

Design and Evaluation of a Bioadhesive Patch for Topical Delivery of Gentamicin Sulphate

N.A. El-Gendy^a, G.A. Abdelbary^{b,*}, M.H. EL-Komy^a and A.E. Saafan^c

^aPharmaceutics Department, Faculty of Pharmacy, Beni-suef University, Beni-suef, Egypt; ^bPharmaceutics Department, Faculty of Pharmacy, Cairo University, Cairo, Egypt; ^cMicrobiology Department, Faculty of Pharmacy, Beni-suef University, Beni-suef, Egypt

Abstract: The use of aminoglycoside antibiotics for the topical treatment of gram positive and gram negative infections especially burns and wounds has increased markedly in recent years. Patch formulation for topical delivery can be advantageously used as an alternative to conventional topical dosage forms. The present study aims to prepare and evaluate gentamicin sulphate patches for topical application and to study the effect of different bioadhesive polymers on diverse characteristics of prepared patches. Drug patches were evaluated for weight and thickness uniformity, moisture absorption capacity, tensile strength and percentage elongation. *In vitro* release patterns of these patches were studied and analyzed. Skin irritation and susceptibility testing of gentamicin sulphate formulae were also evaluated and compared to commercially available gentamicin sulphate cream. The thickness of the films was found to be uniform. Tensile strength of the patches prepared using HPMC as bioadhesive polymer was the lowest compared to the other patches. The *in vitro* release of the patches followed a pattern close to diffusion model. Patches formulated using HPMC gave the most superior results as compared to other compositions.

Keywords: Gentamicin sulphate, bioadhesive, patch, topical delivery.

1. INTRODUCTION

Topical use of antibiotic drugs has occupied a large area of the pharmaceutical field and markets. The majority of these agents exist in markets as ointments and creams. The design of new dosage forms that increase the effectiveness of the drug and/or improve the characteristics of the already existing dosage forms is one of the trends of pharmaceutical technology [1].

Gentamicin sulphate is an aminoglycoside antibiotic used topically in the control of gram positive and gram negative infections especially in burns and wounds. Gentamicin sulphate is commonly used for treating bone and soft tissue infection [2]. Topical gentamicin is often used in the treatment of impetigo, infected bed sores, burns, nasal staphylococcal carrier state, pyoderma, in infections of the external eye and adenexa [3].

Topical absorption of gentamicin sulphate for wound care in a concentration of 0.1% in water miscible bases induces defective protein synthesis with the advantages of painless application, good penetration and low incidence of toxicity of the used dose. Moreover, aminoglycosides are bactericidal while most other antibiotics which interfere with protein synthesis are bacteriostatic [4].

Upon the topical application of gentamicin sulphate formulated as a 0.1 % (w/w) cream or ointment, there is a considerable variation in the mass of applied cream per unit area of skin, driven presumably by the clinical preference and

experience of those using the formulation. The variability in cream thickness, and hence applied dose, is likely to be even greater. It is conceivable that variability in gentamicin dose may lead to observed inconsistencies in the clinical response [5].

Compared to the common formulations such as ointments, creams, lotions and emulsions, patch types are more convenient for application and can prevent active components from being washed out [6]. Since bioadhesive formulations are capable of adhering to body tissues in moist environments, the use of a bioadhesive patch system may allow extension of the clinical environment [7]. Thus, in the scope of all the above, the aim of this work is to design a gentamicin-loaded bioadhesive patch and evaluate its physicochemical characteristics in an attempt to achieve predictable and reproducible gentamicin delivery. Since the patch delivery system possesses a fixed dimension, thus, no variations in its thickness or the applied footprint occur. It takes the form of a topical dosage system, containing a defined drug loading available for release within a defined surface area. Such a system would ideally be self-adhesive, backed with a protective material and capable of delivering a drug dose comparable to that delivered by the proprietary cream.

The use of polymers in skin preparations is manifold. Requirements of such polymers are dependent on the formulation type. The most applied polymers on skin belong to various classes, for example cellulose derivatives, chitosan, carageenan, polyacrylates, polyvinylalcohol, polyvinylpyrrolidone and silicones. These polymers may be gelating agents, matrices in patches and wound dressings and penetration enhancers [8].

*Address correspondence to this author at the Faculty of Pharmacy, Kaser el aini street, Cairo University, Cairo, Egypt; Tel: (20) 12 316 54 88; Fax: (202) 236 258 65; E-mail: gabelbary@gmail.com

after derivatization with o-phthaldialdehyde reagent by Zhang's method [10].

Briefly, the o-phthaldialdehyde reagent was formulated by adding 2.5 g o-phthaldialdehyde, 62.5 ml methanol and 3 ml 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 upto 3 days. Gentamicin sulphate solution, o-phthaldialdehyde 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-phthaldialdehyde 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 isoindole whose absorbance was then measured at 332 nm [2].

2.3.2. Uniformity of Weight and Thickness

Three randomly selected patches of each formula were weighed and the mean was calculated. Patch thickness was determined using a caliper (Vernier Caliper, Shanghai, China) and recorded as the mean of five measurements representing the four corners and the center of each patch.

2.3.3. Determination of pH of 10% w/w Solutions

Each prepared formula was dissolved in distilled water except those prepared using chitosan (LM, HM) which were dissolved in acetic acid to obtain a 10% w/w solution. The pH of the prepared patch solutions was measured by pH meter (Griffin and George Ltd., England).

2.3.4. Moisture Absorption Capacity of the Patches

Moisture absorption capacity was determined as reported by Lin *et al.* [11]. Patches were conditioned by placing them in a desiccator containing anhydrous calcium chloride for 24 hours before use. The conditioned patch strips (1x1 cm) were then suspended in humidity-controlled desiccators by means of fine thread. The thread is made to pierce each strip in such a manner as to permit free access of the moist air to all surfaces of the films. Three desiccators were set up to produce relative humidities (R.H.) of 33, 65 and 97% using saturated solutions of magnesium chloride, sodium nitrite and potassium sulphate, respectively.

The conditioned patch strips were weighed before placing in the humidity-controlled desiccators and then removed and reweighed every 48 hours for 14 days. The percent moisture absorption was calculated by the following equation [12]:

$$\% \text{ moisture absorption} = \frac{\text{weight of the exposed film} - \text{weight of the conditioned film}}{\text{weight of the conditioned film}} \times 100$$

2.3.5. Mechanical Properties of Gentamicin Sulphate Skin Patches

The tensile strength, percentage elongation at break of films and modulus of elasticity (Young's Modulus) were determined using tensile strength tester (TN-30 code N 9112-ID, India). Patch strips of 1cm width were grasped using an upper and lower flat-faced metal grip laminated with a smooth rubber grip. The distance between the grips

was set at 2 cm and this distance, therefore, represented the length of film under stress. A speed of 5 mm s⁻¹ was used for all measurements [5].

The load applied to the patch was automatically increased at specific rate until the patch broke. The percentage elongation at break, E_b, of tested films was determined as follows [13]:

$$E_b = [E/L_o] \times 100$$

Where E is the extension to break of the film and L_o is its original length. The break strength, B, of tested films was determined using the following equation [13]:

$$B = F/A_R$$

Where F is the break force of the film and A_R is its cross sectional area. The modulus of elasticity (M_E) of the patch was calculated from Hook's law [14].

$$B = M_E / (E/L_o)$$

Results were reported as the mean (±S.D.) of five replicates.

2.3.6. In Vitro Release of Gentamicin Sulphate from the Prepared Patches

The release pattern was performed according to a modified USP 23 apparatus 5, paddle over disc method, using a dissolution apparatus (Hanson research, chatsworth, USA) containing 250 ml Sorensen's phosphate buffer (pH 5.5). The plastic substrates carrying the patches were covered with equally sized stainless steel screen (mesh size 100µm) and placed at the bottom of the dissolution vessels. The assembly is prevented from floating and hitting the rotating paddle by attaching a glass disc to the bottom of the plastic substrate [15,16].

The paddle speed was set at 50rpm and the dissolution medium was maintained at a temperature of 32°C ± 0.5 in order to simulate skin temperature. At predetermined time intervals for a total period of 3 hours, aliquots were withdrawn and the drug content was determined spectrophotometrically at 332 nm after derivatization with o-phthaldialdehyde reagent, as previously mentioned. The results were the mean values of three runs. The obtained data was subsequently analyzed to determine the order of release.

2.4. Skin Irritancy Test of the Prepared Patches

Skin irritancy test is briefly described in the literature [8,17]. In the present study, skin irritation was assessed by placing the topical patches containing the drug and a piece of cotton wool soaked in saturated drug solution on the back of albino rats, secured firmly in place with adhesive plaster. 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.

2.5. Susceptibility of Gentamicin Sulphate Skin Patches

2.5.1. Bacterial Isolates

Three selected standard isolates were chosen which were: Gram-positive nonsporulated bacteria *Staphylococcus aureus* NCTC 6571, Gram-negative *Escherichia coli* K-12 (C-600), and *Pseudomonas aeruginosa* PAOI.

2.5.2. Susceptibility Testing

The test was performed according to the guidelines of the National committee for clinical laboratory standards [18]. This test was carried out for the prepared gentamicin patches P2 and P3 as well as the commercially available gentamicin sulphate cream (Garamycin® cream). A 50 ml flask of molten Lauria Bertani (LB) agar was inoculated with 1 ml suspension of each one of the selected organisms. After mixing, the seeded agar was poured in separate plates and then left to cool. The prepared inoculated agar plates were allowed to harden by placing in an incubator at 37 ± 0.5 C for 10 minutes. Using a cork borer, plugs were made and removed by a sterile loop to obtain holes for filling the different formulae. For the tested patch formulae as well as the commercially available cream, 10 mg of each was accurately weighed and inserted in a corresponding hole. The diameter of the inhibition zone (I.Z.) for each formula was recorded after 18 hours incubation period at 37 ± 0.5 C.

3. RESULTS AND DISCUSSION

3.1. Physical Characterization, pH and Drug Content of Gentamicin Sulphate Topical Patches

Physical parameters such as visual inspection, thickness and weight uniformity, as well as pH and drug content of the prepared formulae are shown in Table 2. All formulae were homogeneous and elastic except formula P7 containing Carbopol 971 which was highly brittle. All the prepared formulations complied with the pharmacopoeial limits for content uniformity [19]. Patches with an average weight ranging from 0.538-0.561 gm and an average thickness ranging from 0.142-0.166 mm were obtained. The pH values ranged from 6.22-7.01. These ranges are suitable for application to the skin as reported by Clearly [20], and insure the maximum stability for polyacrylate containing formulae [21].

3.2. Moisture Absorption Capacity of the Patches

Moisture absorption of polymeric patches affects both the mechanical properties and the drug release pattern. Moisture absorption capacities under different humidity conditions,

(Fig. 1-3), revealed that moisture uptake of patches depended on the type of the bioadhesive polymer used. The ability of these polymers to absorb water may be attributed to their hydrophilic characters [22].

Moisture absorption in 97% R.H. chamber is relatively high as shown in (Fig. 3) and the weight of most patches was significantly increased compared to the other relative humidities, 33% and 65%, (Figs. 1 and 2), respectively. The % moisture absorption varied from 1.801 (HPMC) to 4.001 (Na CMC) at 33 % R.H. Regarding the moisture absorption in 33% R.H., gentamicin topical patches can be arranged in descending order as follows: P3 (4.001%) > P6 (3.63%) > P8 (3.214%) > P1 (2.84%) > P4 (2.831%) > P5 (2.3677%) > P2 (1.801%).

The highest % moisture absorption capacities in two weeks were 6.561 and 9.344 for Na CMC patches while the lowest % (4.163 and 6.111) were recorded for the patches made using chitosan (HM) at 65 % and 97% R.H., respectively. Gentamicin sulphate patches can be arranged according to their moisture absorption capacity in descending order as follows: P3 > P8 > P6 > P1 > P4 > P2 > P5, in both 65% and 97% R.H.

It is clear that patches prepared using Na CMC absorbed water to a greater extent followed by patches containing polyacrylates in the three relative humidities. This may be due to the fact that Na CMC is a water absorbing agent and is commonly used in self-adhesive wound care, and dermatological patches to absorb wound exudate or transepidermal water and sweat [22].

Polyacrylates are hygroscopic in nature and their typical equilibrium moisture content at 25°C and 50% relative humidity is 8-10% w/w. Their moisture content does not affect their thickening efficiency, but an increase in the moisture content makes them more difficult to handle. HPC, HPMC and chitosan absorb moisture from the atmosphere, where the amount of water absorbed depends upon the initial moisture content, temperature and relative humidity of the surrounding air [22].

Table 2. Physical Characterization, pH and Drug Content Of Gentamicin Sulphate Topical Patches

Formula	Visual inspection	Drug content (% \pm S.D.)	Uniformity of weight (gm) (average \pm S.D.)	Uniformity of thickness (mm) (average \pm S.D.)	pH
P1	Homogeneous, white, transparent, slightly brittle	90.52 \pm 2.71	0.538 \pm 0.003	0.142 \pm 0.004	6.64
P2	Homogeneous, white, highly elastic, slightly opaque	96.31 \pm 2.4	0.559 \pm 0.003	0.144 \pm 0.005	6.312
P3	Homogeneous, flexible, yellowish, elastic, slightly opaque	94.47 \pm 1.55	0.558 \pm 0.004	0.154 \pm 0.005	6.53
P4	Homogeneous, elastic, transparent	89.34 \pm 3.66	0.555 \pm 0.005	0.146 \pm 0.007	7.01
P5	Homogeneous, elastic, transparent	87.52 \pm 0.96	0.561 \pm 0.022	0.152 \pm 0.004	6.72
P6	Homogeneous, sticky, slightly elastic, transparent	93.61 \pm 2.15	0.548 \pm 0.003	0.166 \pm 0.009	6.22
P7	n/a	n/a	n/a	n/a	n/a
P8	Homogeneous, slightly elastic, slightly transparent	88.35 \pm 1.41	0.538 \pm 0.010	0.148 \pm 0.008	6.71

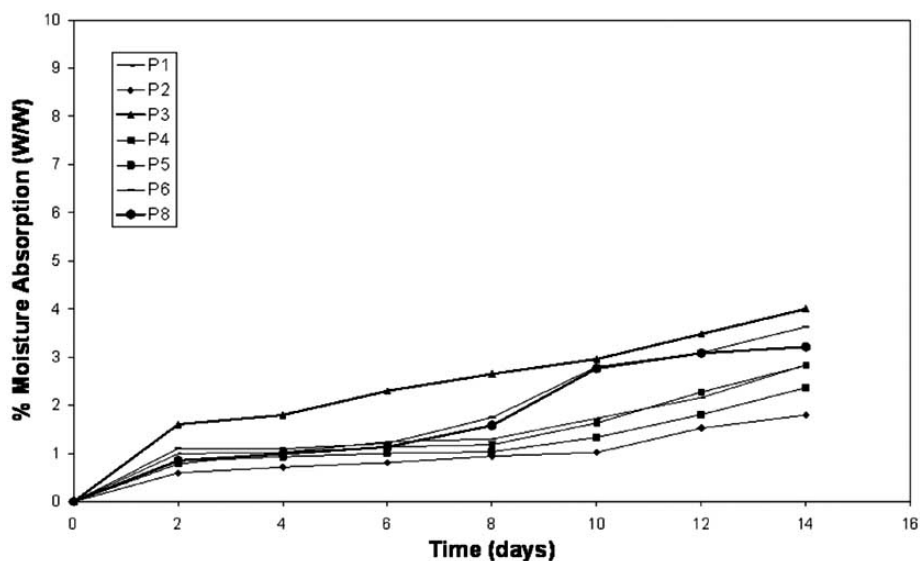


Fig. (1). Moisture absorption capacity of gentamicin sulphate topical patches at 33 % RH.

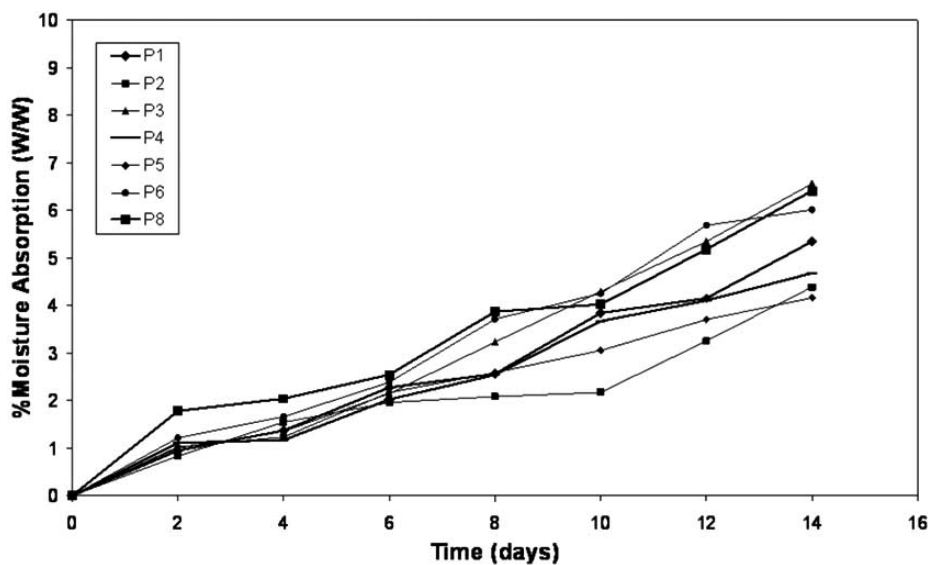


Fig. (2). Moisture absorption capacity of gentamicin sulphate topical patches at 65 % RH.

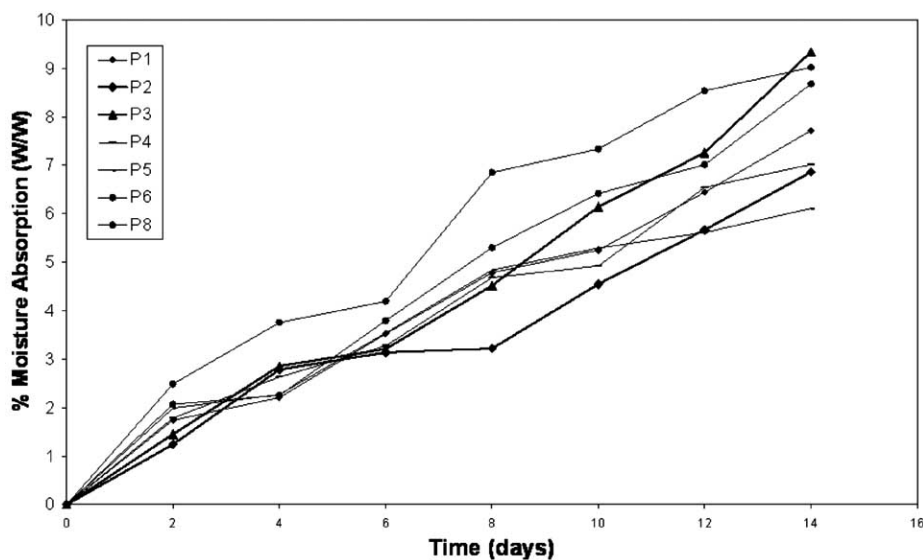


Fig. (3). Moisture absorption capacity of gentamicin sulphate topical patches at 97 % RH.

Table 3. Mechanical Properties of Gentamicin Sulphate Topical Patches

Formula	Elongation % (average \pm S.D.)	Tensile strength (Kg/cm ²) (average \pm S.D.)	Modulus of elasticity (average \pm S.D.)
P1	16.04 \pm 0.003	0.64 \pm 0.0169	3.99 \pm 0.13
P2	208.8 \pm 4.38	0.0475 \pm 0.002	0.0228 \pm 0.0014
P3	94.27 \pm 1.79	0.065 \pm 0.0017	0.0689 \pm 0.0037
P4	21.98 \pm 0.901	0.595 \pm 0.02	2.707 \pm 0.132
P5	27.3 \pm 0.85	0.0825 \pm 0.0025	0.302 \pm 0.0155
P6	23.42 \pm 1.19	0.125 \pm 0.0032	0.5337 \pm 0.0213
P7	n/a	n/a	n/a
P8	13.858 \pm 0.637	1.05 \pm 0.036	7.576 \pm 0.169

3.3. Mechanical Properties of Gentamicin Sulphate Skin Patches

The physicochemical properties of patches are among the factors, which determine the suitability and acceptability of polymeric patches. The tensile strength, percentage elongation and modulus of elasticity were determined for the prepared bioadhesive patches except P7 which is very brittle. All results of mechanical properties are shown in Table 3. The tested patches can be arranged in a descending % elongation and ascending tensile strength order as follows: P2 > P3 > P5 > P6 > P4 > P1 > P8. It is obvious that the best mechanical properties were obtained from topical gentamicin sulphate patches containing 2% HPMC. This could be due to the cross-linked structure of HPMC that attributes to its elongation ability.

3.4. *In Vitro* Release of Gentamicin Sulphate from the Prepared Patches

In the development of topical patches, a drug release testing is very important to assure batch-to-batch uniformity of each drug delivery system and to evaluate the release rate of the drug from the prepared formulae [23]. Even though, the body temperature is maintained at 37°C, the temperature of the skin surface is 32°C [24]. That is why the temperature of the dissolution medium was kept at 32 \pm 0.5°C. Sorensen's phosphate buffer of pH 5.5, used as dissolution medium, simulated the pH of the skin surface [25]. The dissolution results are shown in Fig. (4).

It has to be mentioned that the amount of gentamicin sulphate released from patch P2 was higher than that released from the other patches as it reached ~100% within 3

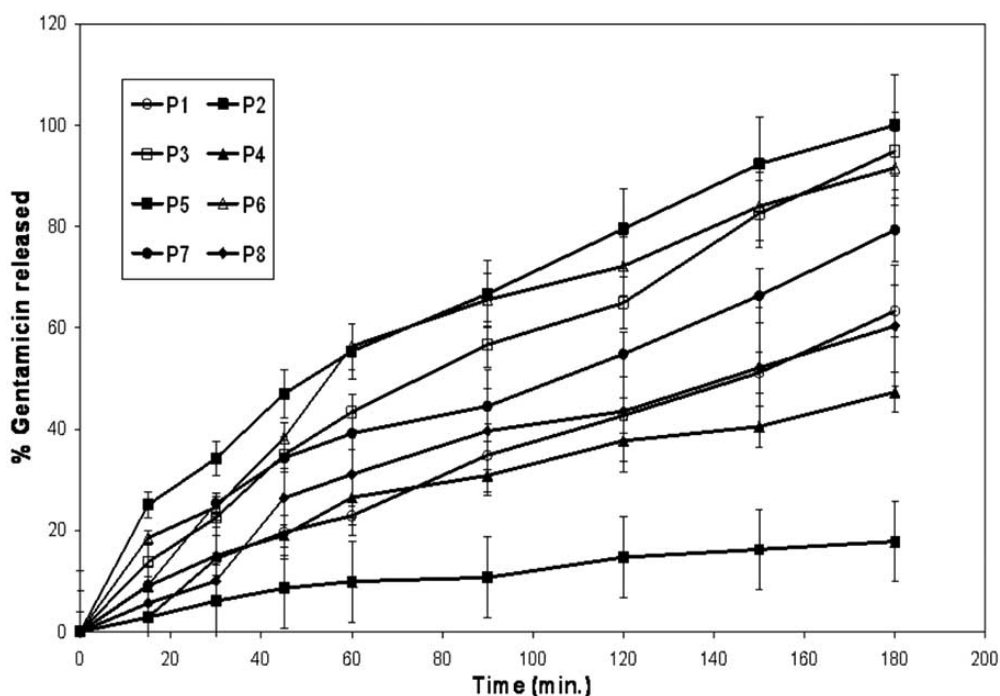


Fig. (4). *In vitro* release profiles of gentamicin sulphate in Sorensen's phosphate buffer (pH 5.5) from patch formulation (P1-P8) n=3.

Table 4. Determination of the Order of Release of Gentamicin Sulphate from Different Topical Patches using the Correlation Coefficient Parameter (r^2)

Topical patches	Zero-order	First-order	Diffusion	Order of release
P1	0.993	0.991	0.993	Diffusion
P2	0.988	0.924	0.998	Diffusion
P3	0.993	0.944	0.994	Diffusion
P4	0.982	0.991	0.995	Diffusion
P5	0.974	0.979	0.990	Diffusion
P6	0.968	0.987	0.988	Diffusion
P7	0.981	0.974	0.987	Diffusion
P8	0.967	0.986	0.987	Diffusion

hours. This might be due to the high solubility of the used polymer (HPMC) in solutions of pH 5.5 [22]. On the other hand, patches formulated using chitosan P4 and P5 exhibited the lowest release rates of the drug (47.31 and 17.83%, respectively). This could be attributed to the fact that chitosan is extensively employed in pharmaceutical industry for its potential in the development of controlled release drug delivery systems [26]. Regarding the *in vitro* release, gentamicin topical patches can be arranged in descending order within 1 hour as follows: P2 (~100%) > P3 (94.87%) > P6 (91.58%) > P7 (79.35%) > P1 (63.33%) > P8 (60.37%) > P4 (47.31) > P5 (17.83%).

Linear regression analysis for the release data was done to determine the proper order of release. Zero-, first- and Higuchi diffusion controlled model equations were applied to all *in vitro* release results [27]. From the results shown in Table 4, it could be concluded that the drug is released by a diffusion controlled mechanism from all the topical patches.

3.5. Skin Irritancy

The majority of the polymers, which are considered in dermal formulations, are probably non-irritant to the skin [8,28]. The animals subjected for primary skin irritation test did not show any signs of erythema or oedema while observing for a period of 7 days. Signs of acute toxicity usually occurring with systemic absorption of polyacrylates and chitosan derivatives were not observed suggesting that these polymers were not absorbed. These characteristics favor the conclusion that both types of polymers are safe [29]. HPMC,

Na CMC and HPC were found to be non-irritating. The absence of irritation of the tested polymers to skin was confirmed by the Draize test as reported in the literature [30]. The available data suggests that irritancy problems on skin with the prepared gentamicin sulphate topical patches are not to be expected.

3.6. Susceptibility Testing of Gentamicin Sulphate Skin Patches

Table 5 shows the inhibition zones (I.Z.) obtained for the different formulae. P3 was the most active formula against *St.aureus* (I.Z.= 3.6 cm), followed by P2 (I.Z.= 2.8 cm) against the same organism. On the other hand, both patch formulae P2 and P3 were moderately active against *Ps. aeruginosa* (I.Z.= 1.7 cm and 2 cm, respectively), the former was slightly active against *E.coli* and the latter was inactive against it. Commercial gentamicin sulphate cream was moderately active against *St.aureus*, but *Ps. aeruginosa* and *E.coli* were completely resistant to it. It is clear that P2 was the only patch which is effective against *E.coli*. This could be explained by the difference in formulae composition that led to different released amounts of gentamicin. The amount released should exceed the minimum inhibitory concentration (MIC) for each organism in order to exhibit an inhibition zone [31,32]. Thus, for P2 the release of gentamicin was enough to exceed the MIC of all three organisms used.

CONCLUSIONS

The results of this study showed that the change in the type of bioadhesive polymer used for the preparation of gen-

Table 5. The Inhibition Zone of each of the Tested Formulae with each of the Tested Microorganisms

Tested microorganisms	Inhibition zone (cm \pm S.D.) of the different formulae		
	commercial	P2	P3
<i>Staphylococcus aureus</i> (<i>St.aureus</i>)	2 \pm 0.021	2.8 \pm 0.012	3.6 \pm 0.014
<i>Escherichia coli</i> (<i>E.coli</i>)	-	1.2 \pm 0.03	-
<i>Pseudomonas aeruginosa</i> (<i>Ps</i>)	-	1.7 \pm 0.015	2 \pm 0.0249

gentamicin sulphate topical patches was accompanied with changes in physical characteristics of the formulated patches. The lowest moisture absorption capacity was obtained with patch formula P2 under the three humidity conditions studied. The prepared patches containing 2% HPMC (P2) imparted the highest elongation percent and lowest tensile strength compared to the other formulae. The present investigation suggests that the drug release from all systems follows Higuchi kinetics and the higher rates were achieved from P2 patch within three hours as compared to the other patches. All topical patches showed no irritation to the skin. Formula P3 was the most active formula (the widest inhibition zone), while patch formula P2 was the broadest in the spectrum of activity (the three organisms were susceptible to it) comparing to the commercially available gentamicin sulphate cream. Thus, it could be concluded that low moisture absorption capacity, better mechanical properties, higher release rates and broad spectrum of activity can be achieved from HPMC patches. Bioadhesive patches may be considered as a promising delivery system for the topical application of gentamicin sulphate.

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