Preparation And Characterization of Sodium Alginate/Acrylic Acid Composite Hydrogels Conjugated To Silver Nanoparticles As An Antibiotic Delivery System

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Research Article

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Abstract

Hydrogels are specific groups of polymers that are highly swellable in aqueous solutions, despite their water-in-soluble structures. Thus, they are promising drug delivery systems attributable to their unique characteristics such as high hydrophilicity, high controllability, facile production routines and, good biocompatibility. The aim of this research was the preparation of sodium alginate/acrylic acid composite hydrogels conjugated to silver nanoparticles to deliver the cephalexin as a model antibiotic compound. The reduction of silver ions into silver nanoparticles as well as the stabilization of created nanoparticles ensued simultaneously with hydrogel backbone formulation during microwave irradiation and monomer cross-linking processes. The impact of acrylic acid and silver ions concentrations and also the radiation time of microwave were then investigated on the main characteristics of hydrogels, namely, swelling ratio, gel fraction, cephalexin load and, antibacterial activity. The results indicated that the hydrogels' characteristics could be significantly predicted by studied all independent parameters, through various second-order polynomial models. The multiple optimization analysis suggested that the prepared hydrogels using 7.8 g acrylic acid and 1.5 g silver nitrate and 1 min microwave radiation could give the best hydrogels with the highest swelling degree, gel fraction, cephalexin absorption and, antibacterial activity. The morphology and either absorption or release kinetics of cephalexin by/from the optimum prepared hydrogels were also investigated. No significant differences between the experimental and predicted data was confirmed the suitability of the suggested models.

Introduction

A hydrogel consists of an elastic cross-linked polymeric network with fluid fillable matrices that let molecules hold the liquid in place. The hydrogels can absorb a considerable fraction of water from 10% to 100 times of the hydrogel weight within its structure, but that will not dissolve in water (parhi 2017; Mori and Anarjan 2018).

Hydrogel nanocomposites are one type of biomaterials that have newly attracted a lot of consideration for their applications in medical and pharmaceutical areas. They have been investigated for various biological applications including drug delivery, tissue engineering, antimicrobial agents, and thermal therapy (Yang et al. 2018). Hydrogel nanocomposites are obtained by incorporating different types of nanoparticles, such as metallic, clay, and ceramics, into a hydrogel structure, which can offer various characteristics and capabilities to hydrogel systems (Gaharwar et al. 2014). The particulate material can be merged into hydrogels in different ways such as addition into monomer solution and followed by polymerization or direct mixing into hydrogel matrix after polymerization (Dannert et al. 2019). It should be noted that the polymerization process in hydrogel formation can be occurred either chemically using cross-linking agents or physically using ionizing irradiations (parhi 2017).

Various either synthetic or natural polymers or monomers can give single or composite hydrogels with improved release control capabilities. For example, Sodium alginate, an anionic macromolecule, that can be elicited from marine algae or produced by bacteria, is plentiful, renewable, non-toxic, water-soluble and biocompatible polymer, with great gelation capacity. It can be readily modified by other monomers or polymers to give the most desired hydrogels for certain application (Mori and Anarjan 2018). Acrylate based hydrogels are another group of the most talented systems in delivery of pharmaceutical constituents like antibacterials in wound wearing, bone cements, contact lenses and etc. formulations. However, due to some deficiency of their physical and biological properties, especially their hydrated state at the body temperature, such as anti-fouling activity, rigidity, water diffusion, novel acrylic-based hydrogels with composite structures have been developed, in order to improve their characteristics and give them superior applicability in biomedical and bioengineering uses. For example, poly acrylic acid and alginate composite were produced in interpenetrating polymer network form and incorporated with silica nanoparticles, or graphene nano-sheets were integrated into poly acrylic acid/gelatin composite hydrogels, in order to produce metrics with the most desired physical and biological characteristics (Serrano-Aroca and Deb 2020).

While antibiotic compounds have been used to stop the bacterial infections, most of them are not effective against multi-drug-resistant pathogenic strains. Therefore, the inorganic antibacterial agents such as silver with great activities against all wild and multi-drug-resistant pathogens was proposed as a new class of antibacterial agents. The more stable and long-lasting antimicrobial activities of silver nanoparticles as compared to silver ions and micro-structured silver have been confirmed in most of the previous researches (Tang and Zheng 2018). Consequently, the size reduction of silver particles into nano-metric scales or renovation of silver ions to silver nanoparticles can improve their antibacterial activities, considerably. It was hypothesized that the silver ions can be reduced into silver nanoparticles and also stabilized during hydrogel formation process through monomers' polymerization (Patra and Baek 2017; Sharma et al. 2019).

The previous researches have confirmed the synergistic antibacterial effect of silver with other antibiotic compounds (Patra and Baek 2017). Thus, due to a perfect ability of hydrogels in either absorption or release of bioactive compounds, it was anticipated that the composite hydrogels conjugated to silver nanoparticles could effectively absorb various antibiotics such as cephalexin, and so, offer efficient, stable and strong bactericidal actions in microbial infection therapies (Zhang et al, 2016).

Therefore, in this research, the effects of main formulation and process parameters, namely, acrylic acid and silver ion (silver nitrate) concentrations, as well as the microwave irradiation time, were investigated on characteristics of produced composite hydrogels. The various polynomial models were suggested to predict the hydrogels' characteristics by selected independent parameters. Finally, the process parameters were optimized, aimed to develop the best product with the most desired properties such as the greatest swelling degree, gel fraction, drug load, and the least drug release. The morphology and either absorption or release kinetics of the best prepared hydrogels were studied, as well.

Materials And Methods

2.1. Materials

All acrylic acid (AAc), sodium alginate, calcium chloride and silver nitrate were obtained from Merck (Gernsheim, Germany). All solvents and deionized water were also acquired from Dr. Mojallali's company (Tehran, Iran). Cephalexin was donated from Daana Pharmaceutical Company (Tabriz, Iran).

2.2. Methods

The hydrogels were produced by simultaneous chemically cross-linking of sodium alginate by calcium chloride and physically polymerization and cross-linking of acrylic acid monomers by microwave irradiation.

Silver nitrate was dissolved in 5 mL deionized water and slowly added into a 50 mL aqueous solution of acrylic acid. various quantities of silver nitrate and acrylic acid were used in the preparation of each sample according to experimental design (Table 1). The mixture was extra stirred magnetically for 30 min before subjecting to microwave irradiation (LG NeoChef™ countertop microwaves with Smart Inverter). The duration of microwave irradiation was done according to the design of the experiment (Table 1), with the difference that the time of irradiation was intermittent and between every 30 s, 1 min of rest was given to prevent overheating of the samples. After the microwave irradiation process, the sample was dropwise added into an aqueous solution of sodium alginate (3.5 g dissolved sodium alginate in 100 mL deionized water) and mixed completely under a magnetic stirrer for an extra 1 h. At last, the calcium chloride solution (11 g dissolved calcium chloride in 50 mL deionized water), was added into the system at the rate of 5 mL/min under a magnetic stirrer. The mixing of samples was continued for an extra 30 min and then they were dried in an electrical oven at 55°C for 3 days.

Table 1
Design of experiment, independent and response variables in preparation of alginate / acrylic acid composite hydrogels conjugated to silver nanoparticles

sample	Acrylic Acid (g)	Silver Nitrate (g)	Microwave time (min)	Swelling ratio (%)	Gel fraction	Cephalexin load (%)	Clear zone (mm)
1	7.97	1	4.1	148.4	0.070	92.00	21
2	0	0.5	3	108.7	0.310	59.25	13
3	0.05	0.5	3	105.6	0.230	57.05	13
4	7.97	1	1.8	506.7	0.520	98.75	21
5	0.05	1	3	124.4	0.440	64.50	16
6	2.02	0.5	4.1	257.7	0.340	43.75	16
7	2.02	1	1.8	392.2	0.690	58.75	17
8	0.1	0.3	3	10.0	0.236	57.75	12
9	0.05	1.5	1.8	50.0	0.910	53.00	21
10	7.97	0.3	1.8	107.0	0.200	55.00	19
11	2.02	0.5	1.8	203.7	0.340	46.00	20
12	2.02	1	4.1	70.0	0.230	58.50	19
13	0.05	0.5	1	135.3	0.280	56.75	16
14	0.05	0.5	3	105.7	0.230	60.25	12
15	0.05	0.7	3	170.5	0.290	66.00	14
16	0.05	0.5	5	165.1	0.260	51.00	10
17	0.05	1	3	30.0	0.490	72.75	15
18	7.97	0.3	4.1	261.3	0.250	51.25	17
19	0.05	0.5	3	106.0	0.210	61.50	12
20	0.05	0.5	3	105.4	0.240	59.25	13

Experimental design

The response surface method with the central composite design was used in order to analyze of variance (ANOVA), multiple regression analysis, and multi-goal optimization in order to get the most desired hydrogels with the maximum swelling degree, gel fraction, cephalexin load, and antibacterial

activities (response variables). The significant effects of independent variables, either in linear, quadratic, or interaction forms, at a 95% confidence interval (p-value < 0.05) were also determined based on their obtained p-value and F-ratio through ANOVA analysis. By multiple regression analysis, the polynomial models were provided for response variables' in order to predict their changes by selected independent variables. The terms with the smaller p-values (less than 0.05) and greater F-ratio are considered as the more significant effect. The selected independent variables were the acrylic acid and silver nitrate (silver ions) contents and the radiation time of microwave. Consequently, 20 composite hydrogels were prepared at different five levels of acrylic acid and silver ions concentrations and microwave irradiation time. The sample preparation order was randomized and the center point was repeated several time in order to prevent the systematical errors and confirm the repeatability of samples. In addition, the response variables' contour plots were also provided in order to visualize their changes by selected formulation and process parameters (Anarjan et al. 2013).

2.3. Analysis

2.3.1 Swelling ratio

1 g of each dried sample was immersed in deionized water for 8 hours. The remained solution was completely removed from swollen hydrogels by

filter paper and then the swelling ratio of hydrogels was calculated using Eq. 1. Swelling ratio
$$(w/w) = \frac{w_2 - w_1}{w_1} \times 100 = (g / g)$$
 (Equation 1) where w_1 and w_2 are initial dry hydrogel and swelled hydrogel weights, respectively (Kondaveeti et al. 2018).

2.3.2. Gel fraction

The swelled hydrogels were placed in aluminum plates and put into oven for 10 h at 55°C until their completely dehydration. Then, they were carefully weighted. Gel fraction calculated from Eq. 2.

$$\text{Gel fraction} = \frac{w_3}{w_2} \times 100 \quad (\text{Equation 2})$$

where w_2 and w_3 are the swelled hydrogel and dried ones weights (Kondaveeti et al. 2018).

2.3.3. Cephalixin load

1g of each dried hydrogel was dipped into 15 mL NaOH solution (0.1N) containing 0.5 g cephalixin for 24 h in order to complete the absorption process of cephalixin. The cephalixin solution were then centrifuged, neutralized and the light absorption intensity of supernatant was measured using a UV-Vis spectrophotometer (DR5000, Hack, Canada) at $\lambda = 320$ nm, in order to find the remained (un-adsorbed) cephalixin of solution. The un-adsorbed cephalixin content in solution were determined based on previously provided standard curve (c_r). The cephalixin load of samples were then calculated by subtraction of obtained cephalixin content (c_r) from its initial content (0.5), according to Eq. 3. The NaOH aqueous solution (0.1N) were considered as blank sample.

$$\text{Cephalixin load (\%)} = \frac{0.5 - c_r}{0.5} \times 100 \quad (\text{Equation 3})$$

2.3.4. Antibacterial test against Staphylococcus aureus (S. aureus)

The antibacterial activity of hydrogels were also tested against *S. aureus* (aerobic gram-positive bacteria) using the agar-well diffusion technique. Various 8 mm-diameter wells have introduced aseptically to inoculated nutrient agar with *S. aureus* in certain concentration (0.5 McFarland). Then, 10 mg of each hydrogels was dissolved in 10 μ L deionized water and poured into the wells. The agar plates were incubated at 37 °C for 48 h. The cephalixin diffuses into the agar medium and hinders the growth of bacteria around the wells, and can be observed as a clear zone. The clear zone diameter is a good indicator of the antibacterial activity of hydrogels, as a bigger clear zone corresponds to the greater antimicrobial activity. The experiments were performed in triplicate, and mean clear zone radial were reported as the antimicrobial activity of samples. The antibacterial activities of hydrogels with no incorporated cephalixin were evaluated as a blank sample (Wang et al. 2020).

2.3.5. Cephalixin absorption kinetics

In order to determine the cephalixin absorption kinetics, 0.5 g of optimum hydrogel was added into 50 mL NaOH solution (0.1N) containing 0.25 g/L cephalixin. The light absorption intensity of solution were measured in 4 min intervals for 5 h, at $\lambda = 320$ nm using a UV-Vis spectrophotometer (DR5000, Hack, Canada). The light absorption intensity of solution were converted to solution cephalixin content using standard curve. The loaded cephalixin by hydrogels in each time was coded as C_a and calculated as $C_a = I_{\text{initial}} - C_t$. I_{initial} is 0.25 g/L and the C_t is the measured concentration of cephalixin in solution at time t.

2.3.6. Cephalixin release Kinetics

For investigation of cephalixin release, 0.5 g of optimum hydrogel was dipped into 15 mL NaOH solution (0.1N) containing 0.5 g cephalixin for 24 h. Then, the loaded hydrogel was placed in an aqueous solution with pH of 7.4 and room temperature ($25 \pm 4^\circ\text{C}$). The light absorption intensities of this solution were measured in 4 min intervals for 5 h, at $\lambda = 320$ nm using a UV-Vis spectrophotometer (DR5000, Hack, Canada). The light absorption

intensity of solution were converted to cephalixin content, using standard curve, and marked as released cephalixin from hydrogel into solution at time t.

2.3.7. Fourier transform infrared spectroscopy (FT-IR)

FT-IR was used in order to determine the functional groups in the optimum product chemical structure. The dried hydrogel was ground and the spectra was achieved against potassium bromide (KBR) using JUSCO spectrophotometer (JUSCO 4100, Japan) at λ ranged from 4000 to 400 cm^{-1} . The analyses were performed in transmittance mode.

2.3.8. UV-Vis Absorption Spectra

In order to confirm the formation of silver nanoparticles into sodium alginate/acrylic acid composite hydrogels networks The UV-Vis absorbance spectra of optimum product were recorded. The optimum product was first dispersed in deionized water, and then its absorption spectra was recorded at 25°C using UV–Visible spectrophotometer (DR5000, Hack, Canada), in wavelength from 250 to 800 nm.

2.3.9. Scanning Electron Microscope (SEM)

The morphology of optimum hydrogel was also evaluated using field emission scanning electron microscopy (FE-SEM, VEGA//TESCAN, Czech Republic). 0.1 g of optimum hydrogel was placed in copper grid and coated by gold in order to increase the conductivity of samples. The micrographs were then provided.

Results And Discussion

3.1. Swelling ratio and gel fraction

The sodium alginate/acrylic acid composite hydrogels conjugated to silver nanoparticles were successfully synthesized through a simultaneous chemically ion cross-linking and physically microwave polymerization of sodium alginate acrylic acid monomers, respectively. However, based on used acrylic acid and silver ion contents and applied microwave time, their characteristics were differed considerably. For instance, the swelling ratio of the obtained hydrogels varied from 10 to 506.7%, and their gel fraction laid between 0.07 and 0.91. All gained hydrogels exhibited noticeable bactericidal activities due to the presence of silver in their matrice, and were able to encapsulate and load various compounds such as cephalixin, from 36.89 to 92.58%. The characteristics of all samples prepared in different conditions were shown in Table 1 and the ANOVA analysis results were summarized in Table 2.

Table 2
ANOVA analysis and p-values and F-ratio of linear, quadratic and binary interaction terms in suggested models for prepared sodium alginate/acrylic acid composite hydrogels conjugated to silver nanoparticles

Characteristics		Linear effects	X ₁	X ₂	X ₃	Quadratic effects	X ₁₁	X ₂₂	X ₃₃	Interaction effects	X ₁₂	X ₁₃	X ₂₃
swelling	P-value	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.008	0.000	0.234	0.458	0.000
	F-ratio	302.91	320.91	166.80	534.15	163.31	46.40	321.43	11.11	199.71	1.61	0.60	565.49
gel fraction	P-value	0.000	0.000	0.000	0.000	0.025	0.007	0.708	0.756	0.000	0.021	0.135	0.000
	F-ratio	4106	10846	76.6	31.63	4.8	11.65	0.15	0.10	34.68	7.5	2.64	90.07
cephalexin load	P-value	0.000	0.000	0.000	0.355	0.000	0.000	0.013	0.017	0.001	0.000	0.373	0.507
	F-ratio	39.23	107.20	33.69	0.94	26.59	60.42	9.03	8.27	12.11	35.81	0.87	0.47
clear zone	P-value	0.000	0.000	0.001	0.671	0.24	0.005	0.078	0.681	0.167	0.800	0.291	0.071
	F-ratio	15.31	35.85	20.23	0.19	4.93	12.60	3.84	0.18	2.07	0.07	1.24	4.08

The ability to be swollen in thermodynamically compatible media is the most favorable characteristic of hydrogels. The water molecules penetrate into the polymeric network of hydrogels and the rubbery phase of hydrogels, which is first be expanded and filled by penetrated molecules of solvent and detach from glassy segments. In contradiction of the favorable osmotic force, the opposite elasticity force occurs that stopovers the extending and deformation of the hydrogel networks. The equilibrium in swelling will be occurred as the elasticity and osmotic forces balance (Ganji et al. 2010).

The ANOVA analysis for swelling ratio of prepared hydrogels indicated that all selected process parameters affected the swelling ratio of hydrogels significantly, especially in linear form. The interactions of acrylic acid with silver nitrate and microwave time were the insignificant terms on this characteristic. Thus, these two terms were removed from initial model, and the final reduced model were offered as Eq. 4.

$$\text{Swelling ratio (\%)} = -742.5 + 63.40 X_1 + 2255.1 X_2 + 108.4 X_3 - 4.879 X_1^2 - 876.8 X_2^2 + 9.89 X_3^2 - 314.9 X_2 \cdot X_3 \quad (\text{Eq. 4})$$

The coefficient of determination (R^2) for this model was 97.26%. Thus, it can be concluded that this model can successfully predict about 97% of swelling ratio changes of hydrogels. Moreover, the insignificant p-value of lack of fit (p-value = 0.824), can confirm the suitability of this model in predicting the swelling ratio of sodium alginate/acrylic acid composite hydrogels. By comparing the F-ratio of significant terms, it could be also concluded that the interaction effect of silver nitrate and microwave time had the greatest effects on this characteristic (Table 2).

The main effects plot of independent variables on swelling ratio of hydrogels were shown in Fig. 1a. Since the interaction of silver nitrate with microwave time was only the significant term on swelling ratio, just the contour plot of the variation of this response by silver nitrate and microwave time at the certain level of acrylic acid (3.985g) was visualized as Fig. 1b. As can be seen in Fig. 1a, using greater acrylic acid contents in formulation of hydrogels, could yield the product with larger swelling ratio. An optimum content was obtained for silver nitrate leading to the hydrogels with the highest swelling ratio. Thus, the swelling ratio of hydrogels increased by rising the silver ions up to certain levels, and greater silver ions decreased this characteristic, considerably. The swelling ratio of hydrogels also decreased by increasing microwave time. However, the effect of microwave time on swelling ratio was different at various levels of silver ion, as simultaneous increases or decreases of microwave time and silver ion content resulted to the production of hydrogels with less swelling ability.

Generally, the microwave exposure of hydrogels for long time lead to a considerable increase in polymerization degree of the acrylic acid monomers. Therefore, the hydrogel network becomes more cohesive and stronger and water penetration into the network will be limited (Makhado et al. 2018; Kretschmann et al. 2007). Furthermore, the previous researches also confirmed that the presence of silver ions has positive impact on construction of stable acrylic acid hydrogel matrices, by accelerating the polymerization of acrylic acid. Thus, the gained stable hydrogel matrices would have greater water absorption and swelling ability. However, at high silver ion contents, various cross-linking can be occurred between silver ions and carboxylic groups of (poly) acrylic acid, leading to form O-Ag-O bonds, in which, with the formation of these bonds, the free hydroxyl groups on (poly) acrylic acid would be reduced. Consequently, the chance for hydrogen bonding between hydrogels carboxylic groups and water decreased, resulted in an extensive decline in water absorption of hydrogels (Serrano-Aroca and Deb 2020; Kowalski et al. 2019). The increase of swelling ratio by acrylic acid content would also be related to the formation of more impregnated poly acrylic acid matrices into sodium alginate networks. Therefore, the amount of empty media of hydrogel networks, which can be filled by water, increased (Serrano-Aroca and Deb 2020; Quintanilla de Stéfano et al. 2020). Moreover, the swelling of hydrogels lasts until equilibrium state, where the Gibbs free energy of hydrogel is minimized. According to the theory of Flory–Rehner, the Gibbs free energy will be minimized if the osmotic and elasticity forces become equal. An increase in hydrogels' cross-linking density causes the formation of smaller chains. The shorter chains have less elasticity force as compared to longer ones. Thus, the equilibrium between elasticity and osmotic forces occurs at less swelling ratios (Quintanilla de Stéfano et al. 2020).

The ANOVA analysis of gel fraction of prepared hydrogels showed that all considered process parameters affected the gel fraction of hydrogels pointedly, particularly in linear form. The quadratic effects of both silver nitrate and microwave time, and the interaction effect of acrylic acid and microwave time, were insignificant terms on gel fraction changes of samples. Thus, these three terms were removed from initial model and the final reduced model for estimating the hydrogels' gel fractions was shown as Eq. 5, with the R^2 equal to 96.90%.

$$\text{Gel fraction} = -0.0627 + 0.09253 X_1 + 0.3005 X_2 + 0.07332 X_3 - 0.001391 X_1^2 - 0.00483 X_1 X_2 - 0.06396 X_2 X_3 \quad (\text{Eq. 5})$$

Moreover, the insignificant p-value of lack of fit (p-value = 0.28), can confirm the correctness of model in predicting the gel fraction of produced composite hydrogels. By comparing the F-ratio of significant terms, it can also be concluded that the linear effect of microwave time had the highest influence on this characteristic (Table 2).

The main effects plot of independent variables on swelling ratio of hydrogels were shown in Fig. 2a. The contour plots of gel fraction changes by both silver nitrate-microwave time (fixed middle level of acrylic acid, Fig. 2b), and acrylic acid-silver nitrate (fixed middle level of microwave time, Fig. 2c) were shown due to the significant effects of acrylic acid-silver nitrate and silver nitrate-microwave time interactions. According to Fig. 2a, increasing of either acrylic acid or silver nitrate and microwave time raised the gel fraction of obtained hydrogels. Moreover, simultaneous increase or decrease of acrylic acid and silver nitrate, at constant microwave exposure time, or simultaneous increase or decrease of silver nitrate and microwave time, at fixed level of acrylic acid, also caused a decrease in gel fraction of samples (Figs. 2b,c).

Increasing the acrylic acid content led to construction the denser and more robust hydrogel matrices, which are insoluble in water. Furthermore, increasing the silver nitrate augmented the O-Ag-O cross-linking between the monomers and thus, made them insoluble in water. The exposure of hydrogels to microwave for extended time also increased the in polymerization degree of the acrylic acid monomers and produced stronger water insoluble matrices (Pourjavadi et al. 2006).

3.2. Cephalixin Load

All synthesized hydrogels could efficiently adsorb cephalexin, ranged from 43.75 to 98.75%. The ANOVA analysis for cephalexin load of samples indicated that the linear effects of acrylic acid and silver nitrate concentrations, the quadratic effects of all studied independent variables and the interaction effect of acrylic acid with silver nitrate contents were significant (p-value < 0.05) on this response. However, the acrylic acid content was the most effective parameter on drug loading of samples. The final reduced model predicting the cephalexin loading of hydrogels was found as Eq. 6. The linear effect of microwave time was not removed from the model due to its significant quadratic effect. The R² for this model was 96.66%.

$$\text{Cephalexin load (\%)} = 24.82 - 11.53 X_1 + 65.8 X_2 + 9.63 X_3 + 1.278 X_1^2 - 35.37 X_2^2 - 1.836 X_3^2 + 5.146 X_1 X_2 \text{ (Eq. 6)}$$

The obtained high R², and insignificant p-value of lack of fit (p-value = 0.179), could confirm the precision of model in calculating the cephalexin load of produced composite hydrogels. By comparing the F-ratio of significant terms, it can also be concluded that the linear effect of acrylic acid had the utmost impact on this characteristic (Table 3).

Table 3. The kinetics of cephalexin adsorption by optimum composite hydrogels (the R² and main coefficients for correlated general absorption models)

Common Kinetic Models	Cephalexin adsorption	
	Equation	C = 0.6612t + 1.4054
Rate=K Linear	R ²	0.9739
	Equation	LnC = 0.2615t + 0.4047
Rate=KC 1 st order	R ²	0.9147
	Equation	1/C = -0.491t + 2.3955
Rate=KC ² 2 nd order	R ²	0.6630
	Equation	1/C = y = 0.2537 (1/t) + 0.9576
Rate= $\frac{KC}{KC+V_m}$ Michaelis–Menten	R ²	0.8767
	Equation	Ln c=0.3096(Ln t) + 0.867
Rate=KC ⁿ Logarithmic	R ²	0.9259

The main effects plots (Fig. 3a) indicated that while increasing the acrylic acid content up to certain level decreased the drug load of samples, further uses of acrylic acid in formulation of hydrogels increased their drug absorption, considerably. The reverse trend was also observed for silver nitrate and microwave exposure time, in which increasing of these two parameters up to certain level improved their drug loading efficiencies, however, additional contents of silver ions or extra duration of microwave irradiation decreased their drug absorption efficiencies. The contour plot of cephalexin absorption changes by acrylic acid and silver nitrate (at fixed middle level of microwave time was also shown in Fig. 3b. According to Fig. 3b, simultaneous increase or decrease of these two parameters could enhance the drug absorption efficiency of hydrogels.

Using high concentrations of acrylic acid (especially at certain microwave exposure time) caused the production of composite hydrogels with weak cross-links. These weak cross-linked acrylic acid-based hydrogels could also be obtained at less silver ions concentrations as well as less microwave irradiation time, since the anionic groups on hydrogels become less protonated, and the hydrogen bonding between the functional groups would be weakened. Thus, the pore size in the hydrogel matrix would be increased, causing a considerable enhance in the efficiency of hydrogels' absorption. Increasing the silver ions also ionized the background polymers, upturned their electrostatic repulsions, weakened their matrices and made them more penetrable against drugs (Quintanilla de Stéfano et al. 2020). Previous researches also have shown that drug diffusion into hydrogels with high cross-linking densities matrices was difficult, because the hydrogels with more cross-links were more compacted and possessed very small less penetrable pores (Kowalski et al. 2019).

3.3. Antibacterial activity

All synthesized hydrogels showed antibacterial activity against *s. aureus*, with growth inhibitory zone ranged from 10 to 21 mm. The ANOVA analysis of antibacterial activity designated that the linear effects of acrylic acid and silver nitrate and the quadratic effect of acrylic acid were just the significant terms, on changes of this characteristic in 95% confidence interval (p-value < 0.05). The quadratic effect silver nitrate content and also its interaction with microwave time were also significant in 90% confidence interval (0.1 > p-value > 0.05). The final reduced model after removing the insignificant terms (p-value > 0.1) was shown as Eq. 7, in order to predict the variation of antibacterial activity of hydrogels.

$$\text{Growth Inhibition zone (mm)} = 21.22 + 2.479 X_1 - 13.92 X_2 - 2.381 X_3 - 0.2239 X_1^2 + 7.90 X_2^2 + 2.35 X_2 X_3 \text{ (Eq. 7)}$$

According to obtained R² for Eq. 7 (R² = 92.39%), it can be concluded that this model can predict more than 92% of antibacterial activities of hydrogels against *s. aureus* in term of their growth inhibited zone diameter.

The main effects plots (Fig. 4a) for changes of this response by selected independent parameters point out that while increasing the acrylic acid content up to certain level increased the antibacterial activity of samples, further uses of acrylic acid in formulation of hydrogels affected reversely, and decreased this ability of samples. Increasing the silver nitrate ions as well as decreasing the microwave time also strengthened the bactericidal activity of hydrogels. The contour plot of growth inhibitory zone of samples changed by silver nitrate content and microwave time (at fixed middle level of acrylic acid) was also shown in Fig. 4b. As can be seen in Fig. 4b, simultaneous increase of both silver nitrate and microwave irradiation time could enhance the bactericidal activity of hydrogels.

According to F-ratio of terms, the silver nitrate content was the most effective agent on antibacterial activity of obtained hydrogels. This result was predictable due to high antibacterial activity of silver. Some previous researches also reported an antibacterial activity for acrylic acid and its di-block copolymers (Gratzl et al. 2014). Thus, it seems that intensifying of hydrogels' antibacterial activities by increasing the acrylic acid could be related to this antibacterial activity of acrylic acid residues in hydrogel matrices. Increasing the irradiation time also decreased the antibacterial activity of hydrogels, due to increasing the cross-linking bonds between acrylic acids and decreasing the monomer residues. Considerable antibacterial activities of prepared samples at high silver nitrate contents and extended irradiation time could be related to the reduction of silver ions to silver nano-particles by microwave emissions and their stabilization between the dense cross-linked matrices of hydrogels (Xia et al. 2012; Singh and Dhaliwal 2020).

3.4. Optimization and model confirmation

The acrylic acid and silver nitrate contents, as well as the microwave irradiation time, were numerically optimized in order to produce the hydrogels with the highest swelling ratio, gel fraction, cephalixin load and, *s. aureus* growth inhibited zone, using multiple goal optimization process. Thus, according to optimization analysis, using the highest contents of either acrylic acid (7.8 g) or silver nitrate (1.5 g) at less microwave exposure time (1 min) can give the hydrogels with the maximum highest swelling ratio (655%), gel fraction (> 0.99), cephalixin load (> 0.99) and, *s. aureus* growth inhibited zone (24.97 mm).

For confirming the presented models, three samples were prepared in obtained optimum conditions (acrylic acid = 7.8 g, silver nitrate = 1.5 g and microwave exposure time = 1 min), and were quantified. The swelling degree, gel fraction, cephalixin load and antibacterial clear zone diameter of these samples were $650.5 \pm 14.5\%$, 0.98 ± 0.07 , $0.99 \pm 0.03\%$ and, 26 ± 2 mm, respectively. The insignificant differences between the experimental data and predicted ones by model (655%, 0.99, 0.99% and 24.97 mm, in turn), confirmed the suitability and correctness of models.

3.5. Complementary characterizations of optimum sodium alginate/acrylic acid composite hydrogels conjugated to silver nanoparticles

3.5.1. The absorption and release kinetics of cephalixin by optimum sodium alginate/acrylic acid composite hydrogels conjugated to silver nanoparticles

The absorption or release of bioactive compounds by/from most hydrogels are occurred mostly due to diffusion phenomena. However, the absorption/release profile for a swelled composite hydrogels are complex, depending on the relative diffusion rates of either water or bioactive compound from/toward hydrogel networks. If the diffusion into the hydrogel takes place in gentle rates as compared to the hydrogel chains' relaxation, the process will be controlled by diffusion. Otherwise, the process will be relaxation-controlled. The irregular pattern between the mentioned states is known as Non-Fickian Diffusion.

In order to determine the cephalixin absorption kinetic model of optimum composite hydrogel, they were immersed into an aqueous solution containing certain amount of cephalixin (0.25 g/L), for 5 h, and cephalixin content of aqueous media was measured at fixed time intervals. The results were correlated to common kinetics models namely, zero-order, first-order, second-order, Michaelis-Menten and logarithmic models. The linearized model coefficients and coefficient of determination (R^2) for each model were calculated. These results were shown in Table 3. Due to the highest obtained R^2 for zero order kinetics, it can be concluded that the cephalixin absorption by synthesized optimum composite hydrogels obeyed zero-order kinetic model. Thus, this model was chosen as the best model to describe absorption behavior of optimum hydrogel for cephalixin at its studied concentrations. In zero-order kinetics, the absorption only depends on time and is constant at various concentrations of active compound. The absorption occurs rapidly until the saturation concentration at equilibrium state reaches (Bhasarkar and Bal 2019).

The release kinetics of cephalixin from optimum hydrogels (into deionized water, pH = 7.0 ± 0.1) were also evaluated after completely saturation and removal of accumulated surface molecules of cephalixin. The cephalixin contents of deionized water were measured at certain time intervals. Similar to cephalixin absorption survey, the results were correlated to common kinetics models and the obtained linearized model coefficients and coefficient of determination (R^2) for each model were summarized in Table 4. From Table 4 and obtained R^2 values for each model, due to the greatest R^2 for Higuchi model, it can be concluded that the release of cephalixin from the synthesized hydrogels, follows this model. Based on Higuchi model, the release is considered as diffusion process based on Fick's law. The diffusion occurs at microscopic or molecular scale through the hydrogel networks and the diffusion rate decreases as the release process continues, since the drug should pass elongated path in order to reach the media and be released. The Higuchi model is valid for most of the water-soluble or water-insoluble drugs which are released from semi-solid matrices like hydrogels (Peppas and Narasimhan 2014).

Table 4

The kinetics of cephalaxin release from optimum composite hydrogels (the R^2 and main coefficients for correlated general release models)

Common release Models		Cephalaxin release	
Zero order	$C-C_0=Kt$	Equation	$C = 0.1009t + 0.0741$
	Linear	R^2	0.9514
First order	$\ln C - \ln C_0 = Kt$	Equation	$\ln C = 0.112t - 0.8406$
	1st order	R^2	0.7979
Higuchi Model	$C - C_0 = Kt^{0.5}$	Equation	$C = 0.3522t^{(0.5)} - 0.173$
		R^2	0.9977
hixson crowell	$C^{1/3} - C_0^{1/3} = Kt$	Equation	$C^{(1/3)} = 0.0533t + 0.5849$
		R^2	0.735
korsmeyer peppas model (power law model)	$\ln C - \ln C_0 = K \ln t$	Equation	$\ln C = 0.8183 \ln t + 1.8078$
	Logarithmic	R^2	0.9872

3.5.2. FT-IR and UV-Vis absorbance Spectrum of optimum hydrogels

The FT-IR spectra of the optimum sodium alginate/acrylic acid composite hydrogels conjugated to silver nanoparticles was shown in Fig. 5A. The absorption spectrum from 3950 to 3500 cm^{-1} was pointed out to stretching sodium alginate NH_2 . The peak at 3431 cm^{-1} was related to the hydroxyl group. The broad peak from 3350 to 2500 cm^{-1} indicated the presence of OH scratching of acrylic acid. The peaks at 1634 and 1433 cm^{-1} were appeared respectively due to symmetric carboxylate group and asymmetric sodium alginate polymer group. The low peak at 1500 cm^{-1} was assigned to stretching C-O. The peaks from 1650 to 1750 cm^{-1} were corresponded to stretching carbonyl group of acrylic acid. The broadband from 1300 to 1000 cm^{-1} was referred to the C-O group of alginate. The peak at 950 cm^{-1} was related to O-H bonding of hydrogel monomers.

Figure 5B shows the UV-Vis absorbance spectra of optimum composite hydrogels conjugated to silver nanoparticles. The observed peak at about 400 nm was related to the surface Plasmon resonance of silver nanoparticles. Thus, the formation of silver nanoparticles from silver ions inside the hydrogels matrices was confirmed (Singh and Dhaliwal 2020).

3.5.3. SEM images of optimum hydrogels

The surface morphology of optimum hydrogel was visualized by scanning electron microscopy images, which were taken in different scales, from $100\text{ }\mu\text{m}$ to 500 nm (Fig. 6). The hydrogels' SEM images obviously showed the relatively wide sized distribution of produced silver nanoparticle on the surface of the hydrogel. The presence of fine pores on hydrogels' surface, which can be seen in TEM images, made them efficient candidate for uptakind the various bioactive compounds. The energy dispersive x-ray spectrum of samples also confirmed the presence of Ag, Ca, Cl, Na, N, O and C on the surface of prepared hydrogels.

Conclusion

The physical microwave radiation and ion cross-linking process were simultaneously applied in order to synthesis the sodium alginate/acrylic acid composite hydrogels and, reduce the incorporated silver ions into stable silver nanoparticles, which were conjugated to hydrogel matrices. Response surface methodology was employed in order to experimental design, statistical analysis, developing the models and, optimizing the fabrication parameters of gained hydrogels. The hydrogels with the highest swelling ratio, gel fraction, cephalaxin absorption and, antibacterial activities were considered as the most desired product, and the fabrication parameters were optimized in order to achieve the best sodium alginate/acrylic acid composite hydrogels. The absorption and release kinetics of cephalaxin from the optimum product were also investigated. It was resulted that while the drug absorption obeys zero-order kinetic model, the release process could explained by Higuchi kinetic model. Due to high antibacterial activity of prepared composite hydrogels, which could be resulted from the synergistic effect of silver ions, acrylic acid monomers and cephalaxin, they can be successfully used in various medical and pharmaceutical applications such as wound healing lotions or wound dressing formulations.

Declarations

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Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Ethics approval

This article does not contain any studies with human participants or animals performed by any of the

Authors.

Conflict of Interest

The authors have no conflict of interest to declare.

References

1. Anarjan N, Nehdi IA, Tan CP (2013) Influence of astaxanthin, emulsifier and organic phase concentration on physicochemical properties of astaxanthin nanodispersions. *Chem Cent J* 7(1): 121, 2-11. [https://doi: 10.1186/1752-153X-7-127](https://doi.org/10.1186/1752-153X-7-127).
2. Bhasarkar J, Bal D (2019) Kinetic investigation of a controlled drug delivery system based on alginate scaffold with embedded voids. *J Appl Biomater* 17(2): PMID: 31230497. [https://doi: 10.1177/2280800018817462](https://doi.org/10.1177/2280800018817462).
3. Dannert C, Stokke BT, Dias RS (2019) Nanoparticle-Hydrogel Composites: From Molecular Interactions to Macroscopic Behavior. *Polymers*, 11(2): 275. [https://doi:10.3390/polym11020275](https://doi.org/10.3390/polym11020275).
4. Francis S, Kumar M, Varshney L (2004) Radiation synthesis of superabsorbent poly(acrylic acid)–carrageenan hydrogels. *Radiat Phys Chem* 69(6): 481-486. [https://doi: 10.1016/j.radphyschem.2003.09.004](https://doi.org/10.1016/j.radphyschem.2003.09.004)
5. Gaharwar A, Peppas N, Khademhosseini A (2014) Nanocomposite Hydrogels for Biomedical Applications. *Biotechnol Bioeng* 111(3): 441-453. [https://doi: 10.1002/bit.25160](https://doi.org/10.1002/bit.25160).
6. Ganji F, Vasheghani Farahani S, Vasheghani-Farahani E (2010) Theoretical Description of Hydrogel Swelling: A Review. *Iran Polym J* 19: 375-398.
7. Gratzl G, Paulik C, Hild S, Guggenbichler JP, Lackner M (2014) Antimicrobial activity of poly(acrylic acid) block copolymers. *Mater Sci Eng C* 38: 94-100. [https://doi: 10.1016/j.msec.2014.01.050](https://doi.org/10.1016/j.msec.2014.01.050).
8. Kondaveeti S, Bueno PVdA, Carmona-Ribeiro AM, Esposito F, Lincopan N, Sierakowski MR, Petri DFS (2018) Microbicidal gentamicin-alginate hydrogels. *Carbohydr Polym* 186: 159-167. [https://doi: 10.1016/j.carbpol.2018.01.044](https://doi.org/10.1016/j.carbpol.2018.01.044).
9. Kowalski G, Kijowska K, Witczak M, Kuterasiński Ł, Łukasiewicz M (2019) Synthesis and Effect of Structure on Swelling Properties of Hydrogels Based on High Methylated Pectin and Acrylic Polymers. *Polymers* 11(1): 114. [https://doi: 10.3390/polym11010114](https://doi.org/10.3390/polym11010114).
10. Kretschmann O, Schmitz S, Ritter H (2007) Microwave-Assisted synthesis of associative hydrogels. *Macromol Rapid Commun* 28:1265-1269. [https://doi: 10.1002/marc.200700117](https://doi.org/10.1002/marc.200700117).
11. Makhado E, Pandey S, Ramontja J (2018) Microwave assisted synthesis of xanthan gum-cl-poly (acrylic acid) based-reduced graphene oxide hydrogel composite for adsorption of methylene blue and methyl violet from aqueous solution. *Int J Biol Macromol* 119: 255-269. [https://doi: 10.1016/j.ijbiomac.2018.07.104](https://doi.org/10.1016/j.ijbiomac.2018.07.104).
12. Mori Z, Anarjan N (2018) Preparation and characterization of nanoemulsion based β -carotene hydrogels. *J food sci tech* 55: 5014-5024. [https://doi: 10.1007/s13197-018-3440-3](https://doi.org/10.1007/s13197-018-3440-3).
13. parhi R(2017) Cross-Linked Hydrogel for Pharmaceutical Applications: A Review. *Adv Pharm Bull* 7(4): 515-530. [https://doi: 10.15171/apb.2017.064](https://doi.org/10.15171/apb.2017.064).
14. Patra JK, Baek KH (2017) Antibacterial activity and synergistic antibacterial potential of biosynthesized silver nanoparticles against foodborne pathogenic bacteria along with its anticandidal and antioxidant effects. *Front Microbiol* 8:167. [https://doi: 10.3389/fmicb.2017.00167](https://doi.org/10.3389/fmicb.2017.00167).
15. Peppas NA, Narasimhan B (2014) Mathematical models in drug delivery: How modeling has shaped the way we design new drug delivery systems. *J Control Release* 190: 75-81. [https://doi: 10.1016/j.jconrel.2014.06.041](https://doi.org/10.1016/j.jconrel.2014.06.041).
16. Pourjavadi A, Barzegar S, Mahdavinia G (2006) MBA-crosslinked Na-Alg/CMC as a smart full-polysaccharide superabsorbent hydrogel. *Carbohydr Polym* 66: 386-395. [https://doi: 10.1016/j.carbpol.2006.03.013](https://doi.org/10.1016/j.carbpol.2006.03.013).
17. Quintanilla de Stéfano JC, Abundis-Correa V, Herrera-FloresSD, Alvarez AJ (2020) pH-Sensitive starch-based hydrogels: synthesis and effect of molecular components on drug release behavior. *Polymers* 12(9): 1974. [https://doi: 10.3390/polym12091974](https://doi.org/10.3390/polym12091974).
18. Serrano-Aroca Á, Deb S (2020) Acrylic-based hydrogels as advanced biomaterials, acrylate polymers for advanced applications. Retrieved from Intech Open. [https://doi: 10.5772/intechopen.92097](https://doi.org/10.5772/intechopen.92097).
19. Sharma AK, Kaith BS, Gupta B, Shanker U, Lochab SP (2019) Microwave assisted in situ synthesis of gum Salai guggal based silver nanocomposites- investigation of anti-bacterial properties. *Cellulose* 26: 991–1011. <https://doi.org/10.1007/s10570-018-2140-5>

20. Singh J, Dhaliwal AS (2020) Water retention and controlled release of KCl by using microwave-assisted green synthesis of xanthan gum-cl-poly (acrylic acid)/AgNPs hydrogel nanocomposite. Polym Bull 77(9): 4867-4893. [https://doi: 10.1007/s00289-019-02990-x](https://doi.org/10.1007/s00289-019-02990-x).
21. Tang S, Zeng J (2018) Antibacterial Activity of Silver Nanoparticles: Structural Effects. Adv Healthc Mater 7: 1701503. [https://doi:10.1002/adhm.201701503](https://doi.org/10.1002/adhm.201701503)
22. Wang T, Zhang F, Zhao R, Wang C, Hu K, Sun Y, Politis C, Shavandi A, Nie L(2020) Polyvinyl alcohol/sodium alginate hydrogels incorporated with silver nanoclusters via green tea extract for antibacterial applications. Des Monomers Polym 23(1): 118-133. [https://doi: 10.1080/15685551.2020.1804183](https://doi.org/10.1080/15685551.2020.1804183).
23. Xia B, Cui Q, He F, Li L(2012) Preparation of hybrid hydrogel containing ag nanoparticles by a green in situ reduction method. Langmuir 28(30): 11188-11194. [https://doi: 10.1021/la302011x](https://doi.org/10.1021/la302011x).
24. Yang K, Han Q, Chen B, Zheng Y, Zhang K, Li Q, Wang J (2018) Antimicrobial hydrogels: Promising materials for medical application. Int J Nanomed 13: 2217-2263. [https://doi: 10.2147/IJN.S154748](https://doi.org/10.2147/IJN.S154748).
25. Zhang Y, Xu Q, Fu F, Lio XD (2016) Durable antimicrobial cotton textiles modified with inorganic nanoparticles. Cellulose 23: 2791–2808. <https://doi.org/10.1007/s10570-016-1012-0>

Figures

Figure 1

The effects of acrylate acid, silver nitrate and microwave time on swelling ratio of sodium alginate/acrylic acid composite hydrogels conjugated to silver nanoparticles

Figure 2

The effects of acrylate acid, silver nitrate and microwave time on gel fraction of sodium alginate/acrylic acid composite hydrogels conjugated to silver nanoparticles

Figure 3

The effects of acrylate acid, silver nitrate and microwave time on cefalexin load of sodium alginate/acrylic acid composite hydrogels conjugated to silver nanoparticles

Figure 4

The effects of acrylate acid, silver nitrate and microwave time on growth inhibition of sodium alginate/acrylic acid composite hydrogels conjugated to silver nanoparticles

Figure 5

FT-IR (A), UV-Vis absorbance spectra of the optimum sodium alginate/acrylic acid composite hydrogels conjugated to silver nanoparticles

Figure 6

FE-SEM images of the optimum sodium alginate/acrylic acid composite hydrogels conjugated to silver nanoparticles