

Evaluation of controlled hydroxychloroquine releasing performance from calcium-alginate beads

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ABSTRACT

This study aimed to develop an effective controlled drug delivery system based on alginate beads to treat autoimmune diseases such as Rheumatoid Arthritis (RA) and Systemic Lupus Erythematosus (SLE). The present study describes the drug delivery systems to control the practical uses of hydroxychloroquine (HCQ) by Ca-alginate beads. The characterization techniques were employed to evaluate the physicochemical properties as scanning electron microscopy (SEM), swelling test (S), hydrolytic degradation (weight loss, WL), and Fourier transform infrared-attenuated total reflection (FTIR-ATR). The release studies from alginate beads prepared in various drug doses were carried out in the aqueous solutions at different pH (5–8) and temperatures (4–37°C). The approximately half-amount of HCQ in HCQ-AB3 was released in 12 h, and about 84.38% was released within 8 days. Korsmeyer-Peppas was applied to model the HCQ release kinetic of alginate beads, which corresponded to the non-Fickian transport mechanism.

Keywords:

Calcium-alginate beads; hydroxychloroquine; drug delivery; RA; SLE

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INTRODUCTION

Alginate is a well-known and most favored biomaterial found in many applications such as the food sector, textile industry, waste removal, and pharmaceutical industries [1-4]. Alginate is a polysaccharide composed of D-mannuronic acid and L-guluronic acid monomers [5,6]. One of the essential properties of alginate is its ability to react with divalent cations and form insoluble crosslinked gels due to the carboxyl groups in the structure. Still, the standard method has been calcium ion gelation to obtain alginate beads [7,8]. Calcium alginate is the most commonly employed system for its easiness of gel formation. Once liquid alginate solutions are contacted with polycation (Ca^{2+}), they are immediately transformed into gel binding between guluronic acid blocks in alginate and Ca^{2+} . Ca-Alginate beads are widely used in drug delivery due to their favorable properties such as non-toxic, water-soluble, film-forming, biocompatibility, ease of gelation, and biodegradable [9-11].

Controlled drug release is the technique that provides releasing of a drug at the therapeutic dose to the desired site in the body [12,13]. The importance of intelligent drug delivery systems (DDSs) in biomedical and pharmacological fields is growing daily. The main goal of DDSs is to provide drug release for long periods avoiding the side effects of the drugs, that increase the

efficacy and safety of drug. The delivery system will also be biocompatible or biodegradable so that it is transformed into non-toxic parts that are eliminated harmlessly from the body [14,15].

Chloroquine (CQ) and its derivate hydroxychloroquine (HCQ) are classified as anti-malarial agents [16]. Although, during the Second World War, the use of anti-malarial as prophylaxis by millions of soldiers, these drugs have been shown for the therapeutic effects in numerous other autoimmune diseases such as Rheumatoid Arthritis (RA) and Systemic Lupus Erythematosus (SLE) [17,18]. HCQ is widely used as it effectively controls dermatological complications in SLE, an autoimmune disease. However, it has been reported to effectively manage the symptoms of Sjögren syndrome, a chronic autoimmune disease, and prevent thrombosis in phospholipid antibody (aPL) syndrome. It has been observed that HCQ also reduces the risks of atherosclerosis and cardiovascular disease in RA patients [19]. In RA and SLE, HCQ is preferred over CQ owing to the lower incidence of gastrointestinal adverse reactions and its safer dose-dependent toxicity profile [19-21]. HCQ has known controlling inflammatory processes and immunomodulatory effects. In addition, HCQ has a modulating effect on activated immune cells [17,22]. HCQ has also been widely used worldwide as a candida-

te drug against SARS-CoV-2 infection to treat patients with COVID-19 due to its antiviral effects [23]. This has led to drug shortages and access problems for patients with SLE in many countries and rising anxiety, concerns in patients with SLE [24].

This study is focused on the treatment strategy to a controlled release of HCQ as a model therapeutics. If HCQ is taken in proper doses, it is a safe drug, although its safety margin is narrow, and a single higher dose might be fatal [17]. Therefore, there is a need for randomized, controlled trials with this drug to prove its efficiency and safety for SLE and RA patients due to the potential for drug-induced toxicity.

This study aimed to develop a controlled drug release system for HCQ, selected as a therapeutic model. At the same time, alginate-based beads were chosen as a carrier system. First, alginate was crosslinked with the presence of calcium ions by a peristaltic pump at room temperature for 1 h. Then, obtained alginate beads were characterized by scanning electron microscopy (SEM), swelling test (S), hydrolytic degradation (weight loss, WL) and Fourier transform infrared-attenuated total reflection (FTIR-ATR). The drug delivery experiments were studied to assure safety and appropriate dose use to include well-designed clinical trials in treating SLE and RA disease.

MATERIAL AND METHOD

Materials

Alginic acid sodium salt with medium viscosity (from brown algae) and hydroxychloroquine sulfate were purchased from Sigma-Aldrich (St. Louis, MO, USA). Calcium chloride dihydrate was obtained from Merck (Darmstadt, Germany). All other reagents were analytical grade and obtained from Merck.

Preparation of HCQ loaded alginate beads

Sodium alginate (1%, w/v) was dissolved in deionized water. The alginate solution was stirred to obtain a homogeneous solution without any bubbles at room temperature. Then, the homogeneous mixture was added dropwise into CaCl_2 solution (5%, w/v) via peristaltic pump (1 mL/min, flowrate). The obtained spherical alginate beads were kept in the crosslinking solution for 20 min. After filtering the beads, they were transferred into pure ethanol for post-crosslinking/gelation purposes for 20 min and subsequently washed with deionized water. Finally, spherical alginate beads were placed on petri dishes and dried under two different conditions as room temperature and lyophilization. Drug-loaded alginate beads were prepared via the approach mentioned above with HCQ

in feeding solution in different amounts of 0.05, 0.1, and 0.2 g. According to the three different drug content, the alginate beads were labeled as HCQ-AB1, HCQ-AB2 and HCQ-AB3, respectively.

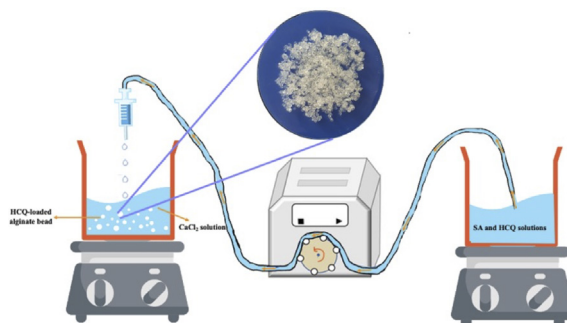


Figure 1. Schematic representation for the preparation of HCQ loaded alginate beads.

Characterization of Alginate Beads

The prepared alginate beads were characterized by the following techniques. The equilibrium swelling degrees of both freeze-dried and air-dried alginate beads were determined by immersing the dried beads (30 mg) into PBS (pH 7.4, 10 mL). The swollen beads were removed from the buffer solution, quickly wiped out with a filter paper, and reweighed (W_s) at defined time intervals. The beads weights (dried and wet) were recorded to calculate the percentage swelling rate of beads by the below equation:

$$S\% = [W_s - W_0] \times 100 \quad (1)$$

Herein, W_0 and W_s designated the weights (g) of beads before and after swelling, respectively.

The hydrolytic degradation behavior was also characterized by investigating the weight loss of freeze-dried alginate beads. The initially beads were immersed in PBS (pH 7.4, 10 mL) and incubated by using a temperature-controlled shaking-bath at 37°C at 100 rpm for 8 days. The alginate beads were removed from the PBS and weighted at different intervals. After alginate beads were dried, the hydrolytic degradation behavior was examined. The hydrolytic degradation rate of the developed alginate beads was determined as the percentage of weight loss (WL) by the following equation:

$$WL\% = [(W_0 - W_t) / W_0] \times 100 \quad (2)$$

where W_0 is the weight of the initial dry sample and W_t is the weight of the dry sample for a given time t .

To assess the morphology of dried beads, SEM images were obtained with a GAIA3 (Tescan, Czech Republic) utilized from Hacettepe University, Advanced Technologies

Application and Research Center (HUNITEK, Ankara, Turkey). The dried beads were coated with gold and scanned by SEM with different magnifications at a high vacuum. In addition, FTIR-ATR was conducted to characterize the functional groups of alginate beads before and after the degradation process. The FTIR-ATR (Perkin Elmer, Spectrum Two™) spectrum was recorded over the wavenumber range of 4000-400 cm⁻¹.

HCQ encapsulation efficiency

HCQ encapsulation efficiency was investigated by dividing the trapped HCQ amount in the alginate beads by the total amount of HCQ in the alginate solution. The residual HCQ in the CaCl₂ gelling and washing solutions was collected and measured by UV/Vis spectrophotometer at 342 nm. HCQ encapsulation efficiency of alginate beads was calculated using the following equation:

$$\text{Encapsulation Efficiency (EE)} = \frac{\text{total HCQ} - \text{residual HCQ}}{\text{total HCQ}} \quad (3)$$

In vitro HCQ release studies

The drug-loaded alginate beads with a different weight (20-50 mg) were placed into a glass bottle containing 4 mL buffer solution in the pH range of 5.0-8.0 and at different temperatures (4-37°C). The drug release studies were performed in an incubator shaker at 100 rpm (JSSB-30T, JSR, Gongju, Korea). At the defined time intervals, 0.4 mL of the buffer solution was taken out and replaced with fresh buffer solution. The released HCQ from the alginate beads was determined at 342 nm using a UV/Vis spectrophotometer (Shimadzu, UV-1280, Tokyo, Japan). For statistical purposes, all the drug release procedures were performed in three replicates to calculate the standard deviation.

RESULTS AND DISCUSSION

Characterization studies

Figure 2 shows the swelling ratios of both the freeze-dried and air-dried HCQ-loaded alginate beads in buffer solution at pH 7.4. As shown in the figure, the freeze-dried alginate beads performed higher swelling degrees than the air-dried alginate beads in the equilibrium. The freeze-dried alginate beads showed a moderate swelling degree of 3088% at 6 days, while air-dried alginate beads reached 1142%. These results clearly showed that freeze-dried samples have a higher porosity structure than air-dried alginate beads. In other words, the air-dried alginate beads had a more compact and dense structure than freeze-dried ones. This may be due to the high speed of solvent removal at a high vacuum in the

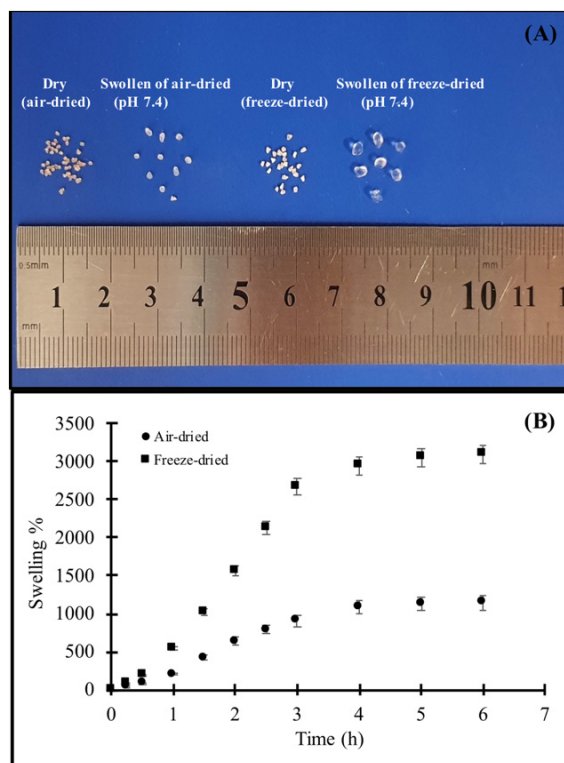


Figure 2. Optical images of the prepared dried HCQ-loaded alginate beads (air-dried and freeze-dried) and swollen sizes of air-dried and freeze-dried HCQ-loaded alginate beads in pH 7.4 (A) and swelling degrees of both the freeze-dried and air-dried HCQ-loaded alginate beads in pH 7.4 at 37 °C (B).

freeze-drying method. In the air-drying method, solvent removal occurs slowly, leading to a less porous structure of the alginate beads [25].

To measure the hydrolytic degradation behavior of the freeze-dried HCQ-loaded alginate beads, they were placed in PBS (pH 7.4) at 37 °C for 8 days (Figure 3). The weight loss of alginate beads gradually increased concerning the time as 27.6% after 8 days at pH 7.4 because of the hydrolytic degradation. Thus, it was shown that the freeze-dried HCQ-loaded alginate beads were exhibited a long-term degradation behavior with good stability that makes the prepared alginate beads a good alternative for DDSs.

SEM was used to characterize the morphologies of air and freeze-dried HCQ-loaded alginate beads. The drying method had a significant effect on the beads' regularity and surface morphology. As shown in Figure 4, the air-dried beads (4A) appear more compact, dense, and smoother than freeze-dried beads (4B). Freeze-dried beads had relatively larger sizes and highly porous structures in comparison with air-dried beads due to the lyophilization procedure. The enclosed water in the beads was quickly removed without major deterioration of the pores during lyophilization. The figure shows that there were relatively rough surfaces and many cavities in the freeze-dried beads integral regions

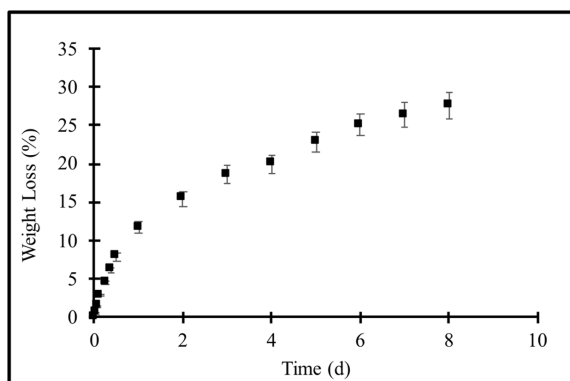


Figure 3. Hydrolytic degradation of HCQ-loaded alginate beads in pH 7.4 at 37 °C.

(4D). The porous structure of the freeze-dried beads is suitable for drug delivery due to provide easy penetration of buffer solution into the structure [25,26].

The morphological changes of degraded alginate beads after incubated for 8 days in pH 7.4 at 37°C were also investigated by SEM. There was not any agglomeration in the structure of alginate beads before incubation in pH 7.4 (Figure 5B). The changes of alginate beads structure were observed as soon as incubation was started with the pH 7.4. The morphological changes were observed at alginate beads after 6 h (Figure 5C), 24 h (D) and, 8 days (E, F). As the incubation continued, the rate of degradation was increased, and more conglutination, shrinkages, and the mass loss of the structure of alginate beads were seen (Figure 5E-F). The weight loss of HCQ loaded alginate beads reached 27.6% at 8 days. The SEM images of degraded alginate beads after incubation were evidence of the weight loss results of alginate beads.

Functional groups of HCQ, alginate beads, and HCQ loaded alginate beads were determined using FTIR-ATR spectrometer (Figure 6). The main characteristics bands for HCQ (Figure 6A), -OH stretching peaks were observed at 3200 cm^{-1} and NH deformations bands at approximately 3300 cm^{-1} . Moreover, the aromatic C=C stretching bands at 1450 and 1610 cm^{-1} and C-N bands at around 1100 cm^{-1} occurred in the spectrum. Similarly, the C-Cl stretching peak is located between 550 and 850 cm^{-1} . The characteristic peaks of HCQ disappear in the HCQ-loaded beads because of the overlap of bands in this region comprising the deformation of -OH, -NH, and -CH. Moreover, there is a decrease in the intensity of 1100 cm^{-1} and 1450 cm^{-1} because of the strong bond stretches of the alginate chain covering the HCQ drug [27]. On the other hand, the spectrum of HCQ loaded alginate beads almost matched up with the spectrum of alginate beads. Only one common peak was detected in HCQ and HCQ-loaded beads different from alginate beads at 1280 cm^{-1} , which was exhibited the presence of the C-O stretch. In addition, when the FTIR-ATR

spectra of alginate beads were investigated before and after the degradation process (6h, 24h, and 8 days), characteristic peaks intensity was decreased around 1600, 1418, and 1030 cm^{-1} , indicating the asymmetric stretching vibration and symmetric stretching vibration of the -COO- group and, C-O-C stretching vibrations, respectively (corresponding figures were also given in Supplementary File, Figure SI-1).

Encapsulation of Hydroxychloroquine by alginate beads

Encapsulation efficiencies of HCQ loaded alginate beads were calculated by considering the crosslinking and washing processes. The encapsulation efficiencies of HCQ-AB1, HCQ-AB2, and HCQ-AB3 were calculated as 43.98%, 55.77%, 81.22%, respectively. In addition, it was observed an increase in EE with increasing the concentration of HCQ in the alginate beads. In the light of these results, HCQ-AB3 labeled beads was used in all release studies.

HCQ release studies

For the optimization studies, the effects of medium pH, drug content, beads amount, and temperature were investigated on the alginate beads. The effect of medium pH on HCQ delivery was performed in the appropriate ranges of pH 5.0-8.0, as shown in Figure 7. The release profiles of HCQ from alginate beads exhibited a biphasic delivery system. It was observed to release HCQ at different rates, initial burst release step within 12 hours followed by a constant rate (ideally) and sustained release. As observed in Figure 7., the medium pH significantly affected the HCQ release properties. As seen from Figure 7A, the amount of HCQ released at pH 5.0 was relatively low and only about 9.5%, while this value was 24.5% for pH 7.4 within 60 min. The low drug release ratio is due to the shrinkage and less swelling of the alginate beads in acidic buffer [1]. HCQ release amounts were 14.48%, 17.89%, 23.89%, 42.36%, and 30.92% in 12 h at pH values of 5.0, 6.0, 7.0, 7.4 and 8.0, respectively. After the immediate releasing phase, the cumulative release was 84.38% at pH 7.4 after 192 h.

The cumulative release of HCQ from alginate beads at different amounts of HCQ loadings is seen in Figure 8. It was seen that the cumulative release of HCQ was increased from 38.76 to 84.38% with the increasing amount of HCQ from 50 to 200 mg. Moreover, the release profile of drugs from HCQ-AB3 realized fast and higher release rates about 2.5-fold than HCQ-AB1 within 12 h. The higher drug amount in the alginate beads resulted in the faster diffusion rate of HCQ from the alginate beads, which cause a higher release rate of HCQ, especially in the early stage of the release

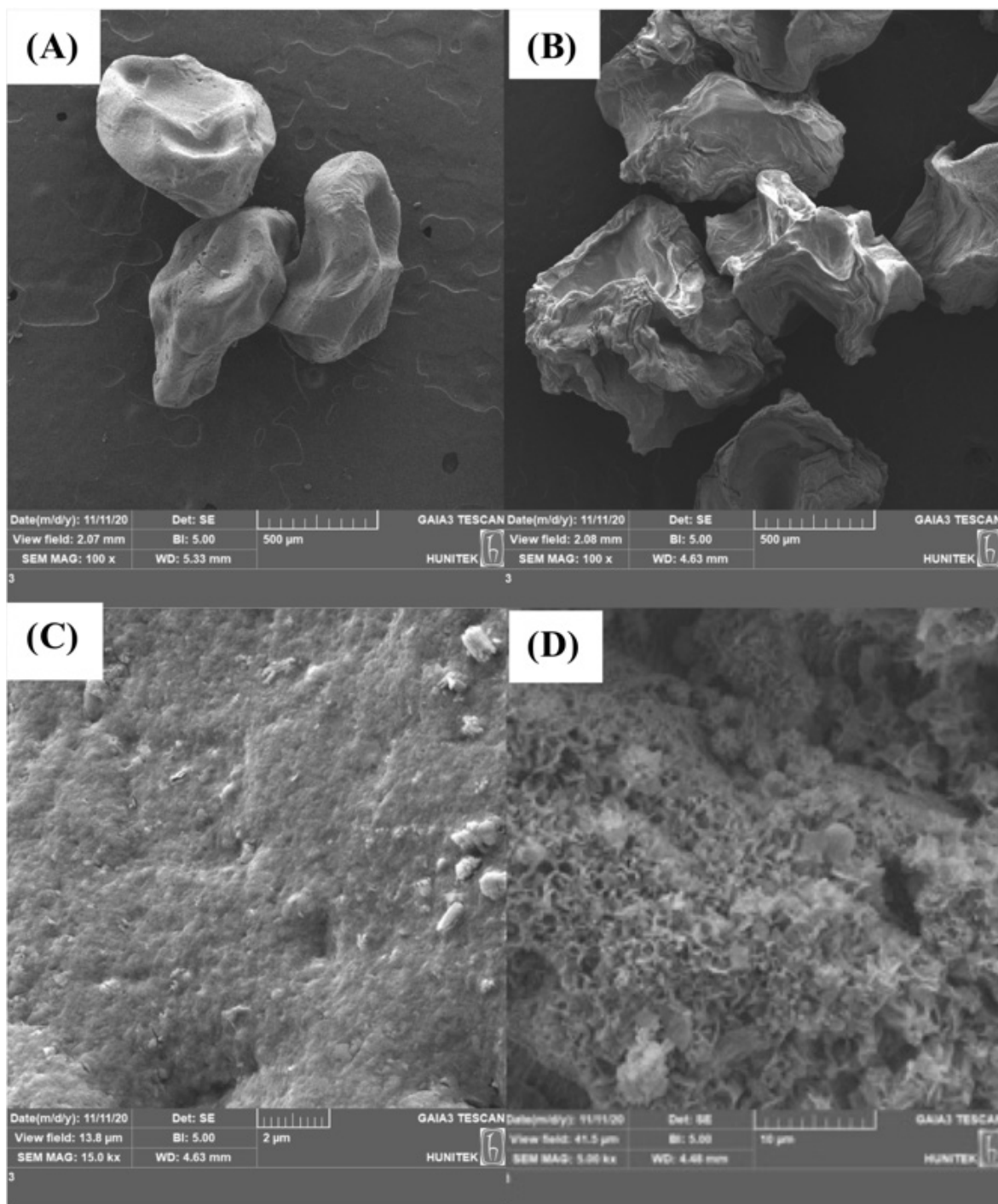


Figure 4. SEM images of air-dried (A, C) and freeze-dried HCQ-loaded alginate beads (B, D).

process [28]. Another reason for the increase in the amount of released drugs is that the drug delivery systems are swelling controlled. Therefore, when the alginate begins to absorb water, it causes a more significant amount of drug diffuses in the mass of the swelling bath [29].

The effect of temperature on the HCQ release profile was carried out at 4, 25, and 37°C, and HCQ release data was presented in Figure 9. The HCQ release was 84.38% af-

ter 8 days at 37°C, whereas the value was less than 32.52% at 4°C with the same treatment. The increasing temperature promotes the penetration of buffer solution into the beads, which enhances swelling of alginate sites in beads and causes the squeezing out of the drug [30].

The effect of HCQ-AB3 bead amounts on the cumulative release was determined by using various bead amounts (20-50 mg beads). As seen from Figure 10, the maximum

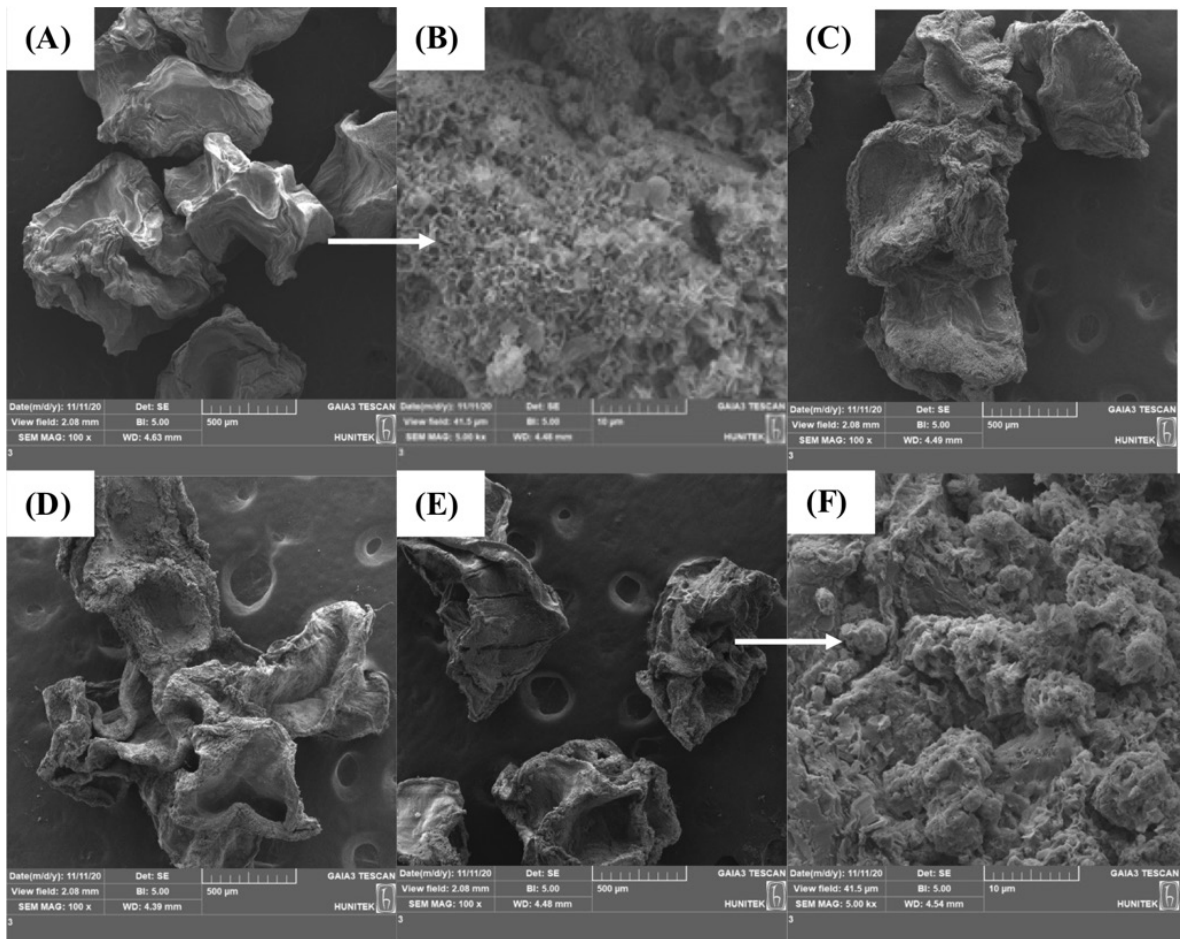


Figure 5. SEM images of freeze-dried HCQ loaded alginate beads before and after incubation at 37°C in pH 7.4. Freeze-dried HCQ loaded alginate beads (A, B), after 6 hours (C), after 24 hours (D), after 8 days (E, F).

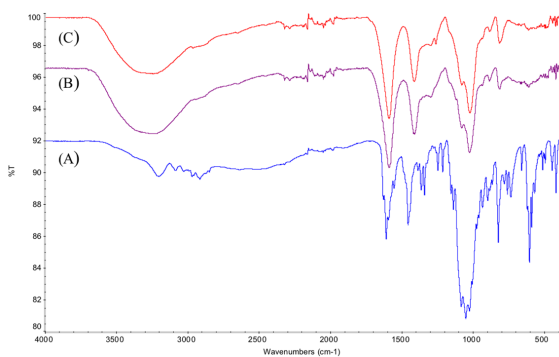


Figure 6. FTIR-ATR spectra of HCQ (A), alginate beads (B), and HCQ loaded alginate beads (C).

cumulative release was realized with 30 mg of HCQ-AB3 alginate beads both the first 12 h and 8 days. The lower cumulative releases of HCQ were obtained at high bead amounts. The amount of the beads and the dose of the drug in the beads are significant to show the effect of the released active substance. Undesirable effects may occur when the dose rate falls below the sufficient amount or rises above the toxic level. The result figured out that the amount of beads to be used is significant for controlling the release performances.

Release kinetic studies of HCQ

To understand the release profile of HCQ from HCQ-Alginate beads, the release data were analyzed using a classic empirical equation:

$$\frac{M_t}{M_\infty} = kt^n \quad (4)$$

where M_t means the amount of drug release at time t , M_∞ describes the amount of drug release at an infinite time, k is the release kinetic constant at the Korsmeyer-Peppas model. The release exponent is n which is related to the geometry of the release system and indicates the release mechanism. The Korsmeyer-Peppas equation can adequately describe the release of drugs from spheres [31,32]. When the value of 0.43 or less than 0.43, the release mechanism is based on diffusion (Fickian diffusion or quasi-Fickian diffusion). If the value of between 0.43 and 0.85, the release mechanism is based on an anomalous diffusion or non-Fickian diffusion (i.e., a mixed diffusion, polymer relaxation). Moreover, the value above 0.85, the release mechanism is based on a Case-

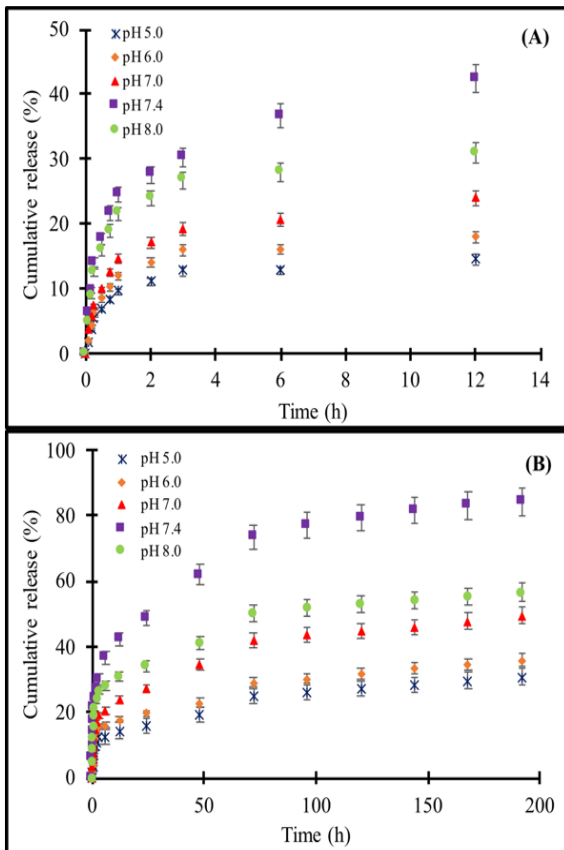


Figure 7. SEM images of freeze-dried HCQ loaded alginate beads before and after incubation at 37°C in pH 7.4. Freeze-dried HCQ loaded alginate beads (A, B), after 6 hours (C), after 24 hours (D), after 8 days (E, F).

II transport related to polymer relaxation on the drug molecules movement during gel swelling [33,34]. The correlation coefficient (R^2) was used to evaluate the fitness of the model.

Table 1 presented the release exponent (n) values and correlation coefficient (R^2) for HCQ-alginate beads. The exponent values ($0.43 < n < 0.85$) demonstrated that the drug release mechanism was anomalous diffusion for each bead. According to the Korsmeyer-Peppas model, the drug release mechanism of HCQ-Alginate beads observed both diffusion and swelling controlled drug release (anomalous diffusion) [33,35]. In general, the release kinetics of alginate beads fitted well with the Korsmeyer-Peppas model ($R^2 > 0.97$).

CONCLUSIONS

In this study, HCQ was encapsulated in alginate beads to use a controlled drug release system. The obtained data demonstrated a significant effect of the drying method on the characteristics of the prepared alginate beads. The highest HCQ encapsulation efficiency (84.38%) was obtained with the HCQ-AB3 formulation, which exhibited biphasic delivery systems at different rates to assure appropriate dose use and safety. Alginate beads loaded with HCQ showed drug release for 8 days with a moderate

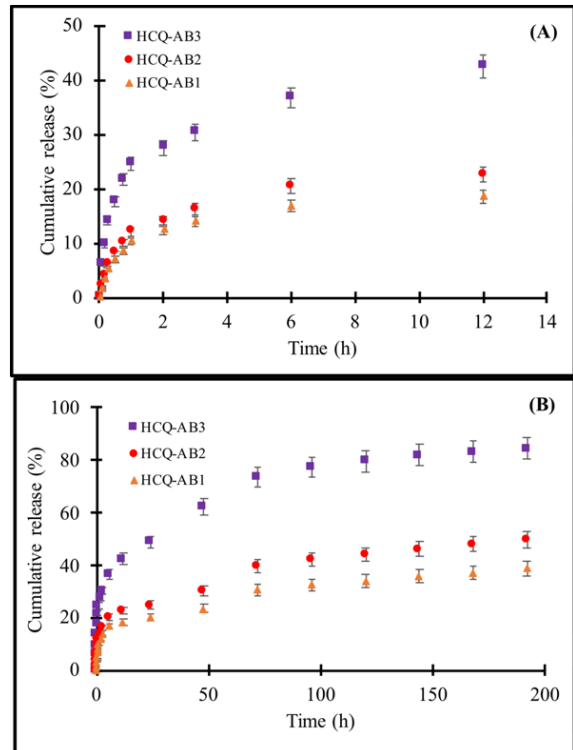


Figure 8. Cumulative release of HCQ from alginate beads containing different drug amounts at pH 7.4 at 37°C. HCQ release (A) for 12 h and (B) for 8 days.

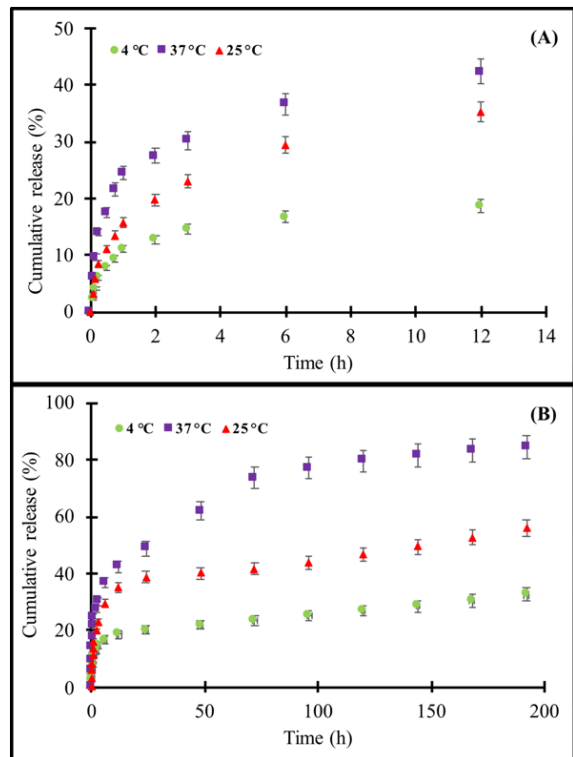


Figure 9. Cumulative release of HCQ from HCQ-AB3 at a different temperature at pH 7.4. HCQ release (A) for 12 h and (B) for 8 days.

burst release of 42.36% within 12 h. Korsmeyer-Peppas model showed that the drug release mechanism was ano-

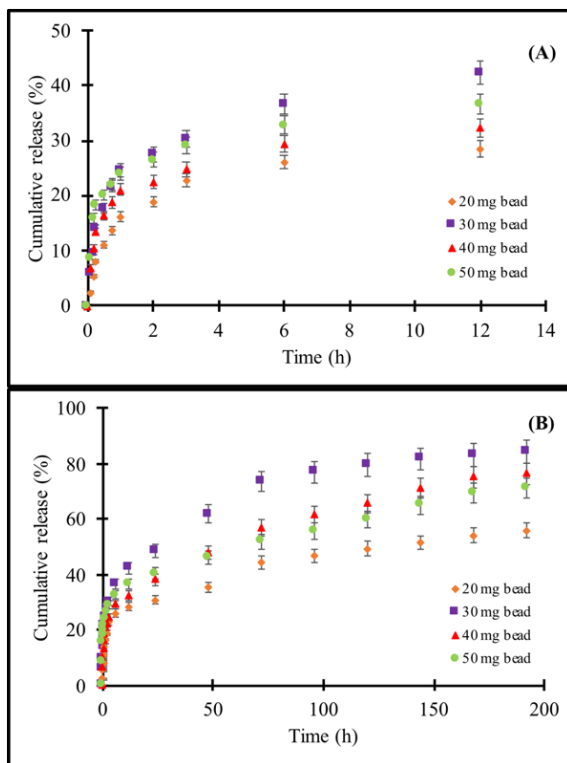


Figure 10. Cumulative release of HCQ from HCQ-AB3 at various bead amounts at pH 7.4 at 37 °C. HCQ release (A) for 12 h and (B) for 8 days.

Table 1. Values of the release exponent (n) and correlation coefficients (R^2) for HCQ-Alginate beads at pH 7.4 at 37 °C.

Polymer code	n	R^2
HCQ-AB1	0.7332	0.9774
HCQ-AB2	0.6936	0.9897
HCQ-AB3	0.5587	0.9969

malous diffusion, i.e., HCQ could release at pH 7.4 in a controlled manner.

CONFLICT OF INTEREST

Authors approve that to the best of their knowledge, there is not any conflict of interest or common interest with an institution/organization or a person that may affect the review process of the paper.

AUTHOR CONTRIBUTION

Sena Piskin: Writing - original draft, Visualization, Investigation. Canan Armutcu: Supervision, Conceptualization, Writing - review & editing.

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