

Preparation and characterization of gatifloxacin-loaded sodium alginate hydrogel membranes supplemented with hydroxypropyl methylcellulose and hydroxypropyl cellulose polymers for wound dressing

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Abstract

Introduction: The aim of this study is to evaluate gatifloxacin-loaded sodium alginate hydrogel membranes, supplemented with glycerol (a plasticizer), glutaraldehyde (a cross-linking agent), and hydroxypropyl methylcellulose (HPMC) or hydroxypropyl cellulose (HPC) polymers, as potential wound dressing materials based on their physicochemical properties and the sustain-release phenomenon. **Materials and Methods:** The physicochemical properties of the prepared hydrogel membranes were evaluated by several methods including Fourier transform infrared and differential scanning calorimetry. Different techniques were used to assess the swelling behavior, tensile strength and elongation, % moisture absorption, % moisture loss, water vapor transmission rate (WVTR), and microbial penetration for the hydrogel membranes. *In vitro* gatifloxacin release from the hydrogel membranes was examined using the United States Pharmacopeia XXIII dissolution apparatus. Four kinetics models (zero-order, first-order, Higuchi equation, and Korsmeyer-Peppas equation) were applied to study drug release kinetics. **Results:** The addition of glycerol, glutaraldehyde, HPMC, and HPC polymers resulted in a considerable increase in the tensile strength and flexibility/elasticity of the hydrogel membranes. WVTR results suggest that hydrated hydrogel membranes can facilitate water vapor transfer. None of the hydrogel membranes supported microbial growth. HPMC-treated and HPC-treated hydrogel membranes allow slow, but sustained, release of gatifloxacin for 48 h. Drug release kinetics revealed that both diffusion and dissolution play an important role in gatifloxacin release. **Conclusions:** Given their physicochemical properties and gatifloxacin release pattern, HPMC-treated and HPC-treated hydrogel membranes exhibit effective and sustained drug release. Furthermore, HPMC-treated and HPC-treated hydrogel membranes possess physicochemical properties that make them effective and safe wound dressing materials.

Key words: Gatifloxacin, hydrogel membrane, *in vitro* drug release, sodium alginate, wound dressing

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INTRODUCTION

Nowadays, wound dressings have a major role to play in the management of wounds, whether they are open wounds (usually chronic wounds of various etiologies) or closed (usually sutured wounds of surgical or traumatic origin). In general, moist wound environment promotes better epithelialization of superficial wounds compared to dry bandaged wounds, and hence, there has been a progressive increase in the numbers and types of occlusive dressings that allow moist wound healing environment.^[1-3] Hydrogels and soft physiological tissue share very similar physicochemical properties such as high water content, soft and rubbery consistency, high molecular and oxygen permeability, good moisturizing and mechanical properties, and low interfacial tension with water or biological fluids.^[4] Hydrogels have become suitable drug delivery systems because they allow molecules of different size to diffuse into and out of them very effectively, offering a useful tool for drug loading and drug release.^[5] Hydrogels are highly permeable to water-soluble drugs and substances, and hence, diffusion is the most common mechanism of drug loading and drug release into and out of hydrogel-based drug delivery system. Several factors control the release rate and release mechanism from hydrogels including water content, crystallinity, polymer composition, and cross-linking density.^[6] Due to their physicochemical and biological properties, polysaccharides with hydrogel-forming potential are considered advantageous in their employment as wound dressing material.^[7] Since hydrogels began to be employed in wound healing, the use of dressings that keep wound tissues moist has been associated with increased healing rates.

Sodium alginate is a sodium salt of alginic acid, a naturally occurring nontoxic polysaccharide found in brown algae. Alginate has been widely used as a food additive, a tablet disintegrant, a pharmaceutical agent, and a gelling agent.^[8] Alginate consists of 1→4, linked D-mannuronic acids and L-glucuronic acid residues arranged as blocks of either type of unit or as a random distribution of each type.^[9] Sodium alginate film has been previously prepared and some of its physical properties, such as water vapor transmission, have been investigated. It was demonstrated that sodium alginate is a potential candidate for wound dressing material.^[10] Alginate has been used in a number of biomedical applications, such as wound dressing, tissue engineering, and drug delivery. Several reports have suggested that certain alginate dressings can enhance wound healing by stimulating monocytes to produce elevated levels of cytokines such as interleukin-6 and tumor necrosis factor- α .^[11] Production of these pro-inflammatory cytokines in the wounded tissue is vital for tissue repair mechanisms that ultimately lead to wound healing. Different types of alginate-based wound dressing material have been commercialized and they have been widely reviewed.^[9,12-15] Hydroxypropyl methylcellulose (HPMC) and hydroxypropyl cellulose (HPC) are nontoxic biocompatible polymers that have been demonstrated to exhibit minimal cell adhesion potential, good chemical stability, film-forming capability, and high hydrophilicity.^[16,17]

Nowadays, antimicrobial agents are incorporated in wound dressings, giving advantage of the property of sustained release over a period of days to protect the wound effectively against infection.^[18] Hence, topical antimicrobial agents have an important therapeutic role in the treatment of wounds and burns because they maintain the wound flora at very low levels. Gatifloxacin is an antibacterial drug that belongs to the fourth-generation fluoroquinolone family of antibiotics, and it is very commonly used for the treatment of skin infections. Gatifloxacin is a unique antibiotic with a broad spectrum of activity against Gram-positive and Gram-negative aerobic and anaerobic bacteria.^[19] A wounded skin tissue is exposed to a wide range of bacteria, and therefore, gatifloxacin is suitable for treating complicated and uncomplicated skin infections due to its broad spectrum and rapid bactericidal activity.^[20] Oral administration leads to side effects. Hence, the local delivery of the drug by topical administration may enable the maintenance of a high local antimicrobial concentration for an extended duration of release without causing systemic toxicity. Polysaccharide hydrogels having good strength and elasticity are expected to be serve as better more effective dressing materials.^[21] Based on the above, gatifloxacin is considered to be a potential drug candidate for topical wound dressing, and polymeric hydrogel dressings usually provide a continuous and sustained release of the antimicrobial agent at the wound surface to bring about a long-lasting antimicrobial action in combination with maintenance of physiologically moist environment for faster wound healing.^[22] However, there is no information available on the topical wound dressing of gatifloxacin-loaded hydrogel membranes. Our study focuses on evaluating the physicochemical properties of different gatifloxacin-loaded sodium alginate hydrogel membranes, with varying combinations of glycerol (a plasticizer), glutaraldehyde (a cross-linking agent), and HPMC or HPC polymers, as suitable wound dressings.

MATERIALS AND METHODS

Chemicals and reagents

Gatifloxacin (IP grade) was kindly provided by Wockhardt Pharmaceuticals as a gift sample (Aurangabad, India). Dry powder of sodium alginate (viscosity of 80-120 mPa in 10 g/L at 20°C) was purchased from the National Institute of Fisheries Technology (Cochin, India). HPMC was purchased from Fisher Scientific (Mumbai, India). HPC was purchased from Rolex Lab (New Delhi, India). All other chemicals and reagents of analytical grade were purchased from standard deviation Fine Chemicals (Mumbai, India).

Fourier transform infrared spectroscopy

The compatibility between gatifloxacin and sodium alginate, HPMC, and HPC was evaluated by Fourier transform infrared (FT-IR). Different physical mixtures of gatifloxacin and the polymers (HPMC or HPC) or excipients (1:1) were separately mixed with three parts of potassium bromide and they were compressed to form pellets with a hydraulic press at 10 tons

pressure. The FT-IR absorption spectra of all samples were recorded in the range of 400-4000/cm by potassium bromide disc method using FT-IR spectrophotometer. Similarly, the FT-IR absorption spectra of all individual components were also recorded. Physical appearance of the samples and the appearance (or disappearance) of peaks in the spectra were observed to access any possible physical or chemical interactions.

Differential scanning calorimetry

The compatibility between gatifloxacin and sodium alginate, HPMC, and HPC was evaluated by differential scanning calorimetry (DSC) using an automatic thermal analyzer system (Mettler Toledo, USA). Five milligrams of gatifloxacin and gatifloxacin with polymers (1:1) were sealed in perforated aluminum pans in the temperature range of 50-480°C and a heating rate of 10°C/min under nitrogen flow of 20 ml/min. Temperature calibrations were performed using indium as a standard. An empty pan, sealed in the same way as the experimental samples, was used as a control.

Preparation of gatifloxacin-loaded hydrogel membranes

The hydrogel membranes were prepared using casting and solvent evaporation techniques as previously described.^[23] In brief, to prepare gatifloxacin-loaded hydrogel membrane formulation, weighed quantity of gatifloxacin was dissolved in distilled water. Sodium alginate (2% w/v) was then mixed in distilled water to dissolve the sodium alginate and agitated for 1 h, followed by the addition of glycerol (0%, 0.5%, or 1% w/v) and/or glutaraldehyde (0% or 1% w/v) to the polymeric solution under gentle agitation. To prepare HPMC-based gatifloxacin-loaded sodium alginate hydrogel membrane formulation, HPMC solution (0.5% w/v) was prepared by dispersing HPMC in distilled water, added to the sodium alginate solution (1.5% w/v), and agitated for 1 h. Subsequently, and under gentle agitation, glycerol (0%, 0.5%, or 1% w/v) and/or glutaraldehyde (0% or 1% w/v) were added to the polymeric solution. To prepare HPC-based gatifloxacin-loaded sodium alginate hydrogel membrane formulation, HPC solution (0.5% w/v) was prepared by dispersing HPC in distilled water, the sodium alginate solution (1.5% w/v), and agitated for 1 h. Subsequently, and under gentle agitation, glycerol (0%, 0.5%, or 1% w/v) and/or glutaraldehyde (0% or 1% w/v) were added to the polymeric solution. The resultant mixtures were then poured into polyurethane coated Petri dishes, which were subsequently kept in an oven at 60 ± 2°C for 24 h. Next, the hydrogel membranes were washed thoroughly with distilled water to wash off remnant glutaraldehyde and they were air-dried at room temperature

for 72 h. The hydrogel membranes were removed from the Petri dishes and stored in desiccators. The resultant hydrogel membranes were of 2 cm² area, 2.1-2.4 mm thickness, 6.73-7.21 pH, and good appearance. The exact composition of all examined formulations is shown in Table 1.

Measurement of swelling behavior

The hydrogel membrane (2 cm²) was taken and then placed in agar gel plate (2% m/v agar in STF, pH 7.4) and incubated at 37 ± 1°C.^[24] The hydrogel membrane was removed from plate after 30 min, surface water was removed with help of filter paper, and hydrogel membrane was reweighed.

Swelling was expressed by the % swelling index (SI) as shown below.

$$\% \text{ Swelling index} = \frac{W_h - W_d}{W_d} \times 100$$

where W_h is the weight of the product after 30 min hydration, and W_d is the weight of the dried product.

Measurement of tensile strength and elongation

The tensile strength of hydrogel membranes was measured by Hounsfield H10KS tensile testing machine (Horsham, PA, USA) equipped with a 5 kg load cell. The initial grip separation was set to 30 mm and the grips were moved at a cross-head speed of 10 mm/min until the dermal patch broke at 25 ± 0.5°C and 75 ± 2% relative humidity (RH). During the measurement, the strips were pulled by the top clamps, and the elongation and force parameters value were measured when the hydrogel membrane broke.^[25] The stress-strain curve was obtained, and the compression modulus of the hydrogel membrane was calculated from the initial slope of stress-strain curve.

Measurement of % moisture absorption and % moisture loss

The % moisture absorption test was carried out to check the physical stability (or integrity) of the dermal hydrogel membranes at humid conditions.^[26] At 80 ± 2% humidity, the hydrogel membranes were placed in desiccators containing saturated solution of aluminum chloride. After 72 h, the hydrogel membranes were taken out and weighed. On the other hand, the % moisture loss was carried out to check the physical stability (or integrity) of the dermal hydrogel membranes at dry conditions.^[27] The hydrogel membranes were placed in desiccators containing

Table 1: Composition of the gatifloxacin-loaded sodium alginate hydrogel membranes

Composition	S ₁ F ₁	S ₁ F ₂	S ₁ F ₃	S ₁ F ₄	S ₁ F ₅	S ₁ F ₆	S ₁ F ₇	S ₁ F ₈	S ₁ F ₉	S ₁ F ₁₀	S ₁ F ₁₁	S ₁ F ₁₂
Gatifloxacin (%)	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Sodium alginate (%)	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
HPMC (%)	—	—	—	—	0.5	0.5	0.5	0.5	—	—	—	—
HPC (%)	—	—	—	—	—	—	—	—	0.5	0.5	0.5	0.5
Glycerol (%)	—	0.5	1.0	1.0	—	0.5	1.0	1.0	—	0.5	1.0	1.0
Glutaraldehyde (%)	—	1.0	1.0	—	—	1.0	1.0	—	—	1.0	1.0	—
Distilled water q.s (ml)	40	40	40	40	40	40	40	40	40	40	40	40

HPMC: Hydroxypropyl methylcellulose, HPC: Hydroxypropyl cellulose

25 g of anhydrous calcium chloride. After 72 h, the hydrogel membranes were taken out and weighed.

The % moisture absorption was calculated as per the following equation:

$$\% \text{ Moisture absorption} = \frac{\text{Final weight} - \text{initial weight}}{\text{Initial weight}} \times 100$$

The % moisture loss was calculated as per the following equation:

$$\% \text{ Moisture loss} = \frac{\text{Initial weight} - \text{final weight}}{\text{Initial weight}} \times 100$$

Measurement of water vapor transmission rate

To determine the water vapor transmission rate (WVTR), the dermal hydrogel membranes were cut into 3.5 cm diameter discs and then put as a cap at the mouth of vials with an internal diameter of about 3 cm (7.07 cm²) containing 25 ml of distilled water. Then, the vials were weighed, kept at constant temperature (35 ± 0.5°C) and humidity (35 ± 2%) for 24 h. Subsequently, the vials were taken out and weighed, as previously described.^[28] The weight loss of the system was considered as an index of WVTR. The WVTR of each sample was calculated as per the following equation:

$$\text{WVTR (g/m}^2\text{/h)} = \frac{W_i - W_f}{24 \times A} \times 10^4$$

Where A is the area of vial mouth (cm²), W_i is the initial weight of the vial with hydrogel cap, and W_f is the final weight of the vial with hydrogel membrane cap.

Assessment of microbial penetration

The ability of the hydrogel membranes to prevent microbial penetration was assessed as previously described.^[29] Briefly, the hydrogel membranes were cut into 15 mm diameter discs and then used as caps at the open mouths of 10 ml vials with a diameter of about 10 mm, each containing 5 ml of nutrient broth (Merck, Darmstadt, Germany). An open vial containing 5 ml of nutrient broth served as a positive control, while a vial containing 5 ml of nutrient broth and closed with a tightly packed cotton ball served as a negative control, while. All vials were kept in an open environment for 7 days to allow for contamination. The cloudiness (or turbidity) of the nutrient broth in the vials was considered a sign of microbial contamination.

In vitro gatifloxacin release study

In vitro release studies were carried out for the formulated hydrogel membranes using the United States Pharmacopeia XXIII dissolution apparatus as previously described.^[30] Phosphate buffer solution (pH 7.4) was used as a dissolution medium. The paddles set at 50 rpm rotation at 37 ± 0.5°C. The apparatus was set at the above experimental conditions, the hydrogel membranes were placed in dissolution vessels, and the dissolution test was carried out. Samples were withdrawn at fixed time intervals (0.5, 1, 2, 4, 6, 8, 12, 24 and 48 h). The amount of

gatifloxacin dissolved was determined by measuring ultraviolet absorption at λ 292 nm.

Drug release kinetic studies

To examine the drug release kinetics and mechanism of drug release for the optimized formulation, the cumulative release data were fitted to models representing zero-order (Q vs. t), first-order (log[Q₀-Q] vs. t), Higuchi square root of time (Q vs. t_{1/2}) and Korsmeyer-Peppas double log plot (log Q vs. log t), respectively, where Q is the cumulative percentage of drug released at time t and (Q₀-Q) is the cumulative percentage of drug remaining after time t. The results obtained from the *in vitro* drug release studies were plotted in four kinetics models.

Statistical analysis

For multiple comparisons, statistical significance was determined using paired *t*-test, Student's *t*-test, and ANOVA coupled with two-sided Dunnett's *post-hoc* test. **P* < 0.01, and ***P* < 0.001 are considered statistically significant.

RESULTS AND DISCUSSION

Fourier transform infrared spectroscopy

The individual spectra and the physical mixture spectra were recorded and analysed. Observation of the finger print region and absorbance values relevant to functional groups prove the absence of any interaction between gatifloxacin and alginate, HPMC, or HPC [Figure 1]. As shown in Figure 1, FT-IR spectra of gatifloxacin and gatifloxacin with sodium alginate, HPMC, or HPC exhibit absorbance patterns that correspond in position and relative intensity to those in the FT-IR spectra of the individual components. The characteristic absorption bands of the studied physical mixtures are listed in Table 2. The findings reveal that there is no obvious change in FT-IR spectra before and after the treatment with sodium alginate, HPMC, and HPC, indicating that there is no physical or chemical interaction between gatifloxacin and sodium alginate, HPMC, or HPC.

Table 2: FT-IR characteristic peaks of gatifloxacin and the physical mixtures of gatifloxacin, sodium alginate, and HPMC/HPC

Sample	Frequency cm ⁻¹
Gatifloxacin	2972.2, 2839.2, 1629.3, 1615.5, 1543.1, 1443.9, 1392.3, 1356.7, 1320.6, 1279, 1208.2, 1141.4, 1056.9, 995.6, 938, 911.6, 890.5, 848.6
Gatifloxacin: Sodium alginate	3370.2, 2839.4, 2341.5, 2167.8, 2025.4, 2017.8, 2012.4, 1987.9, 1981.7, 1943.5, 1629.5, 1545.5, 1444.3, 1392.8, 1365.4, 1320.9, 1279.3, 1207.3, 1094.2, 1062.2, 995.8, 937.9, 890, 820.4
Gatifloxacin: Sodium alginate: HPMC	3388, 2838.4, 2358.9, 2340.8, 2322.4, 2200.9, 2171.6, 2043.8, 2016.8, 1999.2, 1959.7, 1936.9, 1629.4, 1614.1, 1538.4, 1502.2, 1444.9, 1391.1, 1362.4, 3120, 1281, 1207.3, 1059.8, 995.8, 889
Gatifloxacin: Sodium alginate: HPC	3374.3, 2356.9, 2340.5, 1630.1, 1546.4, 1444.3, 1392.3, 1367.4, 1321, 1280.7, 1207.9, 1092.7, 1066, 996.3, 937.6, 889.5, 820.6

HPMC: Hydroxypropyl methylcellulose, HPC: Hydroxypropyl cellulose, FT-IR: Fourier transform infrared

Differential scanning calorimetry

DSC thermograms of gatifloxacin and the physical mixtures of gatifloxacin, sodium alginate, and HPMC/HPC exhibit peaks that correspond in position and relative intensity to those in the thermograms of the individual components [Figure 2]. As shown in Figure 2, the DSC thermograms reveal that the characteristic thermogram peaks did not shift noticeably, suggesting lack of any physical or chemical interaction between gatifloxacin and sodium alginate, HPMC, or HPC.

Swelling behavior

The potential effects of glycerol (a plasticizer), glutaraldehyde (a cross-linking agent), and HPMC or HPC polymers on the swelling of hydrogel membranes in phosphate buffer (pH 7.4) were evaluated. Overall, and as shown in Table 3, the hydrogel membranes that were treated with glycerol (a plasticizer), glutaraldehyde (a cross-linking agent), and HPMC or HPC polymers exhibited a considerable amount of swelling in the hydrogel membranes when compared with the control hydrogel

membranes containing no additives. The SI was found to be between $65.32 \pm 3.43\%$ for S₁F₁ to $130.51 \pm 4.65\%$ for S₁F₈ at 0.5 h. As shown in Table 3, the increased glycerol concentration caused a noticeable increase in the swelling of the hydrogel

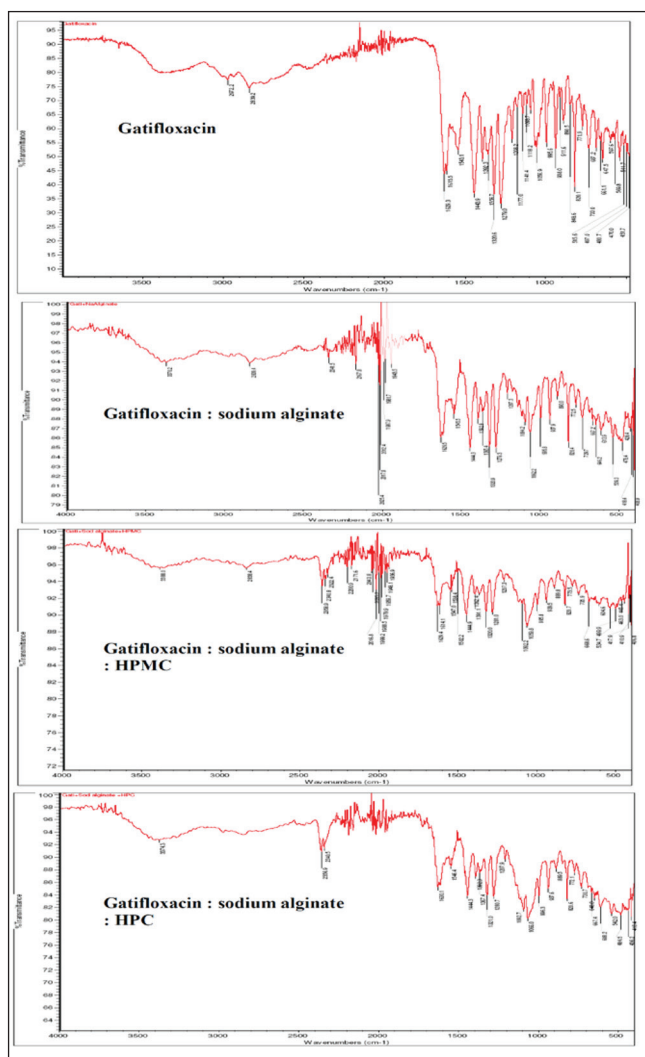


Figure 1: Fourier transform infrared spectroscopy peaks of gatifloxacin and the physical mixtures of gatifloxacin, sodium alginate, and hydroxypropyl methylcellulose/hydroxypropyl cellulose

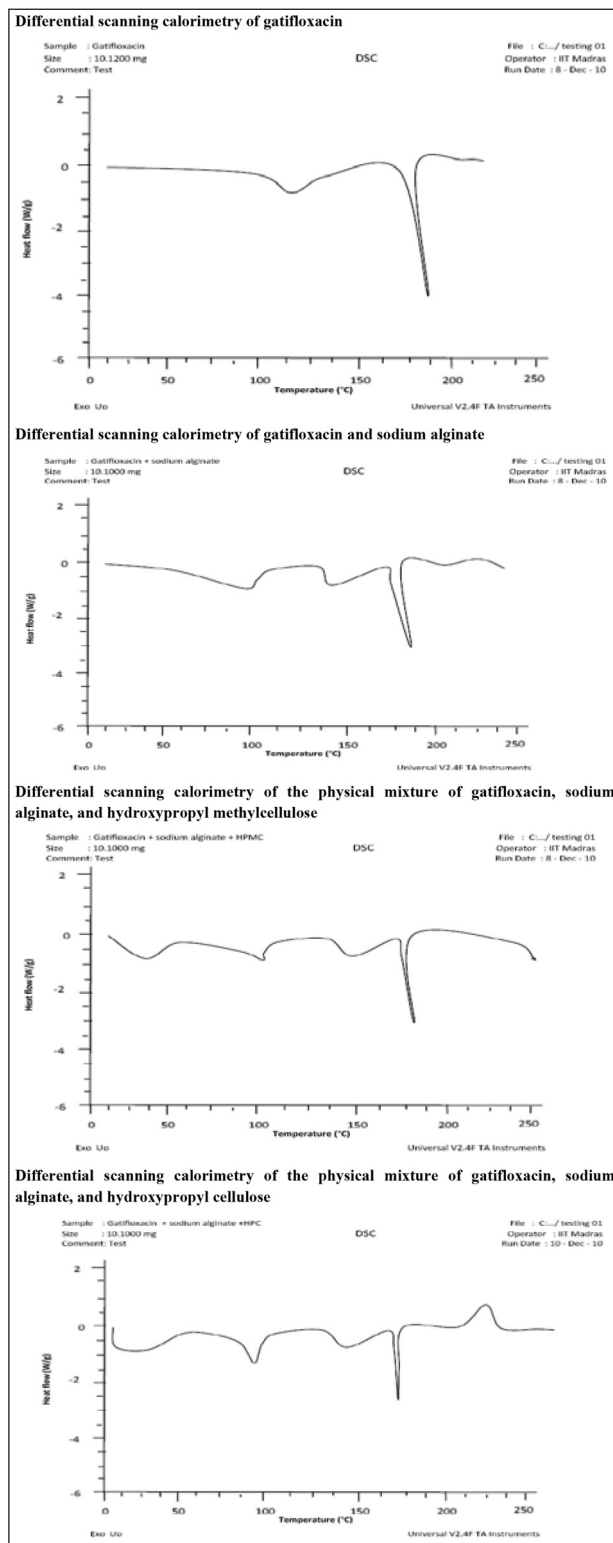


Figure 2: Differential scanning calorimetry of gatifloxacin and the physical mixtures of gatifloxacin, sodium alginate, and hydroxypropyl methylcellulose/hydroxypropyl cellulose

membranes in comparison to the control hydrogel membranes without glycerol (S₁F₁, S₁F₅, S₁F₉). Increasing the concentration of glycerol increases the swelling effect due to more absorption and retention of water. Hydrogel membranes plasticized with glycerol displayed increased percentage of swelling due to polymer glycerol linkage and the increased total hydrophilic nature.^[31] The extent of swelling was lower in cross-linked hydrogel membranes due to the reduction in water absorption as the glutaraldehyde-treated hydrogel membranes absorbed less water than hydrogel membranes without glutaraldehyde (S₁F₄, S₁F₈, S₁F₁₂) [Table 3]. In addition, the extent of swelling was less profound in cross-linked hydrogel membranes due to decreased polymer chain mobility. The hydrogel membranes prepared with HPMC (S₁F₅, S₁F₆, S₁F₇, S₁F₈) and HPC (S₁F₉, S₁F₁₀, S₁F₁₁, S₁F₁₂) polymers displayed markedly increased swelling compared to hydrogel membranes prepared in absence of HPMC and HPC polymers (S₁F₁, S₁F₂, S₁F₃, S₁F₄) [Table 3]. The addition of HPMC and HPC polymers led to a marked increase in swelling most likely due to a hydroxyl group effect.

Tensile strength and elongation

The tensile strength test was used as a tool to assess the effect of additives on the tensile strength property of the hydrogel membranes. From the mechanical properties of the gatifloxacin-loaded hydrogel membranes, it is observed the maximum values of tensile strength were found to vary between 31.34 ± 2.43 MPa for S₁F₄ to 71.21 ± 1.67 MPa for S₁F₅, while the breaking

elongation (flexibility and elasticity) mean values were found to vary between 14.24 ± 2.73% for S₁F₁ and 71.58 ± 2.78% for S₁F₈ [Table 3]. As shown in Table 3, hydrogel membranes that were treated with the plasticizer (glycerol) exhibited a considerable decrease in tensile strength in comparison to the control hydrogel membranes without glycerol (S₁F₁, S₁F₅, S₁F₉). Indeed, hydrogel membranes in presence of the plasticizer displayed a higher degree of flexibility and elasticity in comparison to the control hydrogel membranes (S₁F₁, S₁F₅, S₁F₉) [Table 3]. These observed effects are largely due to the ability of the plasticizer to decrease the intermolecular forces along the polymer chains, leading to decreased tensile strength and increased flexibility. The effect of the plasticizer on the tensile strength and flexibility of the hydrogel membranes was more profound at higher concentration of the plasticizer [Table 3]. Unlike the plasticizer, the cross-linking agent (glutaraldehyde) increased the tensile strength while decreasing the flexibility and elasticity of the hydrogel membranes compared to those without the cross-linking agent (S₁F₄, S₁F₈, S₁F₁₂) [Table 3]. Moreover, the hydrogel membranes prepared in presence of HPMC and HPC polymers exhibited increased tensile strength and decreased flexibility and elasticity compared to the control hydrogel membranes without polymer treatment (S₁F₁, S₁F₂, S₁F₃, S₁F₄) [Table 3]. Overall, the addition of plasticizer, cross-linking agent, and HPMC and HPC polymers resulted in a considerable increase in the tensile strength and flexibility/elasticity of the hydrogel membranes in comparison to the hydrogel membranes prepared in absence of the additives [Table 3]. Based on the swelling behaviour and mechanical properties findings, the following three samples were selected for further analysis: S₁F₃ (control), S₁F₇ (HPMC treatment), and S₁F₁₁ (HPC treatment).

Table 3: Swelling behaviour and mechanical properties of sodium alginate hydrogel membranes

Sample	Swelling index (%)	Tensile test	
		Tensile strength (MPa)	Breaking elongation (%)
S ₁ F ₁	65.32±3.43	67.45±2.24	14.24±2.73
S ₁ F ₂	73.74±4.72	53.44±1.82	43.61±4.23
S ₁ F ₃	89.43±3.07	41.73±2.02	49.22±3.62
S ₁ F ₄	114.37±4.94	31.34±2.43	61.44±2.45
S ₁ F ₅	71.25±4.63	71.21±1.67	18.72±3.41
S ₁ F ₆	104.56±3.87	56.44±2.63	54.66±4.08
S ₁ F ₇	116.78±3.35	46.72±1.42	62.37±2.65
S ₁ F ₈	130.51±4.65	35.94±1.84	71.58±2.78
S ₁ F ₉	67.24±4.72	69.52±2.42	16.42±2.16
S ₁ F ₁₀	97.32±3.88	54.73±2.09	50.73±3.18
S ₁ F ₁₁	114.64±4.23	43.86±2.47	60.34±2.78
S ₁ F ₁₂	121.76±4.87	33.21±1.94	67.35±2.83

All values are expressed as mean ± SD (n = 6), SD: Standard deviation

Table 4: Microbial penetration in sodium alginate hydrogel membranes (n = 6)

Sample	Daily visual observation of the culture medium						
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Positive control	—	+	++	++	+++	+++	+++
S ₁ F ₃	—	—	—	—	—	—	—
S ₁ F ₇	—	—	—	—	—	—	—
S ₁ F ₁₁	—	—	—	—	—	—	—

—: No turbidity, +: Mild turbidity, ++: Moderate turbidity, +++: High turbidity

% moisture absorption and % moisture loss

As shown in Figure 3, the % moisture absorption values for hydrogel membranes S₁F₃, S₁F₇, and S₁F₁₁ are 10.63 ± 0.21%, 18.72 ± 0.14%, and 16.84 ± 0.13%, respectively. The % moisture absorption is significantly (P < 0.05) higher in the formulations in which the hydrophilic polymers (HPMC and HPC) were incorporated (S₁F₇ and S₁F₁₁) compared to the control sample (S₁F₃). Indeed, hydrogel membranes containing HPMC (S₁F₇) demonstrated a greater potential to absorb moisture compared to

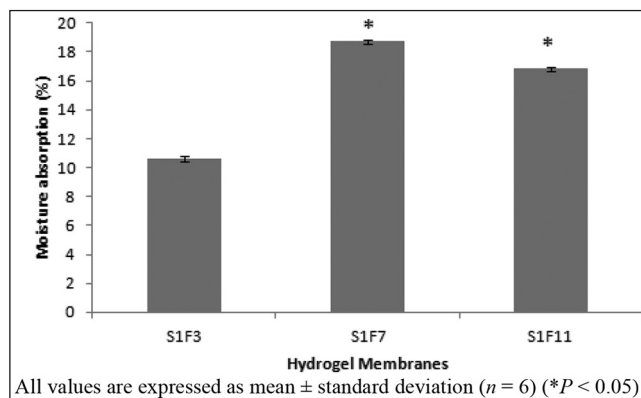


Figure 3: % moisture absorption of hydrogel membranes

HPC-treated hydrogel membranes (S_1F_{11}). At 80% RH, there was more moisture absorption but no change in integrity, as judged by its physical appearance. Consistently, the formulations with HPMC (S_1F_7) and HPC (S_1F_{11}) polymers had less tendency to lose moisture in comparison to the control sample (S_1F_3) [Figure 4]. The % moisture loss values for hydrogel membranes S_1F_3 , S_1F_7 , and S_1F_{11} are $19.31 \pm 0.18\%$, $16.24 \pm 0.12\%$, and $17.43 \pm 0.13\%$, respectively. S_1F_7 and S_1F_{11} hydrogel membranes significantly ($P < 0.05$) retained moisture in comparison to the control sample (S_1F_3). Noteworthy, keeping the hydrogel membranes at a very dry condition led to the maximum moisture loss.

Water vapor transmission rate

Trans epidermal water loss (TEWL) is a sign of the normalization of the stratum corneum barrier function.^[32] For this reason, investigating the moisture permeability of the hydrogel membranes, which are to be applied in wound dressings, is of major importance. The WVTR is a distinct factor that reflects the potential to transmit body liquids or wound exudates. Hence, WVTR of the three hydrogel membranes of interest was determined. As shown in Figure 5, the WVTR values for hydrogel membranes S_1F_3 , S_1F_7 , and S_1F_{11} are $4.87 \pm 0.11 \text{ g/m}^2/\text{h}$, $5.84 \pm 0.18 \text{ g/m}^2/\text{h}$, and $5.07 \pm 0.12 \text{ g/m}^2/\text{h}$, respectively. The WVTR values are significantly higher ($P < 0.05$) for hydrogel membranes treated with HPMC (S_1F_7) and HPC (S_1F_{11}) polymers in comparison to the control hydrogel membranes (S_1F_3) [Figure 5]. The higher WVTR values observed with hydrogel membranes treated with the HPMC and HPC polymers are due to the hydrophilic nature of these polymers. The WVTR results suggest that hydrated hydrogel membranes should be able to facilitate water vapor transfer from a moisture-rich environment to a dry environment. Noticeably, the observed WVTR values in our study are similar to the reported TEWL values of healthy human skin ($5\text{-}10 \text{ g/m}^2/\text{h}$).^[33]

Microbial penetration

Daily visual observation of the culture medium showed that no microbes penetrated through the hydrogel membranes for a period of 1 week. The results indicated that hydrogel membranes with 2.1-2.4 mm thickness could protect against

microbial penetration, while it was previously shown that even 64 layers of gauze could not prevent entry of exogenous bacteria into wounded tissue.^[34] Kokabi *et al.* demonstrated that nanocomposite hydrogel membranes with 3 mm thickness were effective in preventing microbial penetration.^[28] In our study, the gatifloxacin-loaded hydrogel membranes also proved good ability to prevent microbial penetration at even smaller thickness (2.1-2.4 mm). The results of the microbial penetration test demonstrated that none of the prepared hydrogel membranes supported microbial growth. Indeed, microbial growth was only observed in the positive control sample (i.e., no hydrogel membrane) [Table 4]. This indicates that the prepared hydrogel membranes can serve as potentially safe wound dressing materials due to their potent antimicrobial properties.

In vitro gatifloxacin release

The *in vitro* gatifloxacin release analysis reveals that the cumulative % of gatifloxacin release from hydrogel membranes S_1F_3 , S_1F_7 , and S_1F_{11} after 48 h of release is $99.40 \pm 0.50\%$, $92.03 \pm 0.05\%$, and $96.19 \pm 0.10\%$, respectively [Figure 6]. Expectedly, gatifloxacin release rate was the highest at the beginning of the experiment, but then started to decrease gradually until $\sim 100\%$ release was achieved [Figure 6]. Gatifloxacin release was significantly ($P < 0.05$) more sustained in the HPMC-treated and HPC-treated hydrogel membranes (S_1F_7 and

Table 5: In vitro gatifloxacin release pattern in sodium alginate hydrogel membranes

Time (h)	Cumulative drug release (%)		
	S_1F_3	S_1F_7	S_1F_{11}
0	0.00±0.00	0.00±0.00	0.00±0.00
0.5	1.52±0.02	1.94±0.06	1.11±0.12
1	8.53±0.22	7.12±0.16	9.44±0.03
2	28.46±0.14	22.14±0.14	17.14±0.14
4	35.64±0.18	34.25±0.08	35.25±0.40
6	44.71±0.85	41.71±0.46	42.34±0.41
8	64.80±0.43	50.42±0.86	48.83±0.72
12	74.52±1.02	58.47±1.15	65.27±1.08
16	88.11±1.52	73.44±1.08	81.25±1.12
24	89.31±1.10	78.20±1.42	87.42±1.10
48	98.22±0.50	85.03±1.51	94.19±1.08

All values are expressed as mean \pm SD ($n = 6$), SD: Standard deviation

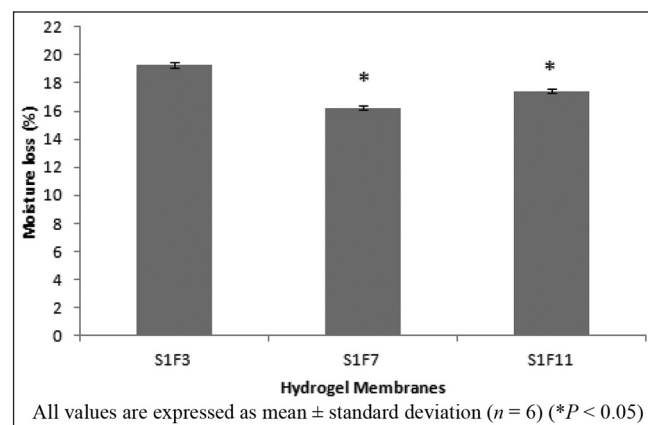


Figure 4: % moisture loss of hydrogel membranes

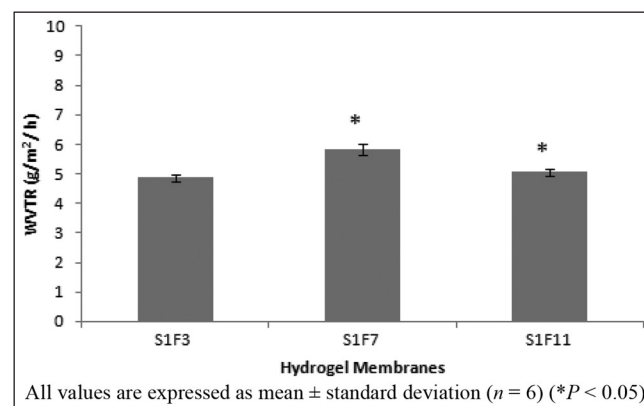


Figure 5: Water vapor transmission rate for hydrogel membranes

S_1F_{11} , respectively) compared to the control sample (S_1F_3). The hydrophilic nature of HPMC and HPC polymers allowed a burst in the release of gatifloxacin in the first few minutes. The presence of cross-linking agent (glutaraldehyde) may have retarded the release of gatifloxacin later on despite the sustained release of gatifloxacin from the hydrogel membranes [Table 5]. The results of gatifloxacin release studies indicate that the presence of copolymers has a substantial effect on the rate of drug release from the hydrogel membranes [Table 5]. The burst release effect is a result of a very quick release of gatifloxacin from the hydrogel membrane wound dressing, ensuring a rapid reduction of bacterial count in the wounded tissue. However, the sustained release effect is due to the slow release of gatifloxacin from the hydrogel membrane due to the action of the cross-linking agent. So, wound dressings supplemented with HPMC-treated and HPC-treated hydrogel membranes seem to be a good option to exert potent anti-microbial activities for at least 48 h postinjury. Several studies reported that applying high concentrations of anti-microbial agents at the wound site can cause tissue toxicity. Such tissue toxicity can be avoided having a sustained release of the antimicrobial agent at sub-toxic concentrations. Our findings reveal that HPMC-treated and HPC-treated hydrogel membranes allow slow, but sustained, release of gatifloxacin for 48 h [Figure 6], which makes such hydrogel membranes suitable and effective wound dressing materials.

Drug release kinetics

The cumulative percentage of drug release data of S_1F_3 , S_1F_7 , and S_1F_{11} hydrogel membranes were fitted to zero-order, first-order, Higuchi, and Korsmeyer-Peppas models to understand the kinetics of drug release from the indicated hydrogel membranes. The gatifloxacin release data were subjected to zero-order of release [Figure 7], and the regression (R^2) values for S_1F_3 , S_1F_7 , and S_1F_{11} samples were found to be 0.741, 0.643 and 0.678, respectively [Table 6]. The gatifloxacin release data were also subjected to first order of release [Figure 8], and the regression (R^2) values for S_1F_3 , S_1F_7 , and S_1F_{11} samples were found to be 0.420, 0.333 and 0.386, respectively [Table 6]. Next, to find out whether diffusion is involved in drug release, the data was subjected to Higuchi equation [Figure 9]. The regression (R^2) values for S_1F_3 , S_1F_7 , and S_1F_{11} samples were found to be 0.936,

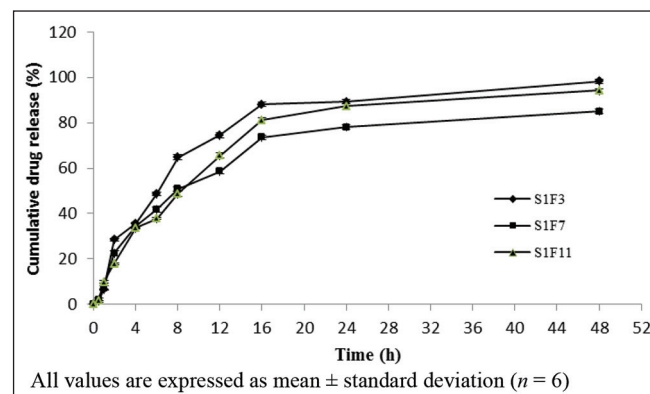


Figure 6: *In vitro* gatifloxacin released from the hydrogel membranes

0.884, and 0.902, respectively [Table 6]. To further elucidate the mechanism of drug release, the data was subjected to Korsmeyer-Peppas equation [Figure 10]. The release exponent (n) values for S_1F_3 , S_1F_7 , and S_1F_{11} samples were found to be 0.834, 0.843, and 0.867, respectively [Table 6]. As evident by the slope values of more than 0.5 but <1.0 for the plot of log cumulative amount release versus log time (Korsmeyer-Peppas plot) [Figure 10], it can be concluded that drug release from the studied hydrogel membranes occurs by the anomalous (nonFickian) type of diffusion, involving swelling of the polymer matrix. Based on the analysis of the correlation coefficient for order of release, the pattern of drug release does not strictly follow the zero-order or the first-order model of drug release. Although the release pattern is closer to the zero-order model, it may indeed be a mixed-order reaction. Further in-depth analysis is required to ascertain the order of drug release from the studied hydrogel membranes. Overall, the drug release kinetics reveal that both diffusion and dissolution play an important role in the release of gatifloxacin from the studied hydrogel membranes.

CONCLUSIONS

Modern wound dressings are designed to limit the spread of infection by delivering antimicrobial agents and providing suitable conditions for faster skin healing. Our study reveals that the formulated gatifloxacin-loaded hydrogel membrane that were treated with HPMC and HPC polymers meet the

Table 6: *In vitro* gatifloxacin release kinetic parameters

Sample	Kinetic models			
	Zero-order	First-order	Higuchi	Korsmeyer-Peppas
	R^2	R^2	R^2	n
S_1F_3	0.741	0.420	0.936	0.834
S_1F_7	0.643	0.333	0.884	0.843
S_1F_{11}	0.678	0.386	0.902	0.867

R^2 is the regression value, while n is the release exponent of Korsmeyer-Peppas model

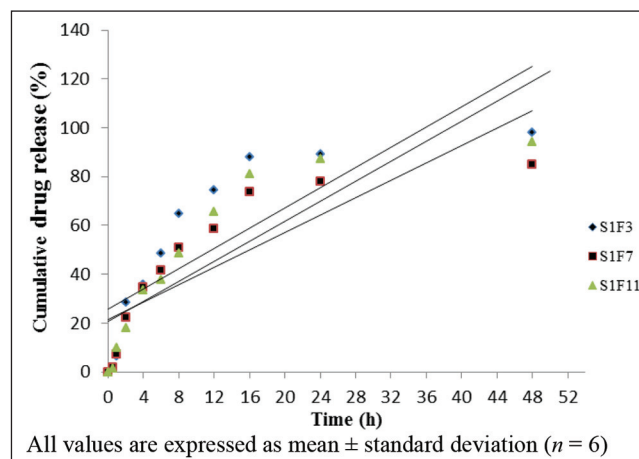


Figure 7: Zero-order model of gatifloxacin release from sodium alginate hydrogel membranes

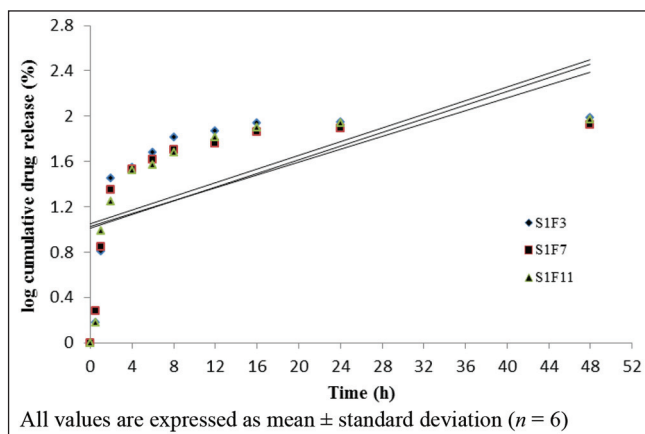


Figure 8: First-order model of gatifloxacin release from sodium alginate hydrogel membranes

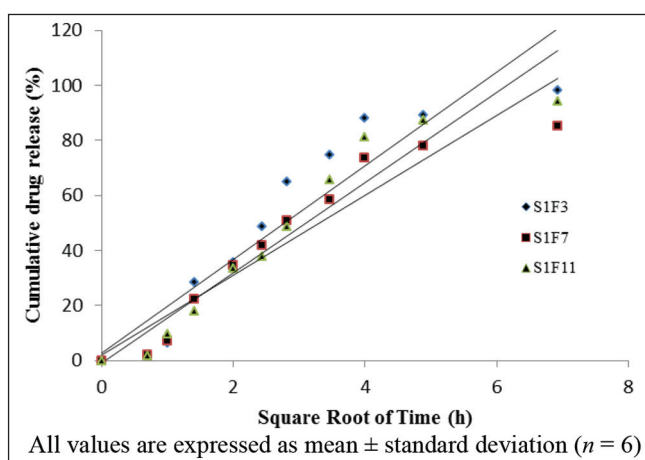


Figure 9: Higuchi model of gatifloxacin release from sodium alginate hydrogel membranes

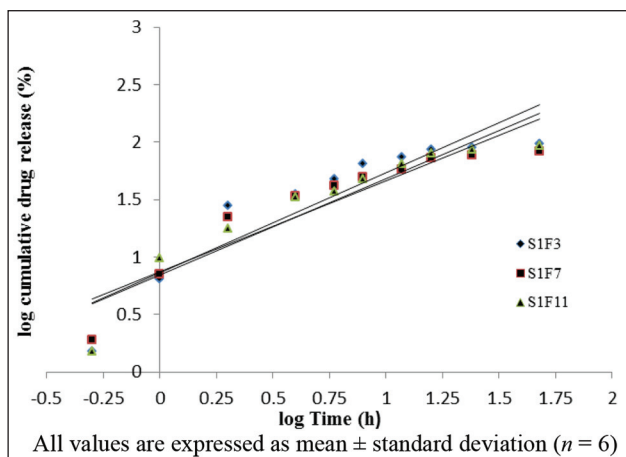


Figure 10: Korsmeyer-Peppas model of gatifloxacin release from sodium alginate hydrogel membranes

essential requirements for a reasonable wound dressing with desirable physiochemical characteristics including better swelling, improved tensile strength and elongation, excellent barrierity, appreciated WVTR, and sustained drug release. In sum, our

experimental approach was successfully undertaken to design a topical drug delivery system in the form of gatifloxacin-loaded sodium alginate hydrogel membranes with desirable wound healing properties.

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Conflicts of interest

There are no conflicts of interest.

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