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Research Article

Preparation of sustained release naproxen sodium loaded microcapsules from alginate and chitosan through experimental design

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ABSTRACT

Naproxen sodium is used as an alternative therapy for arthritis. Though it is administered intra-articular to increase drug concentration in the joint and maximize local effects, naproxen tends to diffuse quickly into the bloodstream. Thus, developing a sustained-release drug delivery system for this application is essential. The study aimed to optimize the parameters to produce microcapsules structured by naproxen encapsulated in an alginate core and coated with a chitosan outer shell. The achieved microcapsules should have the desired size, encapsulated efficiency, and drug release profile in a sustained manner. The cores were prepared using the emulsification method. The preparation was optimized using response surface methodology (Design Expert software v13.0). Independent parameters include alginate concentration, calcium chloride concentration, stabilizer concentration, and stirring speed. The microcapsules were produced by immersing the formerly optimized cores into chitosan solutions. The chitosan concentration, stirring conditions, and immersion duration were investigated. The cores and the microcapsules were evaluated in terms of mean size, encapsulation efficiency, and *in vitro* release. The investigated parameters had simultaneous effects on the alginate core properties. It is determined that the preparation using sodium alginate 4%; calcium chloride 10%, polysorbate 10%, span 10%, and stirring speed at 7200 rpm resulted in optimum core with mean size 6.01 ± 0.51 µm, encapsulation efficiency at 18.03 ± 0.97%. As the cores were immersed into chitosan 1.0% solution at pH 5.0, stirring at 1500 rpm for 30 mins, an outer shell of chitosan would be formed, yielding the microcapsules with a mean size of 7.50 ± 0.16 µm, encapsulation efficiency 14.7 ± 0.52%, and a complete drug release following sustained manner in 24 hours. The optimized conditions for preparing microcapsules containing naproxen sodium from alginate and chitosan were identified. The results of this study can contribute to developing the micro-size drug delivery system.

Keywords:
naproxen sodium, microcapsules, alginate, chitosan, response surface methodology

1. INTRODUCTION

Naproxen sodium, a widely used nonsteroidal anti-inflammatory drug (NSAID)[1], offers therapeutic benefits for various inflammatory conditions, including osteoarthritis and arthritis. Common therapies involve oral, topical application, and intra-articular injections. However, prolonged oral NSAID use is associated with systemic side effects, and repeated intra-articular corticosteroid injections may lead to cartilage degeneration and limited treatment duration. Intra-articular NSAID injection may serve as an alternative therapy by increasing NSAID concentration in the joint, maximizing local effects, and minimizing systemic side effects.

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Unfortunately, the NSAIDs drugs quickly diffuse from the joint cavity into the bloodstream, which makes rapid clearance from the site of administration often necessitates frequent dosing, leading to potential side effects and reduced patient compliance[2]. This necessitates research into developing formulations capable of prolonging the residence time of NSAID active ingredients in the joint for practical therapeutic applications.

Developing drug delivery systems capable of providing controlled release of therapeutic agents has garnered significant interest in the pharmaceutical field. Polymeric microcapsules containing anti-inflammatory agents have garnered attention from researchers in recent decades due to their small size for injection/implantation into joints, local depot formation, and ability to control drug release over an extended period to reduce patient injections. A major challenge in this therapeutic system is the biocompatibility and biodegradability of the polymer components[3]. This requirement limits the applicability of polymers, primarily to groups such as poly (lactic-co-glycolic acid), polycaprolactone, alginate, and chitosan. Among these, sodium alginate (a polysaccharide found in brown algae) is a biodegradable and biocompatible polymer of natural origin, thus receiving more attention for various pharmaceutical applications, particularly in microsphere formulation[4]. The main limitation of alginate microspheres has been demonstrated in their rapid drug release kinetics, which can only be controlled for about 4-6 hours. To overcome this drawback, several studies have shown that coating alginate microspheres (or alginate cores) with chitosan outer shell, a natural polysaccharide, offers the potential to enhance the controlled release properties of these microspheres through surface coating[5-8]. Additionally, alginate-chitosan complex has been demonstrated to have higher stability and improved permeability compared to alginate microspheres containing the corresponding active ingredient[9,10].

Several techniques can be used to create microcapsules from alginate and chitosan[11,12]. In this study, the two-stage procedure was used. The first stage involved preparing alginate cores that were then transferred into a chitosan solution. The polyelectrolyte complex membrane was formed on the surface of the cores[13-15].

In this regard, this study aimed to optimize the preparation of sodium naproxen-loaded microcapsules using water-in-oil (W/O) emulsification. Response surface methodology in combination with I-optimal design was assessed to identify the effects of five independent variables (sodium alginate, calcium chloride, polysorbate 80, span 80, and speed of stirring) on particle size, encapsulation efficiency, and the *in vitro* release of the drug. By systematically exploring the influence of formulation and process parameters on microcapsules properties, this research seeks to advance our understanding of controlled drug delivery systems and contribute to developing more effective therapies for inflammatory conditions.

2. MATERIALS AND METHODS

2.1. Materials

Sodium alginate with medium viscosity (300-400 cps for 1% solution) was purchased from TCI (Japan), medium-molecular weight chitosan (deacetylation degree >90%) was bought from Bio Basic (Canada), sodium naproxen was purchased from Sigma (USA). Calcium chloride, polysorbate 80, span 80, isooctane, acetone, and acetic acid were provided by Xilong (China).

2.2. The preparation of chitosan-coated alginate microcapsules loading sodium naproxen

Microcapsules were synthesized by the two-stage method. In the first stage, alginate cores were prepared using the W/O emulsion technique, followed by external gelation. Alginate was stirred in distilled water to form a solution at a determined concentration. Sodium naproxen was added to the alginate solution at a determined concentration (followed the experiments in table 1) and dissolved completely. Afterward, the alginate solution was added drop wise into the isooctane solution containing span 80 (as a stabilizer) with the volume between water phase and oil phase at ratio 32.5:67.5 (v/v) using a homogenizer (IKA T25-Digital Ultra Turrax, Staufen, Germany) at a determined speed for 5 min to form water-in-oil emulsion. Then, polysorbate solutions were added to the W/O emulsion and continued homogeneous for 3 min. Alginate cores were formed by adding calcium chloride gently to the emulsion under homogeneous for 15 min and then centrifuged at 10000 rpm for 5 min, followed by vacuum filtration using a 0.45 µm filter membrane. The obtained alginate cores were washed triple with acetone and dried at room temperature. In the second stage, the optimal alginate cores were immersed in chitosan solution at determined concentrations containing acetic acid with identified pH under stirring for a determined time. The microcapsules were recovered by centrifugation at 10000 rpm and washed with distilled water three times and with acetone once, followed by vacuum filtration using a 0.45 µm filter membrane. Finally, the microcapsules were stored at room temperature in the glass vials.

2.3. Optimization of alginate microcapsule cores

The Design-Expert software v.13.0 (Stat-Ease, USA) was used with the response surface methodology

study and I-optimal design, including 24 experiments. Five factors were chosen from our screening studied on the influenced parameters of microcapsule preparation process, including (X_1) alginate concentration with the varied range from 2-4% (w/w), (X_2) stirring speed (6000-8000 rpm), (X_3) $CaCl_2$ concentration (5-10%, w/w), (X_4) polysorbate ratio (5-10%, w/w), and (X_5) span ratio (5-10%, w/w). Then, five responses were chosen to determine the relationship with independent factors such as (Y_1) particle mean size (µm), (Y_2) encapsulation efficiency (%), (Y_3), (Y_4), and (Y_5) drug release at 1, 2, and 4 h (%), respectively. Multiple regression models (e.g., Square Root, Natural Log, Inverse Square Root, Inverse, and Power) were used to correlate factors and response interaction and predict the optimal formulation. All analyzed data were shown as mean. One-way analysis of variance (ANOVA) and t-test were used to test the statistical significance, with the significance determined at a level of p=0.05.

2.4. Identify impacts of the process parameters of chitosan-coated alginate microcapsules

The chitosan-coated alginate microcapsules were prepared based on the polyelectrolyte complex formulation. In this study, the polyelectrolyte complex layer was formed on the surface of the alginate cores. According to previous reports, the properties of obtained microcapsules is influenced by different process parameters and chitosan characteristics[13-15]. This study focused on parameters such as the pH of the polymer solutions, chitosan concentration, stirring speed, and coating time and their impacts on microcapsule characteristics.

2.5. Particle size analysis

The mean particle size and the size distribution were evaluated by dynamic light scattering using the Mastersizer MS 3000 (Malvern Instruments, UK). The Dv_{50} value would be determined based on the particle size at which 50% of the cumulative mass is below that value, each sample was diluted with filtered purified water and measured five times (n=5) at 25 ^oC. Results were expressed in terms of mean diameter and Dv_{50}.

2.6. Encapsulation efficiency (EE%)

Accurately weighted amounts (20.0 mg) of alginate cores were dispersed in 100 mL phosphate buffer (PBS pH 7.4) and sonicated in an ultrasonic bath for 30 min. Then, the suspension was centrifuged at 9000 rpm for 5 min. The supernatant was collected and filtered with a 0.45 µm millipore syringe filter. The content of sodium naproxen was determined by the absorbance at 272 nm using a UV-Vis Shimadzu UV - 1601PC (Japan). The measurement was conducted in triple. The encapsulation efficiency (EE%) was calculated as the content of naproxen in alginate cores according to the below equation:

$$EE\% = \frac{\text{Amount of naproxen in alginate cores}}{\text{Amount of alginate cores}} \times 100$$

The amount of naproxen in the alginate core was calculated by the amount of added naproxen in the formulation minus the amount of naproxen detected in the supernatant.

2.7. The *in vitro* release studies

In vitro release of naproxen from alginate cores was examined using the dissolution apparatus with the paddle method (USP apparatus 2) (Labinda DS 1400, India). Approximately 300 mg of alginate cores were suspended in 500 mL PBS pH 7.4 solution and maintained at 37 ^oC under stirring at 100 rpm. At predetermined time intervals during 24 hours, the samples (10 mL) were collected from a release medium, and the same volumes of fresh medium were replaced. Aliquots were analyzed for absorbance using UV-Vis spectroscopy at 272 nm. All experiments were measured triple and the percentage of the cumulative amount of released naproxen was calculated against the time.

2.8. Morphology

The morphology of alginate cores and microcapsules obtained at optimum conditions was examined including interfacial, shape, and porosity by using a scanning electron microscope (SEM) JEOL-JSM–6400 (Japan). The SEM photographs were taken at different magnifications at room temperature and analyzed using an acceleration voltage of 5kV.

3. RESULTS AND DISCUSSION

3.1. Optimization of alginate microcapsule cores

A set of 24 experiments provided by using response surface methodology and I-optimal design were conducted and the responses characteristics of obtained alginate cores were examined, as shown in Table 1.

To evaluate the effect of independent variables on responses, a suitable mathematical model was fitted to investigate the correlated relationships of each response. The analysis linear regression of square root, natural logarithm, inverse square root, inverse, and power were preformed the influence of the variables on the responses and identified statistically significant model. Table 2 provides a suitable linear regression of responses on the independent variables.

From the given sources, it can be inferred that the particle size, encapsulation efficiency, and naproxen release for 1h, 2h, and 4h were suitable for analysis of the influence trends by inverse, inverse square root, and power linear regression, respectively, with the largest R^2 coefficient (>0.96), the largest F-value, and significant p-value<0.05. The coefficients of independent variables on each response were given in the regression equations in terms of coded factors below:

$$\frac{1}{Y_1} = 0.1252-0.0226\times X_1+ 0.0550\times X_2+ 0.0175\times X_3+ 0.0259\times X_5 - 0.0220\times X_1\times X_2 - 0.0129\times X_1\times X_3+ 0.0119 \times X_1\times X_4- 0.0161\times X_1\times X_5 + 0.0193\times X_2\times X_3+ 0.0226\times X_2 \times X_4 - 0.0109\times X_3\times X_4 \quad (1)$$

$$\frac{1}{\sqrt{Y_2}} = 0.2523 - 0.0041\times X_1 + 0.0193\times X_2 - 0.0072\times X_3 - 0.0023\times X_4 + 0.0037\times X_5 \quad (2)$$

$$Y_3 = 27.85 + 6.97 \times X_2 - 0.8104 \times X_3+0.7976\times X_5 \quad (3)$$

$$Y_4 = 48.05-2.13\times X_1+7.60\times X_2-2.28\times X_3- 0.8860\times X_4 +1.94\times X_5 \quad (4)$$

$$Y_5 = 79.86-2.39\times X_1+12.07\times X_2-2.56\times X_3- 0.8339\times X_4 +3.42\times X_5 \quad (5)$$

Following equation (1), the particle mean size was significantly influenced by alginate concentration (X_1), stirring speed (X_2), $CaCl_2$ concentration (X_3), and span ratio (X_5) (p-value < 0.05). Speed of stirring is the most influential factor on the alginate cores mean size due to its control particle size during the continuous (isooctane) phase. The results indicate that increasing the stirring speed generally leads to reduced alginate cores size (as shown in Figure 1), as it produces smaller emulsion drops through stronger cutting forces and increased chaos, which similar to the report of Mahmoud M.A[16]. This result also revealed that the microsphere from alginate polymer loading sodium tolmetin had smaller mean size when increasing the stirring speed in the process. Conversely, the mean size of alginate cores increases as the concentration of sodium alginate increases based on an increase in the viscosity of the sodium alginate solution, increasing the interfacial tension between the sodium alginate droplets and the oil phase, thereby forming larger alginate cores.

Table 1. I-optimal design and value of responses

Trial	X_1	X_2	X_3	X_4	X_5	Y_1	Y_2	Y_3	Y_4	Y_5
F1	3.56	8000	8.50	7.18	5.00	7.81	14.3	33.3	51.6	85.8
F2	4.00	8000	5.00	10.00	7.43	6.00	13.5	36.8	56.0	90.2
F3	2.00	8000	10.00	5.00	5.00	4.91	14.4	33.8	54.2	90.1
F4	2.05	8000	10.00	10.00	5.00	4.57	14.1	33.3	53.0	89.0
F5	2.00	6500	8.45	7.65	5.00	13.22	17.4	23.6	43.8	71.8
F6	4.00	6000	5.75	7.58	10.00	12.21	18.2	20.9	41.7	72.3
F7	3.10	8000	5.00	5.43	10.00	7.81	12.1	35.8	60.0	99.4
F8	2.00	6000	10.00	10.00	10.00	12.37	18.9	21.0	42.1	69.4
F9	2.68	6584	8.55	5.00	10.00	6.57	15.8	26.0	50.1	79.1
F10	4.00	6400	10.00	10.00	5.00	16.81	20.2	19.8	34.1	62.1
F11	2.90	6000	7.45	10.00	7.30	18.04	18.2	20.4	40.2	69.3
F12	2.00	8000	5.00	7.15	5.00	7.70	13.2	36.3	59.3	92.5
F13	2.88	6819	5.80	7.15	8.34	10.92	15.7	26.3	49.2	80.9
F14	3.98	7140	6.475	5.00	7.05	11.50	15.1	27.6	50.2	81.4
F15	3.80	6000	10.00	5.00	5.00	16.84	20.7	20.3	36.0	60.6
F16	2.69	7540	6.75	10.00	6.36	6.16	14.4	30.3	50.1	85.2
F17	2.00	8000	8.10	7.50	8.60	3.76	13.2	36.8	57.1	95.0
F18	3.47	6280	5.00	8.13	5.00	12.73	17.3	22.3	42.3	69.4
F19	4.00	6000	9.00	5.70	8.70	16.12	19.2	22.5	37.5	64.9
F20	4.00	8000	10.00	5.00	10.00	6.48	14.1	34.0	52.1	90.6
F21	3.10	6900	10.00	7.43	7.53	6.46	16.7	25.8	46.2	75.8
F22	2.00	6870	5.00	10.00	9.50	6.98	14.6	27.8	51.6	84.3
F23	2.00	6000	5.00	5.00	7.50	12.64	16.3	23.7	43.2	72.3
F24	3.40	7420	8.75	10.00	10.00	5.46	15.7	31.5	50.9	85.3

Table 2. Analysis of the effect of factors on responses by linear regression

Response	Transform	Model	R^2	Adjusted R^2	Predicted R^2	SD	%CV	F-value	p-value
(Y_1) Particle mean size	Inverse	2FI	0.9813	0.9463	0.8830	0.0132	10.56	8.83	0.0025
(Y_2) Encapsulation efficiency	Inverse square root	Linear	0.9771	0.9771	0.9680	0.0028	1.09	197.59	<0.0001
(Y_3) Drug release for 1h	Power (λ=1)	Linear	0.9724	0.9648	0.9484	1.12	4.03	126.93	<0.0001
(Y_4) Drug release for 2h	Power (λ=1)	Linear	0.9697	0.9613	0.9412	1.43	2.97	115.21	<0.0001
(Y_5) Drug release for 4h	Power (λ=1)	Linear	0.9916	0.9892	0.9843	1.13	1.42	423.94	<0.0001

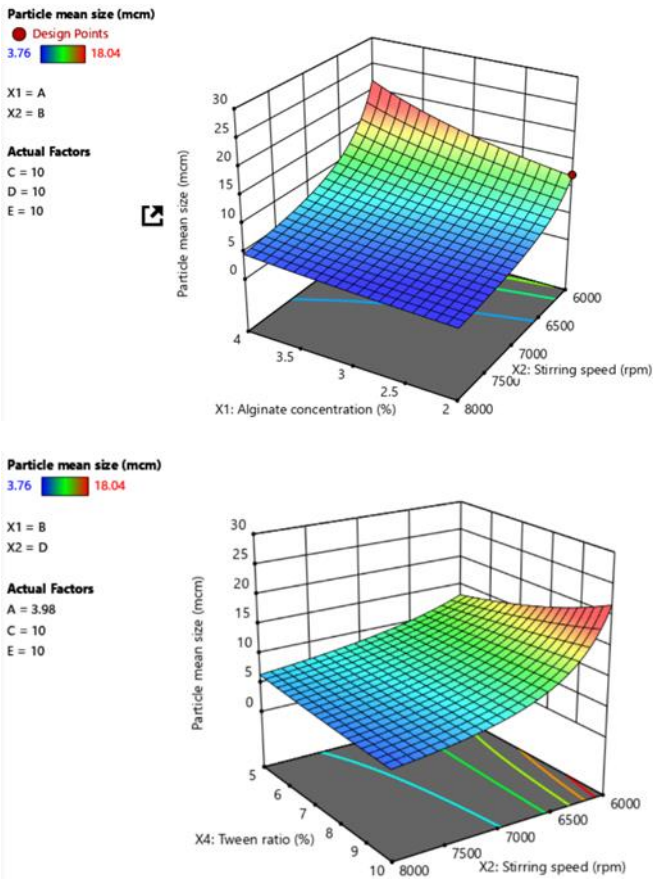

Figure 1. 3D response surface plot showing the effects of alginate concentrations, speed of stirring, and polysorbate ratio on the mean size of alginate cores

According to the inverse square root linear regression (equation (2)), all five factors influence the EE% of alginate cores (p-value < 0.05). The alginate concentration is a crucial parameter affecting the EE%. At low concentrations of sodium alginate solution, the network of sodium alginate-Ca^{2+} crosslinks incomplete to form a spatial structure, allowing sodium alginate chains to move freely, creating many large pores. Consequently, sodium naproxen easily escaped during the preparation process of the microsphere. At high concentrations of sodium alginate, the sodium alginate chains are tightly interconnected, forming a dense gel structure with small porous, resulting in a higher EE% of sodium naproxen. Additionally, other research was shown that the concentration of $CaCl_2$ increased leading to more loaded drug onto the cross-linking structure[17]. However, Rastogi *et al*[18] had reported that increasing of $CaCl_2$ should be limited not more than 20%. It could be caused by the instant gelling of polymer alginate and the quickly squeezing out of the aqueous phase in the W/O emulsion, resulted in increase in the microsphere size and decrease in the EE%.

The speed of stirring also has a significant influence on the EE% of alginate cores. Reducing the stirring speed leads to an increase in the level of interaction between Ca^{2+} ions and the polymer, forming larger-sized alginate cores and increasing the EE% of the drug.

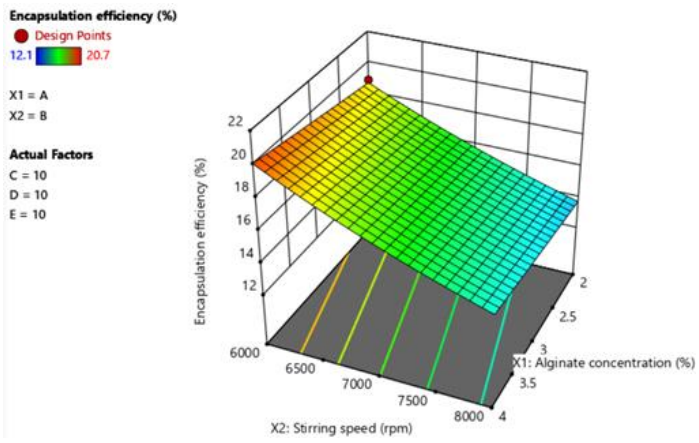

Figure 2. 3D response surface showing plot the coefficients of alginate concentrations and speed of stirring on the EE% of alginate cores

According to the power linear regression with lamda = 1 (equation (3), (4), (5)), alginate concentration (X_1), stirring speed (X_2), $CaCl_2$ concentration (X_3), polysorbate ratio (X_4), and span ratio (X_5) have a clear influence on the in vitro drug release of alginate cores, especially at 2 and 4 hours.

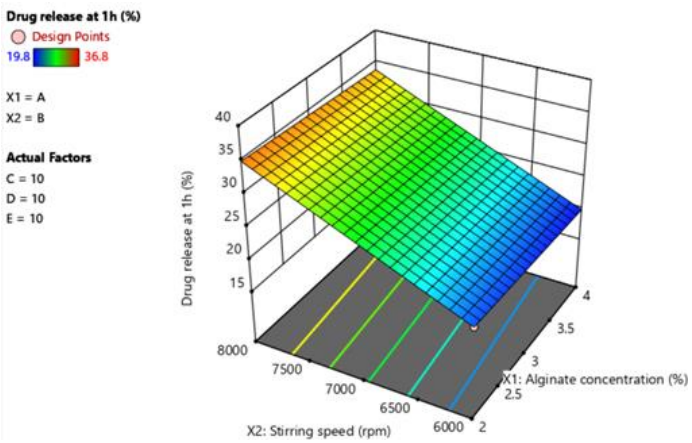

Figure 3. 3D response surface plot showing the efficients of stirring speed and polysorbate ratio on the drug release at 1 h from alginate cores

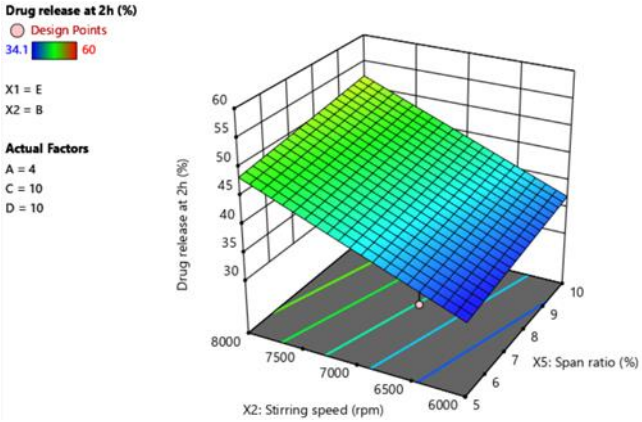

Figure 4. 3D response surface plot showing the efficients of stirring speed and span ratio on the drug release at 2 h from alginate cores

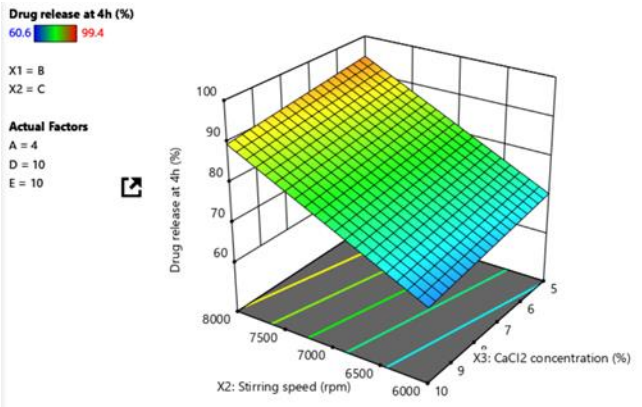

Figure 5. 3D response surface showing the efficients of stirring speed and $CaCl_2$ concentration on the drug release at 4 h from alginate cores

Similarly to EE%, the in vitro drug release from alginate cores was strongly influenced by alginate concentration and $CaCl_2$ concentration. At low concentrations of sodium alginate solution, the alginate - Ca^{2+} cross-linking interactions were looser with more large gaps resulted in differences pore size lead to significant differences in the in vitro drug release. While increased Ca^{2+} concentration caused increased cross-link density, and decreased the permeability of the membrane, hence indirectly reducing the ratio of drug release. In the alginate cores-forming process, polysorbate 80 plays an important role in accelerating Ca^{2+} ions move into the dispersed phase and contact alginate to form cross-linking. This role of polysorbate was reported by Charles J. Thoman et al[19] shown that polysorbate 80 as an ionophore transported Ca^{2+} ions through membranes with rate of 0.72 ± 0.22 ($\times 10^8$ mol/s/m^2). Instead of using the lipophilic dialysis membranes as in the above reference, this study used the emulsion with the continuous phase being the oil phase, so polysorbate 80 could reversibly transport Ca2+ ions, helping them diffuse quickly inside the emulsion droplet to occur cross-linking with sodium a ginate immediately until a complete spatial structure is formed.

Additionally, stirring speed impacts the drug release rate through its influence on particle size. Increasing the drug release from alginate cores occurred while the stirring speed was raised, which was caused by reducing the particle size and the growth of interfacial tension. This was also reproted by Mahmoud M.A[16] that the in vitro release profile was decreased by using lower stirring speed and higher polymer concentration. In other work of Thu et al[15] demonstrated the effect of porous size could lead to significant differences in the drug release rate. Their results shown that alginate-based microsphere obtained from low alginate concentration had bigger porous size and relatively with higher albumin release rate.

According to 24 experimental results of I-optimal design and analyzed regression models, the optimal conditions for alginate cores processing were suggested and predicted by Design Expert software with the maximum combined desirability value was 0.607 (the detail desirability of (Y_2) encapsulation efficiency was 0.607, and others reponse (Y_1), (Y_3), (Y_4), and (Y_5) were 1), as shown in Table 3. These optimal conditions were performed in triplicate to verify the reliability of the model and the consistency of the analytical results.

The results were statistically analyzed, showing that the obtained alginate cores had properties similar to those predicted by Design Experts (t-test, not significant difference, $p>0.05$). The particle size distribution of optimal microspheres is shown in **Figure 6**, with a mean size of 6.01 ± 0.51 µm and a narrow size distribution (span value 1.050). The in vitro release profile is shown in **Figure 7**, indicating that 50% of sodium naproxen was released in the first two hours, and more than 80% of sodium naproxen was released from alginate cores in 4 hours.

Table 3. The optimization conditions of alginate cores preparation process

Factors/Responses	Goal	Design Experts suggestions	Design Experts predictions	Experimental results
(X_1) Alginate concentration	-	3.94%	-	4.00%
(X_2) Stirring speed	-	7194 rpm	-	7200 rpm
(X_3) $CaCl_2$ concentration	-	10.00%	-	10.00%
(X_4) Polysorbate ratio	-	9.92%	-	10.00%
(X_5) Span ratio	-	10.00%	-	10.00%
(Y_1) Particle mean size	In range (12-20 µm)	-	6.64 µm	6.01 ± 0.51 µm
(Y_2) Encapsulation efficiency	Maximize	-	16.47%	18.03 ± 0.97%
(Y_3) Drug release at 1h	In range (20-30%)	-	28.22%	29.81 ± 2.42%
(Y_4) Drug release at 2h	In range (40-50%)	-	46.32%	48.73 ± 2.97%
(Y_5) Drug release at 4h	In range (80-90%)	-	80.00%	86.3 ± 2.55%

The results of this study reveal that cross-linking between sodium alginate and Ca^{2+} ions in a PBS buffer (pH 7.4) was unstable due to the exchange of Ca^{2+} ions in alginate cores with Na^+ ions. This result in the separation of the "egg box" structure, leading to rapid diffusion of sodium naproxen from the alginate cores. Over time, the cross-linking-based spatial structure of alginate cores breaks down completely, releasing all the sodium naproxen into the medium. To address this issue, the researchers investigated chitosan-coated alginate microcapsule which could help reduce the drug release rate during the initial hours and control drug release for 24 hours.

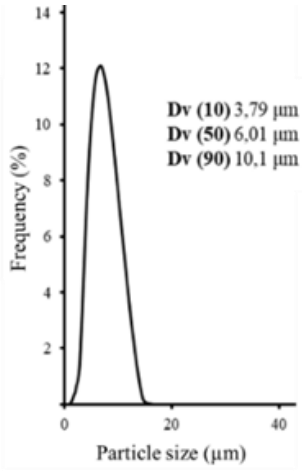

Figure 6. The particle size distribution of optimal alginate cores

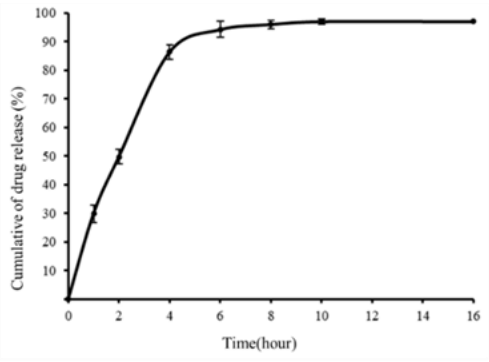

Figure 7. The *in vitro* release of optimal alginate cores at pH 7.4

This study was used the preparation process based on the ideas of Wan et al[20]. From the experiment results, the mechanism of alginate microsphere formation was clarified. First, a sodium alginate solution containing naproxen sodium is homogenized in an isooctane external phase, forming a water-in-oil emulsion. The main advantage of this process is smaller microspheres can be formed, especially in range of micrometer or even nanometers[17]. In contrast with classical methods will create alginate microspheres of large size when dripped direct sodium alginate solution. The water-in-oil emulsion under high stirring speed to create sodium alginate microspheres of the desired size. To solidify sodium alginate microspheres, Ca^{2+} ions are needed to convert sodium alginate into solid calcium alginate by cross-linking structure. To maximum effectiveness contact between Ca^{2+} ions and sodium alginate in the water-in-oil emulsion, the additional coordination agent must simultaneously meet two requirements: (i) distribution on the emulsion droplet surface; (ii) capacity to reversibly transport ions across the membrane. In this process, polysorbate 80 is the chosen substance that plays the above role. Polysorbate transports Ca^{2+} ions into the dispersed phase and contact with sodium alginate, creating a spatial cross-linked structure.

3.2. Identify impacts of the process parameters of chitosan-coated alginate microcapsules

As shown in Table 4, a set of 14 experiments were conducted to evaluate four impacts of chitosan-coated alginate microcapsule through the immersion process, including the pH of the chitosan solution (6 levels), the stirring speed of the coating phase (3 levels), the coating time (3 levels), and the chitosan concentrations (4 levels). All microcapsules obtained from experiments were analyzed based on characteristics similar to those of alginate cores, specifically particle size, EE%, and *in vitro* release profile.

Table 4. Preparation conditions of chitosan coating trials and characteristics of obtained microcapsules

Trial	pH	Stirring speed (rpm)	Time (minute)	Chitosan concentration (%)	Particle mean size (µm)	Encapsulation efficiency (%)
F25	**2**	2000	30	1	5.59 ± 0.11	17.6 ± 0.61
F26	**3.5**	2000	30	1	5.63 ± 0.21	16.5 ± 0.78
F27	**4**	2000	30	1	6.51 ± 0.34	11.3 ± 0.96
F28	**5**	2000	30	1	7.41 ± 0.14	11.7 ± 0.36
F29	**6**	2000	30	1	6.87 ± 0.13	9.3 ± 0.26
F30	**6.3**	2000	30	1	Chitosan did not dissolve completely	
F31	5	**1000**	30	1	8.51 ± 0.35	13.6 ± 0.89
F32	5	**1500**	**30**	**1**	7.50 ± 0.16	14.7 ± 0.52
F33	5	**2000**	30	1	7.55 ± 0.47	11.7 ± 0.72
F34	5	1500	**15**	1	6.39 ± 0.14	14.1 ± 0.80
F35	5	1500	**45**	1	7.38 ± 0.20	13.1 ± 0.66
F36	5	1500	30	**0.25**	6.11 ± 0.13	16.1 ± 0.79
F37	5	1500	30	**0.50**	6.77 ± 0.30	15.3 ± 0.36
F38	5	1500	30	**1.50**	7.61 ± 0.14	14.1 ± 0.44

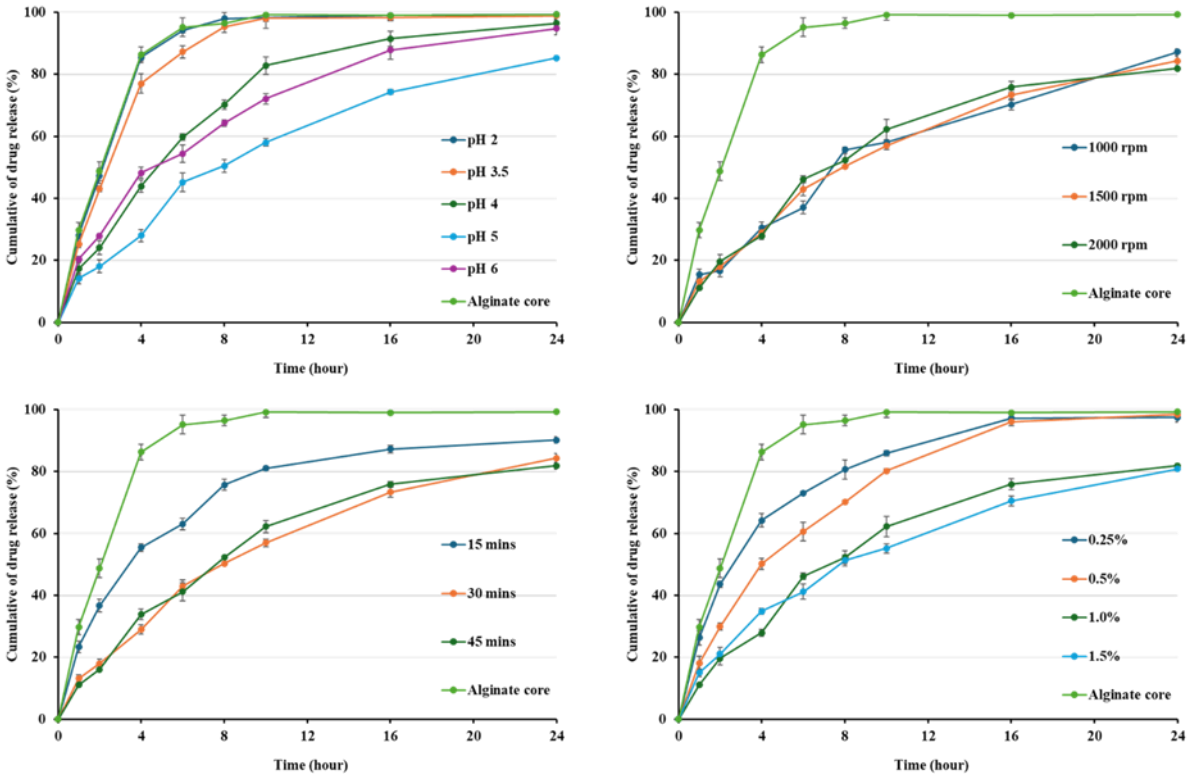

Figure 8. The *in vitro* release of chitosan-coated microcapsule considering different preparation conditions: (a) pH of chitosan solution (pH 2, pH 3.5, pH 4, pH 5, pH 6); (b) stirring speed (1000, 1500, 2000 rpm); (c) coating time (15, 30, 45 mins); and (d) chitosan concentration (0.25%, 0.5%, 1.0% and 1.5%).

The impacts of pH chitosan solution in the immersion process were examined in range of pH from 2.0 to 6.3, due to characteristics of chitosan, an alkaline polymer with a pKa of 6.3, is affected by pH, affecting its charge state and properties. At pH < 3.5, microcapsule size, EE%, and in vitro release rate remain relatively stable compared to alginate cores (t-test, $p > 0.05$). At pH from 4 to 6, the in vitro release rate decreases, and the microcapsule size increases (t-test, $p < 0.05$).

The formation of a polyelectrolyte complex membrane between chitosan and sodium alginate reduces porosity, reducing active ingredient leakage. Specially, at pH 5, the *in vitro* release rate occurs the slowest, indicating that pH 5 is the optimal pH for creating electrostatic complexes between chitosan and alginate (**Figure 8**). This result is. similar to the report of Sevgi Takka *et al*[21] in evaluation of chitosan/alginate beads containing bovine serum albumin by ionotropic gelation

The *in vitro* release profile did not significantly change with stirring speed but increased stirring speed from 1000 rpm to 1500 rpm led to a decrease in microcapsule mean size from 8.51 ± 0.35 µm to 7.50 ± 0.16 µm (t-test, $p < 0.05$). Higher stirring speed caused more disturbance during the encapsulation process, resulting in smaller microcapsules. However, increasing stirring speed from 1500 rpm to 2000 rpm resulted in a decrease in the EE% from 14.7 ± 0.52% to 11.7 ± 0.72 (t-test, $p < 0.05$), indicating that high stirring speed increases active ingredient loss.

The *in vitro* release rate decreased with an increase in chitosan coating time from 15 minutes to 30 minutes, resulting in an increase in size from 6.39 ± 0.14 µm to 7.50 ± 0.16 µm. However, when the coating time increased to 45 minutes, the *in vitro* release rate did not decrease further (t-test, $p > 0.05$), and EE% decreased slightly. Therefore, a shorter coating time (30 minutes) was chosen to investigate the effect of chitosan concentration on the *in vitro* release profile. C. H. Zheng *et al*[8] also reported the similar coating time in the research on chitosan-coated alginate microcapsules containing albumin.

The *in vitro* release rate in microcapsules depends on the chitosan concentration used for coating. The chitosan solution at 0.25% significantly decreased the in vitro release profile of the obtained microcapsule compared to the alginate cores without chitosan. Increasing chitosan concentration from 0.25% to 1% led to a reduction in the *in vitro* release rate. No valuable effect was seen in using chitosan concentration at 1.5%. Low chitosan concentrations create a thin membrane of polyelectrolyte complex, while higher concentrations form a stable complex. This could be explained by the interaction between amin groups in chitosan structure and carboxyl groups in sodium alginate structure to form polyelectrolyte complex membrane. Misirli et al[22] was point out that the microcapsule prepared by using spraying method using an extrusion device containing mitomycin-C had their mean size increased when using higher chitosan concentration in range of 0.5% to 1%. The mechanism of polyelectrolyte complex was studies for decades[10,15]. It could be clearly to see that in the lower chitosan concentration, the interaction between chitosan and alginate was not completely, so the thinner polyelectrolyte complex membrane was formed. Furthermore, the result demonstrated that chitosan concentration was not effect in the microcapsule in vitro release in range of 1% to 1.5%. As a result, the 1% concentration of chitosan was suitable for the encapsulation process. This result was similar to the research of C. H. Zheng et al[8]. Beside, the changing of chitosan concentration was not effect on the microcapsule EE%, which was desmontrated by Mahmoud M. A[16].

As a result, a chitosan solution with a pH of 5, 30 minutes of stirring, 1500 rpm of stirring speed, and 1% chitosan concentration were used to develop the alginate-chitosan microcapsule preparation process. The microcapsules that were generated have a mean size of 7.50 ± 0.16 µm with a narrow size distribution (span value 0.964).as displayed in **Figure 9** and EE% of around 14.7 ± 0.52% for sodium naproxen. The *in vitro* release profile of sodium naproxen-loading microcapsules was significantly reduced after coating with chitosan; after 8 hours, the release reached 50%; thereafter, the *in vitro* release profile slowed down; after 24 hours, the release reached 84.3 ± 4.16% as show in **Figure 10**.

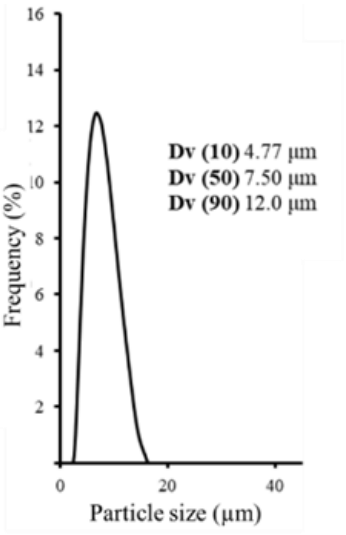

Figure 9. The particle size distribution of optimized alginate-chitosan microcapsules

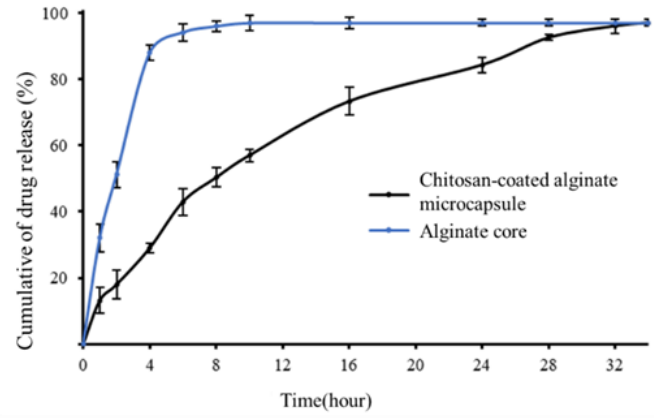

Figure 10. The *in vitro* release of optimized alginate-chitosan microcapsules

The alginate-chitosan microcapsules are primarily spherical and have a generally smooth and uniform surface, according to SEM data in **Figure 11**.

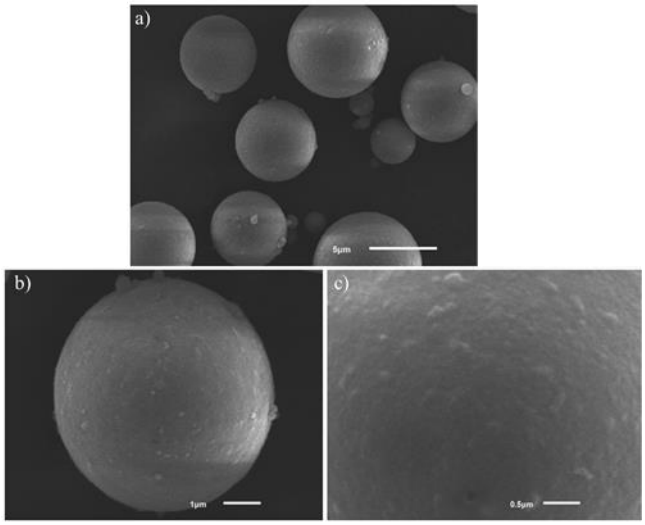

Figure 11. SEM photographs of alginate-chitosan microcapsules at (a) 2000x, (b) 15000x, and (c) 30000x

4. CONCLUSION

This study investigated the microcapsules containing sodium naproxen from alginate and chitosan through a two-stage preparation method. In the first stage, the alginate cores were optimized using DoE and demonstrated the significant influence of independent variables such as polymer concentration, stirring speed, $CaCl_2$ concentration, polysorbate ratio, and span ratio on dependent variables like particle mean size, encapsulation efficiency, and *in vitro* release profile. The optimum conditions for alginate cores were identified. In the second stage, the chitosan-coated alginate microcapsule process was developed and the impacts of parameters such as the pH of the chitosan solution, stirring speed, coating time, and chitosan concentrations were pointed out. The developed microcapsule process has potential for controlling the release of drugs such as sodium naproxen.

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