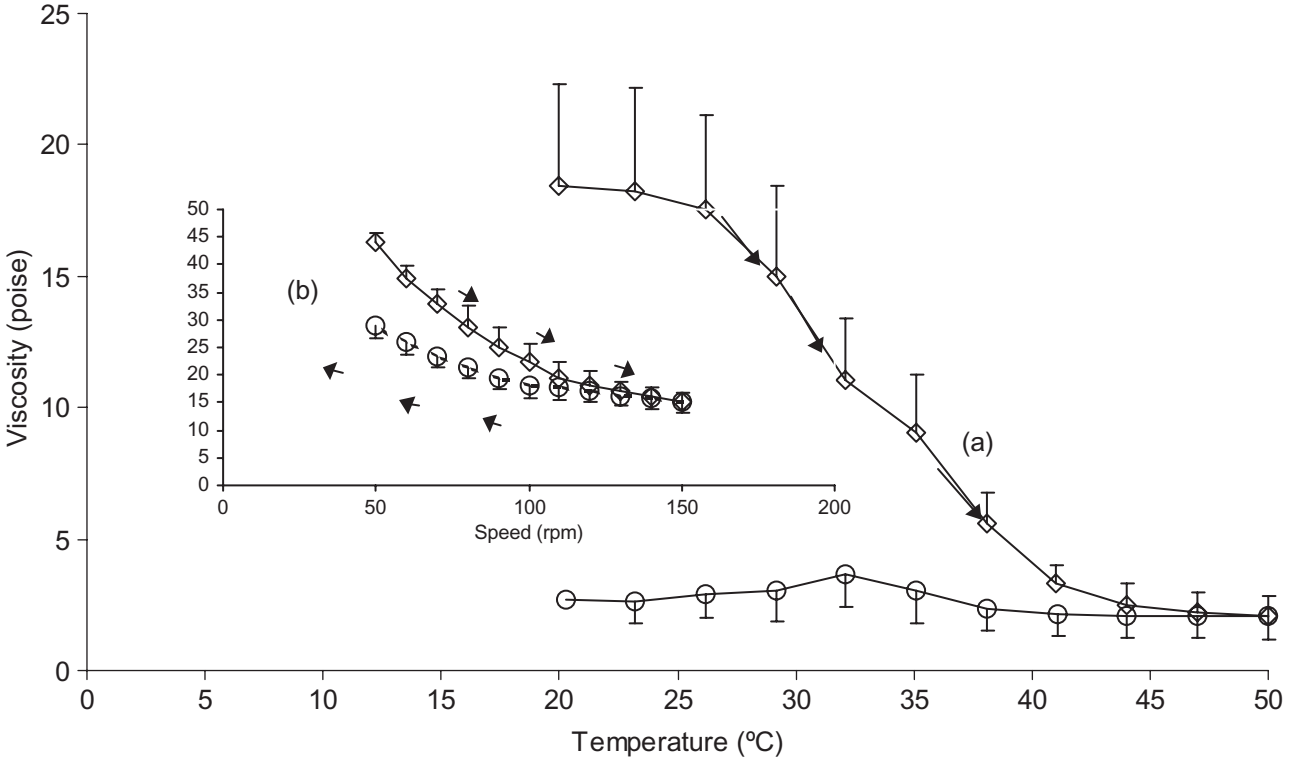

Figure 6. (a) Viscosity change upon emulgel temperature modification at a speed of 50 rpm, n = 6, —◇— initial, —○— final and (b) viscosity change with speed variation, at a temperature of 25°C, for emulgel, n = 6.

Advantages of the proposed systems

Treatment of actinomycetoma involves regimens combining different antibiotics[10,11,27,28]. However, although some regimens have shown to be effective[29], most treatment schedules are very long (mean duration of therapy greater than 1 year) and may require oral and/or parenteral medication. Unfortunately, in many cases, once the patients finish the treatment, they relapse. More aggressive measures include surgical intervention, whether for tissue debridement or for amputation[3].

In the particular case, mycetoma caused by *A. madurae* has been treated successfully with intramuscular kanamycin[8]. However, once the patient is discharged home, the risk of relapse is high. A topical treatment should be advisable to extend or even prevent the risk of relapse. In this sense, Palma-Ramos et al.[14,15] developed a kanamycin containing emulgel, having good results in patients previously treated in the hospital and discharged home. In this work, we optimized the emulgel originally prepared by these authors and propose a patch formulation. To our knowledge, this is a brand-new proposal. Both systems offer the possibility of a local delivery of kanamycin directly in the affected site, avoiding physician participation. They could be a complement to systemic medication, reaching a high drug concentration in the affected site. This is important, taking into account that poor countries are among the most affected.

Emulgel is an o/w emulsion included in a gel. It shares the properties of both systems. It is viscous enough so as to be readily spread on the skin without leakage. Furthermore, the polymer used to prepare the emulgel of this work (Carbopol 934P) has bioadhesive properties, which ensures its resting on the skin[30,31]. Once de emulgel has been spread, water evaporates, favoring the formation of a thin film. This film can act as a barrier. Emulgel is not greasy, leaves a refreshing sensation, and avoids skin dryness[32]. The drawbacks, as with other semisolid formulations, are an inaccurate dosing and the possibility of sticking to the clothes (loss of formulation)[33,34].

With regard to the patch, it presents the following advantages: (i) it acts as a protective barrier; (ii) it can be cut to fit a required size; (iii) it releases a more precise dose, controlling release rate; (iv) because of its semi-occlusive properties, it likely enhances the absorption effect; (v) it is a noninvasive treatment; (vi) it can serve as a complement to the systemic medication; (vii) it is not sticky or greasy; and (viii) it does not stick to the clothes. A drawback of the patch is that it can adhere only in certain areas of the body, provided that they are free of folds and creases[35,36].

Conclusions

With the aim of protecting the areas affected by mycetoma and at the same time ensuring the release of kanamycin to these sites, two different systems were developed: (i) transdermal patches (with freely dispersed kanamycin and kanamycin adsorbed in silica and dispersed in the patch) and (ii) an emulgel.

The optimal patches were those containing 15.9% of freely dispersed kanamycin and 14.6% of kanamycin adsorbed in silica, which corresponds to the batch 2–0.8. The assessments performed to both pharmaceutical forms (patches and emulgel) show that they have the adequate technological characteristics (such as bioadhesion, postmoisture detachment, rupture distance and strength, gas exchange, water uptake, and dissolution studies in the case of the patches; and pH, viscosity, and penetrability in the case of the emulgel) for being used as an auxiliary in the treatment of actinomycetoma caused by *A. madurae*. By comparing both systems, we can see that both are easy to apply. On the one hand, the patches deliver a more precise dose of the antibiotic at the affected site than the emulgel, which represents an advantage; however, the emulgel can favor a close contact in areas of the body where it is difficult for the patch to adhere (i.e., areas with folds and creases).

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