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Optimization of controlled release Ciprofloxacin dermal hydrogels using different chitosan molecular weights

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ABSTRACT

Ciprofloxacin (CFX) is a broad spectrum antibiotic belonging to fluoroquinolones. The study aims to optimize and formulate chitosan hydrogels loaded with 1% CFX for dermal use. Two factors, three levels (3^2) full factorial design was used to optimize the effect of chitosan molecular weight, MW (X1) and concentration (X2) on both CFX release rate within 3 h (Y1) and diffusion coefficient (Y2). The prepared hydrogels were evaluated for their viscosity and pH values as well as in-vitro drug release. In addition, disc diffusion method was used for antibacterial susceptibility test for the CFX-loaded chitosan hydrogels. The statistical data showed that X1 and X2 have significant antagonistic effects (p-value < 0.05) on CFX release from chitosan gel bases, but the effect of X1 was higher. The drug release from chitosan hydrogels followed Higuchi diffusion kinetic model, and the calculated n values indicated an anomalous (non-Fickian) transport. Furthermore, CFX-loaded chitosan gels exhibited potent antibacterial activity on both gram negative and positive strains. The results concluded that controlled release hydrogels of the antimicrobial agent can result in maximum therapeutic efficacy of the drug along with minimized time required to change wound dressing. This can lead to improved patient compliance.

Keywords: *Ciprofloxacin, Chitosan Molecular Weight, Hydrogel, Optimization, Antimicrobial.*

1. INTRODUCTION

Ciprofloxacin (CFX) is a third-generation of fluoroquinolones, and is considered as one of the most widely used broad-spectrum antibiotics in both human and veterinary medicine.CFX acts against gram negative and gram positive bacteria by the inhibition of bacterial DNA unwinding and duplicating. [1].

Antibiotics administration by the topical route can result in delivering higher antibiotic concentration locally with a minimized systemic drug concentration in relation to the systemic route [2]. Several clinical studies showed that different classes of antibacterial agents can be administered topically to inhibit and eradicate effectively the bacteria infections [3]. The drug delivery system that can effectively deliver continual release of the drug, can effectively improve the effectiveness of the wound dressings and avoid the possibly expected infections. In addition, by reducing the time required to change wound dressing, patient compliance can be increased [4]. Novac et al. [5] synthesized gellan gum derivatives containing quaternary ammonium groups to get particulate transdermal controlled release ciprofloxacin. The In vitro release of CFX studies showed that the drug was released up to 24 h, confirming quaternized gellan–chitosan particles' potential as controlled release systems for topical dermal applications.

Hydrogels resemble an important class of biomaterials particularly used for drug delivery applications, owing to their biocompatibility, good rheological and bioadhesive properties, high capacity for drug loading and modified-release behaviors.

Chitosan is a polysaccharide contains chitin that is obtained from the hard outer skeleton crab, lobster, and shrimp [6]. Also, it is biodegradable, which is used in a wide range of biomedical applications due to its hemostatic, antimicrobial and wound healing properties [7]. The application of chitosan in preventing or treating wound and burn infections is not only attributed to its intrinsic antibacterial properties, but also due to its power to deliver extrinsic antimicrobial agents to wounds and burns. Chitosan can also be utilized as a slow-release drug-delivery vehicle for growth factors to improve wound healing [8].

Varshosaz et al. [9] investigated the effect of chitosan molecular weight (MW) and concentration on performance of lidocaine gel bases. They revealed that chitosan gel bases resulted in prolonging the anesthetic effect of the drug for transdermal delivery. Drug release studies in gels showed that increasing the concentration and MW of chitosan caused an increase in both the rate and extent and also in flux of drug probably because of the increase in repulsive forces between lidocaine and chitosan cations. Their results showed the prospect of attaining controlled drug release by the use of chitosan matrices.

However, Senel et al. [10] observed that increasing the chitosan concentration resulted in an increase in chlorhexidine gluconate release rate from its gel bases. In a certain chitosan concentration however, increasing the polymer MW results in enhanced drug release rates. Therefore, it is possible to adopt chitosan as controlling polymeric matrix for the drug release by tuning its molecular weight and concentration.

The formulation of antibiotics in controlled release chitosan hydrogels could be therapeutically potential in prolongation of its antimicrobial action. Other advantages could be achieved are related to the antibacterial, antifungal and would healing properties of chitosan.

The aim of the present study was to optimize and formulate chitosan hydrogels containing ciprofloxacin for controlled release antimicrobial properties using 3^2 full factorial design. The prepared hydrogels were characterized for their viscosity, pH values and in vitro CFX release.

2. EXPERIMENTAL SECTION

2.1. Materials.

Chitosan of different molecular weights (low MWt grade; 40 kD, medium MWt grade; 480 kD and high MWt grade (the same degree of deacetylation; 96%); 850 kD) were purchased from Sigma-Aldrich Chemie, GmbH (Steinheim, Germany). Ciprofloxacin (CFX) and standard cellophane membrane, molecular weight cut off ≈ 12,000 were purchased from Sigma-Aldrich Chemie, GmbH (Steinheim, Germany). Lactic acid was purchased from Fluka Chemica (Buch, Switzerland). All other materials and solvents used were of reagent or analytical grade and used without further purification.

2.2. Experimental design.

Two factors, three levels (3^2) full factorial design was used to optimize chitosan molecular weight, MW (X1) and chitosan concentration (X2) concentrations using a statistical package (Statgraphics Plus, version 5).

Table 1. Variables in 3^2 full factorial design.

Independent variable, Factor			
	Low (-1)	Middle (0)	High (1)
X1: Chitosan MW (KD)	40	480	850
X2: Chitosan concentration (%)	1	2	3
Dependent variable, Response			
Y1: Release (%) Y2: diffusion coefficient (cm^2/s)			

Table 2. Matrix of 3^2 full factorial design for CFX chitosan hydrogel formulations.

Experiment no.	Chitosan MW (X_1)	Chitosan concentration (X_2)
1	850	2
2	450	3
3	40	3
4	450	2
5	40	1
6	450	1
7	850	3
8	40	2

Statistical models with interaction terms were derived to evaluate the effect of the two factors on CPX-loaded hydrogel release after 3 h (Y1) and diffusion coefficient (Y2).

Two factors have been selected two in different levels, namely chitosan molecular weight (X1) and chitosan molecular concentration (X2). In addition, CFX release percentage (Y1) and diffusion coefficient (Y2) have been selected as the measured responses as illustrated in Table 1 and the factorial design matrix shown in Table 2. The results (responses) are provided in each row in the matrix.

2.3. Preparation of CFX-loaded chitosan hydrogels.

Chitosan hydrogels containing CPX (1% w/w) were prepared using different chitosan molecular weight grades (40, 480, and 850 kDa). In addition, three concentrations (1, 2 and 3% w/w) of each chitosan grade were used in preparing the gel bases. CPX was dispersed in dilute lactic acid solution (2 w/w %) by a slow stirring to form a clear solution was obtained. Thereafter, the formula weight of chitosan was added, and stirring was continued slowly until gel formation. The prepared hydrogel was allowed to stand for 2-3 h to get rid of the air bubbles, and then was kept at 5^oC.

2.4. Characterization of CFX-loaded chitosan hydrogels.

2.4.1. pH determination.

The pH values of the formulated CPX-loaded chitosan hydrogels were measured using pH meter (Mettler Toledo, Greifensee, Switzerland), which was previously calibrated using standard buffers of pH 4 and pH 7 as per the established procedure.

2.4.2. Rheological properties of hydrogels.

The rheological properties of the CFX-loaded chitosan hydrogel bases were determined by using Brookfield DV-II model RV Viscometer (USA) at different rates of shear. The measurement was carried out over range of speed from 0.5-20 rpm with 1 minute between each successive speed and in a descending order.

2.4.3. In vitro release of CFX from hydrogels.

The in vitro release of CFX from its chitosan hydrogel formulations was performed by using a standard semi-permeable cellophane membrane into a saline phosphate buffer (pH=7.4) at 32 + 0.5^oC. The membrane was cut into 4 X 4 cm pieces and soaked in distilled water for 24 hr. prior to its use in the release study. The water was removed from the membrane by pressing it between two filter papers. Accurately weighed one gram of the hydrogel was placed over the membrane. The membrane was placed tightly over the lower end of the dialytic tube (2.2 cm diameter) by means of a cotton thread. The upper opening of the tube was covered with a thinly perforated nylon membrane, to

minimize the evaporation of the liquids, which may be incorporated in the base, and to reduce the hydrostatic pressure. The tube was assembled into a glass vessel containing 50 ml of saline phosphate buffer (pH = 7.4), then submerged into a water-bath shaker previously adjusted to 37 + 0.5°C and 50 stroke/min. At suitable time intervals, 2 ml sample was withdrawn and diluted to a specific volume with distilled water and the cumulative amount of CFX released into the buffer solution was determined spectrophotometrically at 278 nm. It should be noted that, at each sampling time, 2 ml of the buffer at the same temperature was replaced to the release medium to keep its volume constant.

The diffusion coefficient of CFX from different hydrogels can be calculated using the following equation [11]:

$$q = Q/A = 2Co\ (Dt/\ \pi)\ 1/2$$

Where Q is the drug released amount in mg, A is the diffusion membrane surface area in cm^2, Co is the loaded amount of the drug in the base, D is the diffusion coefficient (cm^2/s), and t is the time (s).

2.4.4. Kinetic modeling of the in vitro release of CFX from chitosan hydrogels.

The in vitro release data of CFX from different chitosan hydrogels were fitted using Zero order, First order and Higuchi diffusion models as well as Korsmeyer-Peppas equation to determine the model that describes drug release from these hydrogel formulations. The preference of the release mechanism is based on the correlation coefficient value. In addition, the release exponent (n) was calculated from Korsmeyr equation [12-13]:

$$\frac{Mt}{M\infty} = Kt^n$$

2.4.5. Microbial Cultures.

For comparing the polymeric effect on bacterial growth, different microbial strains were used in the current study. They include Bacillus cereus ATCC 7004, Bacillus subtilis, Staphylococus aureus ATCC 25923, Micrococcus luteus and Enterococcus faecalis representing Gram positive bacteria. While Pseudomonas aeruginosa ATCC 27853, Klebsiella pneumonia ATCC 53657, Escherichia coli ATCC 25922, Salmonella typhi ATCC 0650 and Proteus vulgaris ATCC 29905 represent Gram negative. Candida albicans was used as a fungi representative. These organisms were provided by the microbiology lab, pharmaceutics department, Faculty of Pharmacy, King Saud University. The bacterial strains were cultured in Mueller-Hinton broth MHB (Difco, USA) with moderate shaking at 37°C for 18 h. The bacterial kinetic was detected by determining the absorbance at 620 nm wavelength every hour using a spectrophotometer (Shimadzu UVPC 2000, Shimadzu Co. Kyoto, Japan) to detect the bacterial stationary phase.

2.4.6. Evaluation of antimicrobial activity.

Kirby Bauer disc diffusion method was used for determination of the antimicrobial activities [14-15]. The tested hydrogel sample was prepared and the sterile blotting paper disc (5 mm diameter) was soaked in the prepared gel. To remove the excess of testing gel formula, the prepared disc was dried at suitable controlled temperature (at 37 °C for 18 hs). An adequate amount of culture was aseptically flooded onto the Muller-Hinton Petri dishes surface (23×23 mm) and discs of antibiotic were aseptically located on the upper layer of the MHA plates (adequately divided to keep away from inhibition zones overlapping). The excess inoculum was removed out and the plates were dried for 1 hour at 37°C. In an upright position. The plates were incubated for 24 hs at 37°C. The diameters of the zones of inhibition appearing around the discs when present was measured to the nearest millimeter (mm). The results were recorded according to the average diameter of the clear area. The antimicrobial activity tests were run in triplicate and expressed as an average value.

3. RESULTS SECTION

3.1. In vitro release of CFX from chitosan hydrogels.

The in vitro release patterns of CFX from different chitosan hydrogels are displayed in Figures 1-3.

Hydrogel bases formulated by using low chitosan molecular weight showed the highest in vitro CFX release rate in comparison to other tested chitosan molecular weights, Figure 1. In addition, by increasing chitosan concentration in the hydrogel, a retardation to the CFX release was observed.

For example, 36.71%, 33.43% and 29.66% of the loaded CFX released from hydrogels containing different concentrations of low molecular weight chitosan (1%, 2% and 3%, respectively). Also, increasing chitosan molecular weight from low to medium grade resulted in slowing the drug release rate from the tested hydrogels, in addition to the retarding effect of chitosan concentration, Figure 2.

In addition, the slowest CFX release was exhibited from chitosan hydrogels made of the highest polymer molecular weight, Figure 3.

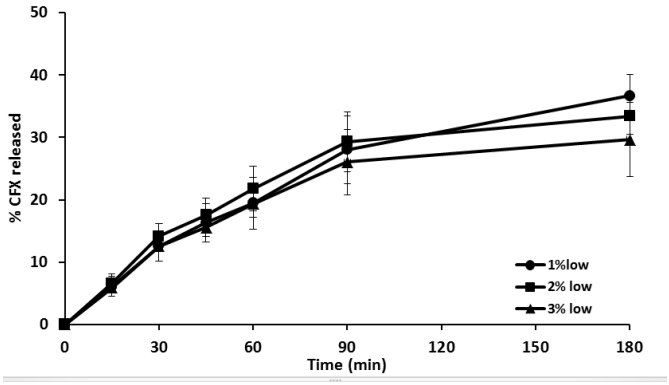

Figure 1. Effect of different concentrations of low MW chitosan on the in vitro release profiles of CFX from hydrogels.

The inverse correlation between CFX in vitro release rate and chitosan molecular weight as well as chitosan concentration can be explained on the basis of the increased viscosity values of chitosan hydrogel by increasing chitosan molecular weight and

concentration, Table 3. This resulted in decreasing the drug diffusion coefficient values from the tested hydrogels, Table 3.

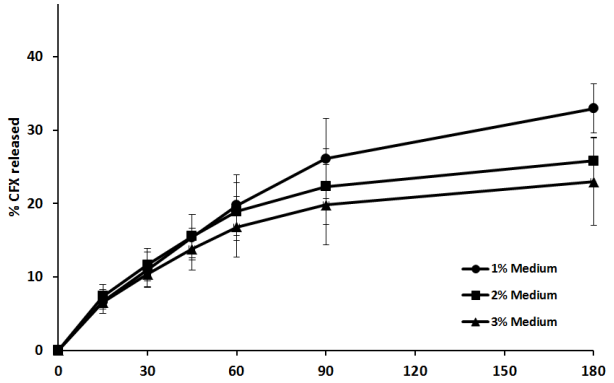

Figure 2. Effect of different concentrations of medium MW chitosan on the in vitro release profiles of CFX from hydrogels.

The drug showed the highest diffusion coefficient (8.37 cm^2/s x 10-6) from chitosan gel base prepared from 1% low molecular weight chitosan, while very slow diffusion (3.65 cm^2/s x 10-6) has been recorded from 3% high molecular weight chitosan gel base.

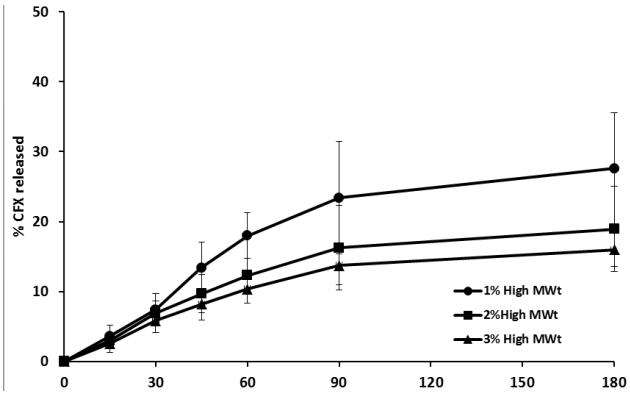

Figure 3. Effect of different concentrations of high MW chitosan on the in vitro release profiles of CFX from hydrogels.

Table 3. Viscosities, pH values and diffusion coefficients of CFX release from different chitosan hydrogel formulations.

Gel Formula		pH	Viscosity (cp)	Diffusion coefficient (cm^2/s) x 10^{-6}
Low MW Chitosan	**1%**	4.16	-	**8.37**
	2%	4.43	542	**7.61**
	3%	4.82	1000	**6.77**
Medium MW Chitosan	**1%**	4.10	166.7	**7.52**
	2%	4.38	3870	**5.93**
	3%	4.60	7000	**5.25**
High MW Chitosan	1%	4.21	250	**6.3**
	2%	4.47	13600	**4.11**
	3%	4.65	48160	**3.65**

Previous authors showed that drug release rate from a gel base is highly affected by its viscosity. As the viscosity of the gel increased, the release of the drug was expected to be slower. [16]. The results of Senyiğit et al. [17] showed that miconazole nitrate and econazole nitrate release rates from mucoadhesive vaginal chitosan gels were significantly influenced by the viscosity of chitosan. Varshosaz et al. [9] showed that the direct effect of polymer MW on lidocaine release is proportional to the number of amino groups available for ionic repulsion of the drugs having the same ionic charges. As the molecular weight increases, more amino groups may become more available for ionic repulsion of the similar charged drug ions.

However, an inverse release profile was recorded when acidic drugs were incorporated into chitosan gel bases. Starýchová et al. [18] found that at higher chitosan concentrations in the gel bases, an enhanced indomethacin release rate was observed. They concluded that the release rate of the drug from these gel bases was affected by raised pH values of the gels by the presence of chitosan.

3.2. Kinetic modeling of the in vitro release of CFX from hydrogels.

The in vitro release of CFX from chitosan hydrogels was characterized for the release mechanism, the preference of which was based on the correlation coefficient value. The data revealed a good fit Higuchi diffusion model. Fitting the data to Korsmeyer-Peppas model, showed that the computed "n" values were found higher than 0.45, but all were less than 1 (Table 4), indicating also non-Fickian or anomalous drug release [12]. Moreover, higher n values were observed in case of gel formulations based on high molecular weight chitosan.

3.3. Experimental design data analysis.

Chitosan gel bases containing 1% CFX were prepared based on the matrix of the design as shown in Table 2, and formulations composition listed in Table 2. Effect of chitosan molecular weight (X1) and concentration (X2) on the in vitro release rate of CFX from hydrogels after 180 min (Y1) is displayed in Figures 1-3. To generate mathematical models for the responses, the release data of CFX were analyzed using a statistical package (Statgraphics Plus, version 5). High value of correlation coefficient were recorded upon analyzing the effects of X1 and X2 on both the % release and diffusion coefficient of CFX from the tested hydrogels (r >98.6), Table 5 A&B. The equations that describe the impact of X1 and X2 on the in vitro release and diffusion coefficient of CFX from chitosan hydrogels are:

CFX release (Y1) = 46.1074 - 0.0086X1 - 9.795X2 - 0.0000013X12 - 0.003X1X2 + 1.56833X22

Diffusion coefficient (Y2) = 10.7247 - 0.0017X1 - **2.50491**X2 - 0.00000056X12 - **0.000**7X1X2 + 0.427X22

The value of the regression coefficients (X1 and X2) are correlated with the effects of these variables on the responses. It is clearly from the statistical data that the two factor X1 and X2 have a highly significant antagonistic effect (p-value < 0.05) on CFX release from chitosan hydrogel bases, but the effect of X1 is more pronounced as seen from Pareto chart, Figure 4A and Table 5A. Moreover, the interactive effects (X1X2) and quadratic effects ($X1^2$ and $X2^2$) on CFX release are very slight and insignificant. The X1 and X2 worked together to slow the drug release rate from the tested chitosan hydrogels as shown in the response surface plot for CFX release, Figure 4A. Similarly, the effect of the two factors X1 and X2 on the diffusion coefficient of CFX from hydrogel

formulations are significantly antagonistic, and the effect of X1 is higher than the effect of X2 as seen from Table 5B and Pareto chart in Figure 5A.

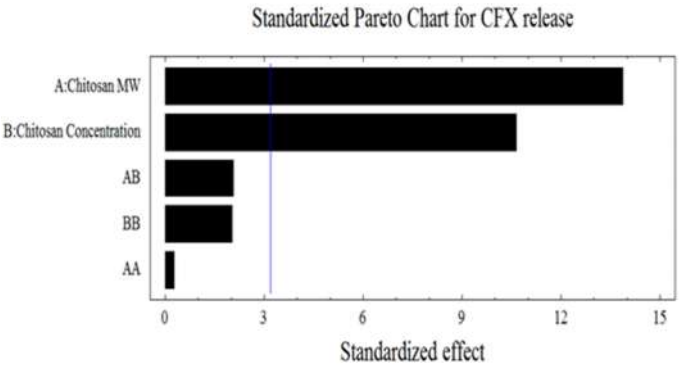

Figure 4A. Standardized Pareto chart for CFX release from chitosan hydrogels.

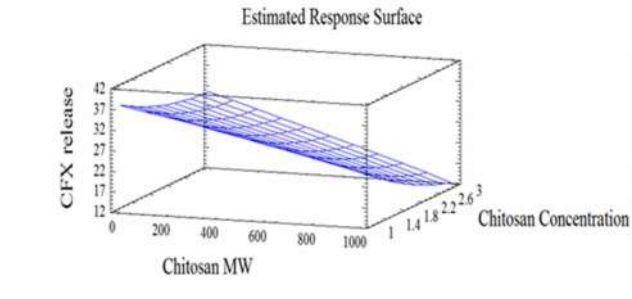

Figure 4B. Response surface plot for CFX release from chitosan hydrogels.

The interactive effects and quadratic effects of the tested variable were found to be insignificantly affecting the drug diffusion. In addition, the response surface plot for CFX diffusion coefficient from chitosan hydrogel formulations revealed the antagonistic effects of X1 and X2 on the drug diffusion, and a stronger antagonism was observed with X1, Figure 5B.

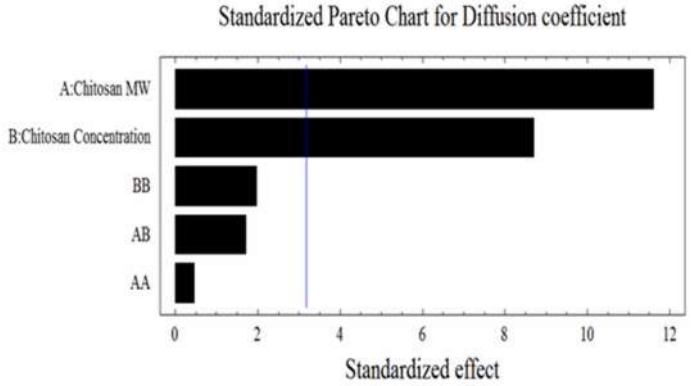

Figure 5A. Standardized Pareto chart for CFX diffusion coefficient from chitosan hydrogels.

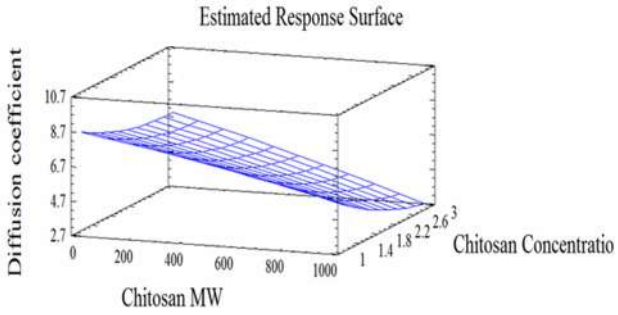

Figure 5B. Response surface plot for CFX diffusion coefficient from chitosan hydrogels.

Table 4. Kinetic modeling of CFX release from different chitosan hydrogel formulations.

Gel Formula		Zero order		First Order			Higuchi		Korsemayer-Peppas
		r	slope	r	slope	R	slope	r	n
Low MW Chitosan	**1%**	0.954	0.19734	0.971	-0.00109	0.987	2.92	0.985	**0.72**
	2%	0.903	0.177	0.923	-0.00096	0.978	2.73	0.965	**0.65**
	3%	0.903	0.1572	0.921	-0.00083	0.978	2.43	0.965	**0.66**
Medium MW Chitosan	**1%**	0.939	0.1758	0.957	-0.00094	0.988	2.64	0.988	**0.67**
	2%	0.878	0.130	0.897	-0.00066	0.980	2.06	0.974	**0.52**
	3%	0.878	0.1552	0.894	-0.00058	0.980	1.83	0.974	**0.52**
High MW Chitosan	1%	0.917	0.1553	0.930	-0.00080	0.965	2.33	0.961	**0.85**
	2%	0.912	0.103	0.922	-0.00050	0.975	1.57	0.962	**0.75**
	3%	0.912	0.0871	0.920	-0.00041	0.975	1.33	0.962	**0.75**

Table 5A. Analysis of variance for CFX release from chitosan hydrogels.

Source	Sum of Squares	df	Mean Square	F- Ratio	P Value
X1: Chitosan MW	231.385	1	231.385	192.20	0.0008
X2: Chitosan Concentration	136.422	1	136.422	113.32	0.0018
$X1^2$	0.085	1	0.085	0.07	0.8072
X1X2	5.153	1	5.153	4.28	0.1304
$X2^2$	4.919	1	4.919	4.09	0.1365

R-squared = 99.05

Table 5B. Analysis of variance for the diffusion coefficient of CFX release from chitosan hydrogels.

Source	Sum of Squares	df	Mean Square	F- Ratio	P Value
X1: Chitosan MW	12.586	1	12.586	134.46	0.0014
X2: Chitosan Concentration	7.085	1	7.085	75.69	0.0032
$X1^2$	0.019	1	0.019	0.21	0.6803

Source	Sum of Squares	df	Mean Square	F- Ratio	P Value
X1X2	0.276	1	0.276	2.94	0.1847
$X2^2$	0.364	1	0.364	3.89	0.1431

R-squared = 98.64

3.4.Antimicrobial activity

The growth and turbidity of different tested microorganisms were measured at 620 nm as optical density. All bacteria were grown in a similar way, but with differences in turbidity. The growth kinetics curves of different organisms were detected. The exponential increasing (the log phase) was recorded for each organism during the first 6-9 h and the stable stationary phase were detected. Different sizes of inhibition zone were observed for the tested gel preparation samples with different MW against the tested microorganisms as represented in Table 6. In vitro antimicrobial activity of CFX from different chitosan hydrogels was determined against the test bacteria and fungi by the Kirby–Bauer disc diffusion method. It is well established that all loaded chitosan gel bases containing CFX showed clear significant bactericidal effects against both Gram-positive and Gram-negative bacteria relative to the positive control. The antimicrobial activity of the tested CFX hydrogel was found to be not influenced solely by only one factor. Chitosan concentration, hydrogel viscosity diffusivity of CFX in these hydrogels were found to be influential parameters on the antimicrobial activity from the tested hydrogels. Hence, it is difficult to draw an exact pattern concerning the antimicrobial activity of CFX from these hydrogels.

Table 6. Zone of inhibition activity (in millimeter) of different ciprofloxacin hydrogels against various microorganisms.

Microorganism	Inhibition zone diameter (mm)							
	Low MW chitosan		Medium MW chitosan		High MW chitosan		Positive control	Negative Control
	1%	3%	1%	3%	1%	3%		
Staphylococcus aureus	13.3±1	19.6±1	17.3±4	15.6±2	16.0±1	13.6±2	24.3±1	0
Klebsiella pneumoniae	12.6±1	14.3±0	22.1 ±5	19.1 ±1	21.5±4	18.6 ±1	22.4 ±1	0
Bacillus cereus	17.3±1	12.6±1	20.2 ±1	18. 2±1	15.6±2	18.2 ±4	21.3 ±1	0
Pseudomonas aeruginosa	16.0±1	15.6±1	18. 2±5	14.3±0	20.3 ±1	18.5 ±1	21.5±3	0
Escherichia coli	17.6±1	13.6±2.	19.1 ±1	22.1 ±3	17.3±4	12.8±1	28.1±1	0
Micrococcus luteus	16.6±1	33.3±1	18.1 ±1	12.6±1	18.6 ±1	15.5±2	22.3 ±1	0
Salmonella typhimurium	22.3 ±1	13.6±2	15.6±2	21.5±1	18. 5±1	17.3±1	23. 5±3	0
Bacillus subtilis	22.1 ±5	17.3±1	16.0±1	14.3±0	14.8±2	13.6±1	22. 5±1	0
Enterococcus faecalis	18.6 ±1	14.3±0	12.6±1	15.0±2	16.6±1	20.1 ±1	22. 6±2	0
Candida albicans	20.0 ±1	18.6 ±1	15.6±3	22.3 ±1	14.5±0	18.3 ±1	25.5±2	0

Values are expressed as mean ± standard deviation of the three replicates

4. CONCLUSIONS

The formulation of antibiotics in controlled release chitosan hydrogels can efficaciously expand the efficacy of the wound dressings and avert future infections. The antimicrobial activity of chitosan itself, in addition to its retarding effect on the CFX release from hydrogels can result in maximized therapeutic action along with reduced time required to change wound dressing, resulting in increased patient compliance.

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