Research Article

Formulation and statistical optimization of dextran-calcium alginate beads for controlled oral drug delivery systems

Kahina Benfattoum^{1*}, Nabila Haddadine^{1*}, Khaled Beyaz¹, Naima Bouslah¹, Ahmed Benaboura¹, Philippe Maincent², Régis Barillé³, Anne Sapin-Minet²

¹Laboratoire de Synthèse Macromoléculaire et Thio organique Macromoléculaire, Université des Sciences et de la Technologie Houari Boumediene, Faculté de chimie, B.P. 32, El Alia, Bab Ezzouar, Alger, 16111, Algérie.

²Université de Lorraine, CITHEFOR EA3452, Faculté de Pharmacie, Nancy, France

³Laboratoire MOLTECH ANJOU, Université d'Angers/UMR CNRS 62002, Bd Lavoisier, 49045 Angers, France.

Received: 14 August 2018

Revised: 3 September 2018

Accepted: 9 October 2018

Abstract

Objective: Novel beads formulations based on, dextran and sodium alginate biopolymer were prepared and evaluated for *in vitro* sodium diclofenac (SD) release. **Material and methods:** Dextran-calcium alginate (Dex-CA) beads, loaded SD were prepared by the ionotropic gelation method using calcium chloride as a cross-linker agent. The effects of biopolymer-blend ratio and the concentration of the cross-linker on the cumulative drug release after 8h ($R_{8h}\%$) and the drug encapsulation efficiency (DEE%), were monitored using the response surface methodology (RSM) based on 3^2 factorial design. **Results and conclusion:** The results showed that particle beads formed under optimal conditions presents a DEE value of $66.94 \pm 2.45\%$, R_{8h} of $61.14 \pm 2.32\%$, mean diameter of 1.08 ± 0.01 mm and density of 2.73 ± 0.31 g/cm³. Additionally, the beads were unaffected in acidic gastric pH; however, they showed a swelling behavior under alkaline intestinal pH. The *in vitro* drug release from the beads followed the (Hixson-Crowell), equation monitoring the super case-II transport mechanism, related to the swelling and the relaxation behavior of the polymeric matrix. Moreover, the important drug encapsulation efficiency and the sustainable drug release of these materials make them promising for the development of novel drug carrier systems.

Keywords: Polysaccharide, beads particles, statistical optimization, drug delivery carriers

Introduction

The release of drug from an appropriate dosage form at prefixed time intervals and predetermined rates represent the primary challenge for scientists involved in pharmaceutical studies (Matricardi et al., 2013). Exactly, the successful formulation of a stable and efficient dosage form depends on the careful selection of excipients (Palanisamy et al., 2013). In this context, it should be mentioned that the use of polymers as excipients in the encapsulation systems and controlled drug

delivery systems has over the years become an essential area of researches and developments (Tønnesen et al., 2002). Among the natural and synthetic polymers that can be used for the formulation aimed to optimize drug targeting and or release rate, hydrophilic polysaccharides from an animal, bacterial or plant-based derivatives represent a class of macromolecules of particular interest (Matricardi et al., 2013). Their gel-forming property is often exploited to entrap drug molecule in an insoluble semi-permeable hydrogel matrix in the form of various shapes. Hydrogels in particulate form, like beads, capsules or particles, are more commonly used for oral drug encapsulation (Laroui et al., 2010; Abdellatif et al., 2016). Alginate, from marine plants, is an anionic and an unbranched polysaccharide, which is composed of β -($\rightarrow$ 14)-linked D-mannuronic acid (M block), and α -L-guluronic acid (G block) units arranged with varying proportion of GG, MG, and GM blocks (Agarwal et al.,

*Address for Corresponding Author:

Prof. Nabila Haddadine

Laboratoire de Synthèse Macromoléculaire et Thio organique Macromoléculaire,

Université des Sciences et de la Technologie Houari Boumediene, Faculté de chimie, B.P. 32, El Alia, Bab Ezzouar, Alger, 16111, Algérie.

Email : n_haddadine@yahoo.fr

DOI: <https://doi.org/10.31024/apj.2018.3.4.1>

2456-1436/Copyright © 2018, N.S. Memorial Scientific Research and Education Society. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

2015). It has the ability to form hydrogel bead in the presence of multivalent cations, such as calcium ions in aqueous media (Goyal et al., 2011). Alginate beads have the advantages of being orally non-toxic and having high biocompatibility (Yotsuyanagi et al., 1987). However alginate beads are sensible to the environmental pH, so acid-sensitive drugs incorporated into the beads would be protected from gastric reactions (Goyal et al., 2011). Recent efforts in drug development focused on the use of alginate as safe drug carriers (Kim et al., 1992; Kato et al., 1993; Shiraishi et al., 1993) and many modifications based on their combinations with other polymers have been investigated to develop novel drug delivery systems (George et al., 2006).

The investigation and the use of dextran polysaccharide have attracted significant interest in developing a different type of encapsulation systems (Cortesi et al., 1999; Volodkin et al., 2004). However, the alginate/dextran combination as new encapsulation systems have not yet been used to establish particle beads formulation. Therefore, it would be interesting to evaluate the performance of this mixture in the Sodium Diclofenac (SD) anti-inflammatory drug protection and its release from particle beads. The dextran was chosen regarding its long-standing and safe record in biomedical/pharmaceutical applications (Cortesi et al., 1999). Dextran is known for its unique properties such as a high water solubility, biodegradability, biocompatibility, non-immunogenicity, and non-antigenicity (Mokhtarzadeh et al., 2016). It is a bacterial polysaccharide, synthesized from sucrose by several lactic-acid bacteria (Vu et al., 2009). These uncharged branched polysaccharides consist of d-glucopyranose residues with α -(1 $\rightarrow$ 6)-linkages and different ratios of linkages and branches such as α -(1 $\rightarrow$ 2), α -(1 $\rightarrow$ 3) and α -(1 $\rightarrow$ 4)-linked side chains, depending on the bacterial strains (Siddiqui, Aman et al., 2014). It can be readily biodegraded by dextranase (α -1-glycosidases) enzyme that exists in different parts of a human body including colon, liver, kidney, and spleen and various bacteria, which live in the colon and release non-toxic metabolites (Sun et al., 2012; Mokhtarzadeh et al., 2016).

The SD, consists of a hydrophilic drug model and a potent non-steroidal anti-inflammatory drug, used in the long-term treatment of inflammation and painful conditions of rheumatic and non-rheumatic origin (Petrucelli et al., 2007). The usual oral dose is 75–150 mg daily in divided doses (Sanchez et al., 2003). It is eliminated rapidly, with a half-life of 1-2 h. Hence, the rapid absorption after oral administration justifies the need for a sustained release profile (Petrucelli et al., 2007). Following all routes of administration, SD causes gastrointestinal disturbances if it is present with large doses in the digestive tract (Chowdary et al., 2006). The formulators, therefore, has the choice of keeping a constant drug dissolution

rate or minimizing the dissolved drug concentration in the local intestine (Chowdary et al., 2006).

The development of new encapsulation systems requires that different parameters must be optimized to prepare stable and efficient encapsulation systems (Morales et al., 2017). In this study, the factorial design, a usual method of response surface methodology (RSM), was used as a tool to determine the optimal process conditions to prepare particle bead formulations with high drug encapsulation efficiency and a retard drug release. It involves the structured data collection process and describes the relationship between important and unimportant factors (Ghosh et al., 2013). Additionally, it is used to find out the ideal operating conditions for a given system or the way in which a particular response is affected by a set of variables over some specific regions of interest (Baş et al., 2007). Finally, the performances of the prepared bead particles regarding drug stability and in vitro release behavior in acidic and alkaline media are compared according to the different formulations. Furthermore, we discuss how the functional properties of different pH-sensitive materials and chemical bonds can be exploited to develop slow down drug delivery systems.

Materials and methods

Materials

Sodium Alginate from brown algae (SA, Sigma-Aldrich), Dextran M_s ~ 6000 (D, Sigma-Aldrich), and Calcium Chloride (CaCl₂, Prolabo), were used for the formulation of particle beads. Sodium Diclofenac (SD, Sigma-Aldrich) was chosen as a drug model to prepare controlled drug delivery systems. All reagents and chemicals used for the experimental procedures were of analytical grade, and all solutions were prepared with ultrapure water.

Experimental Design

The 3² factorial design, a powerful process optimization tool, was used to investigate the optimal process conditions to prepare a matrix beads formulation with a high drug encapsulation efficiency and a retard drug release. Precisely, nine experimental trial formulations were evaluated by varying SA to D ratio from 1 to 4 (w/w) and CaCl₂ concentration from 2.5 to 7.5 (% w/v). The effects of the factors on drug encapsulation efficiency (DEE, %), cumulative drug release after 8 h (R_{8h}, %), particle size (mm), and density (g/cm³) were investigated as optimization response parameters.

Formulation of particle beads

The ionotropic gelation technique using calcium chloride

(CaCl₂), as a cross-linker agent was employed for formulating SD loaded D-CA particle beads. An exact amount of SA and D biopolymers were dispersed in 25 ml of ultrapure water and were stirred for 2 h to get a homogenous solution. Afterward, 5% (w/v) of SD was added to this dispersion mixture in all formulations. The final polymers-blend dispersion mixture containing SD was homogenized for 30 min and then sonicated for 5 min to eliminate the air trapped inside. This dispersion was poured into 25 ml syringe and was dropped, from 10 cm height, into 50 ml of a particular concentration of the CaCl₂ solution, under constant stirring and at room temperature (24±2) °C. After 30 min, the formed beads were removed from the CaCl₂ solution, washed three times with purified water, and were a drought at 37°C until a constant weight was obtained. The dried beads were stored in desiccators until their uses.

Evaluation of particle beads

Yield percentage

The yield percentage of particle bead formulation was calculated using the weight of the final product after drying concerning the initial total weight of the drug and polymers used in the formulation.

The yield percentage (w/w) was calculated by using the following formula (1) (Singh et al., 2009).

$$\text{Yield (\%)} = \frac{\text{Amount of product formed}}{\text{Amount of reactant taken}} \times 100 \quad \dots\dots\dots(1)$$

Surface morphology analysis

The surface morphology of the optimized formula was examined using Scanning Electron Microscopy (SEM) (JSM 6300 Scanning Microscope, Japan). The particle bead was mounted on an appropriate stub and then coated with gold sputter module in a vacuum evaporator in a standard argon atmosphere.

Particle size and density

The particle beads formulations are spherical. From this solid dosage forms, it was important to determine (i) the particle size and (ii) the density of the dried particle beads in order to examine the influence of experimental variables. The particle sizing was measured using an optical microscope (Motic Microscope, China) with a standard stage micrometer. The average volume was calculated by measuring the diameter of 20 beads from each formulation. Then, to estimate the densities of this particle beads, the mean weights and diameters were measured and used in the following equations (Malakar, Nayak et al., 2012):

$$D = M/V \text{ and } V = 4/3\pi r^3,$$

where **D**, **M**, **V**, and **r** were respectively, the density (g/cm³), weight (g), volume (cm³) and radius (cm) of the beads.

Drug entrapment efficiency

The drug encapsulation efficiency (DEE), of the various particle bead over different formulations, was determined indirectly by the following method. 100 mg of dried beads were crushed and placed into 100 ml of phosphate buffer (pH 7.4). The contents were shaken on vortex mixture for one hour to extract the drug. After the stipulated time, the samples were centrifuged and then were filtered through Whatman filter paper (No.40) for removing the polymer debris formed after beads disintegration. The clear solutions obtained were analyzed using a UV-VIS spectrophotometer (Perkin Elmer, USA) at 276 nm for the SD content. From that, the DEE (%) was determined using the formula (2).

$$\text{DEE (\%)} = \frac{\text{Actual drug Content in the particle beads}}{\text{theoretical drug content in the particle beads}} \times 100 \quad \dots\dots\dots(2)$$

Swelling degree

The swelling kinetics was carried out by the gravimetric method in two different aqueous media: (i) in 0.1 N HCl (simulated gastric fluid, pH 1.2) and (ii) in phosphate buffer (simulated intestinal fluid, pH 7.4). The particle beads were immersed in a dry state into a conical flask containing 50 ml of release medium and were incubated at 37±0.5 °C under shaking at 50 rpm. At fixed intervals, samples were taken out from the swelling medium, were blotted with a piece of paper towel to absorb excess water on the surface and then were weighed immediately. The difference in weights gave the amount of water uptake by the particle beads. At each time, the degree of swelling *S* (t) was calculated using the following expression (3):

$$S(t) = \frac{W_t - W_d}{W_d} \times 100 \quad \dots\dots\dots(3)$$

Where, *W_t* and *W_d* are the sample weights at time *t* and in the dry state, respectively. Each experiment was repeated three times.

In vitro drug release studies

Dissolution studies were conducted to determine the in vitro release pattern of the drug from the product formulations. Accurately weighed quantities of SD loaded D-CA particle beads (equivalent to 100 mg) were placed in simulated gastric fluid (200 mL, 0.1 N HCl, pH 1.2) for the first 2 h. Then, the particle beads were filtered and again placed in intestinal fluid (200 mL, phosphate buffer solution, pH 7.4) for 6 h. The dissolution test was carried out in conical flasks incubated in a shaking water bath at 37±0.5°C, with a speed

of 50 rpm. At predetermined time intervals, 5 mL of samples dissolution fluid was collected from the dissolution medium and immediately were replaced with the same volume of fresh media. These samples were filtered, diluted and then analyzed for the drug content using a UV-Visible spectrophotometer (Perkin Elmer, USA) at 276 nm. The percent drug release was calculated.

Drug release kinetics and mechanism

In order to determine kinetics and mechanisms of the SD release from the developed particle bead formulations, the dissolution profile of all formulations was fitted by various mathematical models to ascertain the kinetic modeling of the drug release and then to decide the most appropriate model. The regression analysis was performed, and the equation model that best fits considering release data was chosen based on the correlation coefficient (R^2). Zero-order, first order, Hixson-Crowell, Higuchi, and Korsmeyer-Peppas were used as mathematical models (Malakar et al., 2012).

Stability studies

The optimized formula was submitted to stability test. Samples were placed in high-density polyethylene (HDPE) bottles with induction sealing of aluminum and were stored at $30 \pm 2^\circ\text{C}$ under a relative humidity (RH) controlled at $75 \pm 5\%$ for 6 months. The storage aspect was evaluated visually, and the content of SD was determined using a spectrophotometer and compared with the initial samples.

Drug-excipient compatibility studies

Drug-excipient compatibility is a crucial step in the formulation of stable drug forms (Chadha and Bhandari 2014). In this context, to show the stability of SD in the developed formula, it has been characterized by (i) Powder X-ray diffraction (P-XRD, Philips PW 1729 X-ray diffractometer) to study drug crystallinity; (ii) Thermo-Gravimetric Analysis (TGA, Thermal Analyzer instrument Q500, USA) to evaluate the thermal stability, and (iii) Fourier transform infrared spectroscopy (FTIR, Perkin Elmer Spectrum, USA) to investigate the possible chemical interactions between the different beads components.

Statistical analysis

In this study, the response surface modeling and the evaluation of the quality to fit the models were performed employing the Design-Expert 10 software (Stat-Ease Inc., USA). Polynomial models including interaction and quadratic terms were generated for all the response variables using multiple linear regression analysis. After fitting the response data, the experimental results were analyzed by ANOVA. It displayed b-coefficients, Sum of Squares (SS), F values, and p values of model terms. Other statistical parameters: the multiple correlation coefficients (R^2), adjusted and predicted R^2 that authenticated the suitability of the

models (Malakar et al., 2012). The mathematical model was expressed as follows,

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_1X_2 + b_4X_1^2 + b_5X_2^2$$

Where Y is the response, b_0 the intercept, b_1 - b_5 regression coefficients. X_1 and X_2 individual effects, X_1X_2 the interaction effect, and X_1^2 and X_2^2 polynomial terms of individual effects.

Results and discussion

Formulation of particle beads

The SD loaded D-CA beads were formulated by the ionotropic-gelation method using CaCl_2 as a cross-linker agent. The formation of double helical junction zones was followed by a complexation with divalent Ca^{2+} ions and hydrogen bonding with water, to form a three-dimensional network (Nayak et al., 2014). Based on statistical method, it is evident that the variation of the ratio SA to D polysaccharides and the concentration of the cross-linking agent in the formulation has resulted in the optimum formula. The percentage yield of the production of the formulated particle beads was in the range 63.09-91.81 % (Figure 1). A better yield was observed when the polymer concentration and CaCl_2 concentration were higher (i.e., the concentration of the polymer increased when the ratio of SA to D decreased). At lower polymers concentration, the polymers chains on the exposed surface of the droplet that is exposed to calcium chloride solution are rapidly cross-linked. This prevents further diffusion of calcium ions into the extruded polymers droplet, thereby leaving the inner polymers chains un-crosslinked. The ability of calcium ions to consolidate the alginate-polymer chains into an egg-box structure is dependent on the diffusivity of the cross-linker solution in the polymer droplet (Singh et al., 2009).

Figure 1. Yield percentage for the different formulations, SD loaded D-CA bead particles

Statistical optimization

The selection of the optimal formula of SD loaded D-CA particle beads was enabled by the use of the RSM based on 3^2 factorial (Nayak et al., 2014). Exactly, nine innovative formulations were prepared by ionotropic gelation method taking two process variable factors like polymer blend ratio (SA to D ratio, X_1) and cross-linker (CaCl_2 , X_2) concentration. The DEE (Y_1 , %), R8h (Y_2 , %), particle size (Y_3 , mm), and density (Y_4 , g/cm^3) were evaluated as dependent variables (responses). The Overview of the preliminary plan including investigated responses is presented in table 1. From this experimental conditions and corresponding responses values four models were obtained in the second-order equations. The coefficients of regression equations, the regressions statics of the processing parameters and the analysis of variance (ANOVA) are shown in table 2 and 3. According to the results, the F-values (77.04, 70.07, 61.26 and 56.05, respectively) and the small P-values (both P-values <0.004) suggested that both models were highly significant. Meanwhile, the right relationship between the predicted and the actual values shown in figure 2, indicated that the factors in each

model were well correlated. The high values of R^2 , as well as adjusted R^2 and predicted R^2 for the models fitting, showed that regression models were appropriate and could accurately represent the variables chosen in the experimental region. As well, based on the results of P value, the theoretical simplification was carried out by eliminating the non-significant conditions ($p > 0.05$) in the polynomial equations arising from the multiple regression analysis (Nayak et al., 2011). (i) From the model relating DEE (Y_1 , %) as a response, it can be seen that the coefficients b_1 , b_3 , and b_5 had no statistical significance. (ii) Then, from the model giving R_{8h} (Y_2 , %) as a response, it can be noticed that all the coefficients of this model equation had statistical meanings except the coefficients b_3 and b_5 . (iii) Considering the model relating particle size (Y_3 , mm) and density (Y_4 , g/cm^3), the coefficients b_0 , b_1 , and b_2 had statistical significance in the two models equations excepting the coefficients b_4 had statistical significance just in the model relating density. So, the final mathematical models used was as follow:

Figure 2. Relationship between predicted and actual values for (a) DEE (%), (b) R8h (%), (c) Particle Size (nm), and (d) Density (g/cm^3)

Table 1. Experimental design plan of 3² full factorial design for various SD loaded D-CA particle beads

Experimental formulations	Factors			Responses										
	SA to D ratio (w/w) (X ₁)	CaCl ₂ (%, w/v) (X ₂)	DEE (%)	R _{8s} (%)			Particle Size (mm)			Density (g/cm ³)				
				Actual value ^a	Error ^b (%)	Predicted value	Actual value ^a	Error ^b (%)	Predicted value	Actual value ^a	Error ^b (%)	Predicted value		
D1	4.00	7.50	61.73 ± 0.78	61.90	-0.27	71.98 ± 3.25	71.91	0.09	0.93 ± 0.03	0.93	0	3.11 ± 0.82	3.09	0.64
D2	4.00	5.00	59.75 ± 0.46	59.15	1.01	75.57 ± 3.29	75.34	0.30	1.03 ± 0.05	1.02	0.98	3.04 ± 1.18	3.09	-1.61
D3	4.00	2.50	52.11 ± 0.15	52.54	-0.81	78.30 ± 2.34	78.60	-0.38	1.05 ± 0.03	1.06	-0.94	2.97 ± 0.20	2.94	1.02
D4	2.00	7.50	69.75 ± 0.46	70.28	-0.75	68.17 ± 3.54	68.79	-0.90	1.09 ± 0.06	1.10	-0.90	2.92 ± 0.35	2.92	0
D5	2.00	5.00	66.55 ± 1.13	66.26	0.43	71.85 ± 3.52	71.72	0.18	1.17 ± 0.05	1.16	0.86	2.87 ± 0.39	2.84	1.05
D6	2.00	2.50	58.63 ± 0.57	58.39	0.41	74.96 ± 0.02	74.47	0.65	1.18 ± 0.05	1.17	0.85	2.57 ± 0.62	2.61	-1.53
D7	1.00	7.50	65.63 ± 0.12	64.93	1.07	65.25 ± 3.29	64.70	0.85	1.19 ± 0.03	1.18	0.84	2.63 ± 0.30	2.65	-0.75
D8	1.00	5.00	59.40 ± 0.92	60.29	-1.47	67.01 ± 3.24	67.37	-0.53	1.21 ± 0.06	1.22	-0.81	2.54 ± 0.31	2.53	0.39
D9	1.00	2.50	51.98 ± 0.81	51.79	0.36	69.68 ± 3.95	69.87	-0.27	1.22 ± 0.03	1.22	0	2.26 ± 0.79	2.26	0
O-D	1.44	8.00	66.94 ± 2.45	68.46	-2.22	65.14 ± 3.15	66.12	-1.48	1.08 ± 0.01	1.12	-3.57	2.73 ± 0.31	2.78	-1.83

^aMean ± S.D; n = 3, ^bError (%) = {(Actual-Predicted)/Predicted} x 100**Table 2.** Analysis of variance (ANOVA) of the response surface quadratic model for the response parameters

Sources	DEE (%)			R _{sh} (%)			Size (mm)			Density (g/cm ³)						
	Coefficients	SS	F-Value	P-Value	Coefficients	SS	F-Value	P-Value	Coefficients	SS	F-Value	P-Value				
Model	66.866	295.82	77.04	0.0023	73.257	147.43	70.07	0.0026	1.131	0.078	61.26	0.0032	2.945	0.63	56.05	0.0037
X ₁	-0.57	1.95	2.54	0.2094	3.985	95.28	226.43	0.0006	-0.102	0.062	242.55	0.0006	0.282	0.48	212.83	0.0007
X ₂	5.628	186.63	243.01	0.0006	-2.965	51.84	123.19	0.0016	-0.042	0.011	41.29	0.0076	0.1367	0.11	49.18	0.0060
X ₁ X ₂	-0.944	3.70	4.81	0.1158	-0.3798	0.60	1.42	0.3188	-0.021	0.0018	7.08	0.0763	-0.060	0.015	6.80	0.0799
X ₁ ²	-7.147	77.85	101.36	0.0021	-1.901	5.51	13.09	0.0363	-0.009	0.0001	0.46	0.5478	-0.1375	0.029	12.88	0.0371
X ₂ ²	-1.928	7.44	9.68	0.0528	-0.087	0.015	0.036	0.8622	-0.027	0.0014	5.56	0.0995	-0.0733	0.011	4.81	0.1159
Residual	-	2.30	-	-	-	1.26	-	-	-	0.0007	-	-	-	0.0067	-	-
SS _{Total}	-	298.13	-	-	-	148.69	-	-	-	0.079	-	-	-	0.63	-	-

$$Y_1 = 66.87 + 5.63 X_2 - 7.15 X_1^2$$

$$Y_2 = 73.26 + 3.98 X_1 - 2.97 X_2 - 1.90 X_1^2$$

$$Y_3 = 1.13 - 0.10 X_1 - 0.042 X_2$$

$$Y_4 = 2.94 + 0.28 X_1 + 0.14 X_2 - 0.14 X_1^2$$

The three-dimensional (3D) response surface plots are very useful in learning about the main interaction effects of the factors, whereas two-dimensional (2D) contour plot gives a visual representation of the values of the response (Nayak et al., 2011; Guru et al., 2013). The 3D response surface and their contour plots were presented to evaluate the effects of the independent variables (factors) on each response investigated. In the figure 3, the 3D response surface plot relating to the DEE depicts a curvilinear relationship with 'a region of maxima' lying between the lower to intermediate levels of both factors, but all the 3D response surface plots relating R_{8h}, particle

size and density exhibits a curvilinear relationship in the form of straight line from low to medium level. The 2D contour plots obtained by plotting the independent variables on all measured responses prove this result.

To evaluate the statistical optimization of the mathematical models, optimal values of responses were obtained by statistical analysis based on the criterion of desirability. The selected optimal process variable parameter of the optimized formula was mentioned in table1. Optimized SD loaded D-CA beads (O-D) were evaluated for DEE (%), R_{8h} (%), particle size (mm) and density (g/cm³). Results showed that optimal condition was obtained for DEE of 66.94 ± 2.45 %, R_{8h} (%) of 65.14 ± 2.32%, the particle size of 1.08 ± 0.01 mm and density 2.73 ± 0.31.

Table 3. Regression statistics

Parameters	DEE (%)	R _{8h} (%)	Size (mm)	Density (g/cm ³)
R ²	0.9923	0.9915	0.9903	0.9894
Adjusted R ²	0.9794	0.9774	0.9741	0.9718
Predicted R ² Adeq	0.9127	0.9056	0.8941	0.8565
Precision	25.834	26.245	22.458	21.664

Figure 3. The 3D response surface plots and the 2D contour plots for (a-b) DEE (%), (c-d) R_{8h} (%), (e-f) Particle Size (nm), and (g-h) Density (g/cm³)

Surface Morphology

The shape and morphological analysis of the optimum formulawere visualized by SEM and presented in figure 4. It was observed that this formula possessed a homogenous and compact structure with a spherical shape. Their detailed examination revealed a very rough surface with large wrinkles and cracksseenon their surface which was probably due to the formulation procedure or might be caused by partially collapsing of the polymeric gel network during the drying (Hasnain et al., 2016). The drug crystals observed on the surface were probably formed as a result of their migration along with water to the surface with water during the drying process (Das et al., 2014).

Particle Size and density

The particle size and density of dried SD loaded D-CA are ranged from 0.93 ± 0.03 to 1.22 ± 0.03 mm and 2.26 ± 0.79 to 3.11 ± 0.82 g/cm³, respectively (table 1). An opposite direction observed in figure 5, decreasing the particle size and an increasing of density was found with increasing of biopolymers blend ratio and CaCl₂ concentration used in the formulation process. This could be due (i) to the increase in viscosity of the polymer-blend solution with the incorporation of D biopolymer at an increasing rate that in turn increased the droplet size and decreased the density during the formulation and (ii) to the shrinkage of the polymeric gel by a

Figure 4. Scanning electron microphotograph of optimum formula

Figure 5. Opposite direction of Particles Size and Density from the different formulations, SD loaded D-CA particle beads

higher degree of the cross-linking with the high concentration of the cross-linker (Nayak et al., 2011).

Drug entrapment efficiency

The DEE of the newly formulated SD loaded D-CA particle beads ranged from 51.98 ± 0.81 to 69.75 ± 0.46 % (table 1). It was observed that the DEE (i) decreased with the decreasing of both SA to D ratio (X_1) and then increased by implying an optimum value for DEE concerning CaCl₂ concentration and it (ii) decreased with the decreasing of CaCl₂ concentration. So, the DEE in particle bead formulations can be attributed to several factors. First, the decrease of SA to D ratio may be due to the increase in the biopolymer concentration. Therefore, the DEE significantly changed. This was probably due to the hydrophilic properties of the biopolymers used. Second, the high CaCl₂ concentration has prevented the drug leaching in the beads. Therefore, an insufficient cross-linking in the formulated particle beads might have larger pores, and the drug leaching may occur through the pores (Hasnain et al., 2016). DEE is advantageous since it transports enough drug at the target site and increases the residence time of the drug within the beads matrix.

Drug-excipient compatibility studies

Biopolymers (SA and D) that have been used in this study as particle bead formulation for controlled drug delivery system are referred as the excipients. The interaction between SD drug molecules and these excipients is essential to be studied, as biopolymers might be not compatible and affect the stability of the encapsulated drug. Besides that, we were also interested in the interactions between various functional groups present in the polymer bend that contribute to the stability of the encapsulating matrix bead formulations. Therefore, instrumental methods like FTIR, DSC, TGA/DTG, and P-XRD was employed to investigate the compatibility of the drug-excipients in the particle beads formulation.

In the first step, SD drug molecule, excipients, and optimum formula were subjected to FTIR analysis. The figure 6 a-b showed the FTIR spectra of SA and D polysaccharides as excipients presented similar bands found in the literature (Heyn et al., 1974; Malakar et al., 2012). In the FTIR spectrum of SA polysaccharide, a full band of -OH stretching vibrations appeared around 3486.62 cm⁻¹. The asymmetric and symmetric -C=O stretching vibration of the carboxylate group was observed at 1529.85 and 1415.14 cm⁻¹, respectively. Other characteristic bands attributed to the saccharide structure appeared at 1035.51 and 2915.08 cm⁻¹ due to C-O stretching

vibration and C-H aliphatic stretching vibration, respectively. Indeed, in the FTIR spectrum of D polysaccharide, the high band centered at 3444.55 cm^{-1} is assigned to the stretching vibrations of the hydroxyl group. In addition to these significant peaks, two vibrational modes located at 1151.72 and 2916.71 cm^{-1} are attached to the stretching vibrations of the C-O-C and the stretching vibrations of the C-H band, respectively. The comparison of FTIR spectrum of SA and D polysaccharides with one of blank D-CA particle beads formulation (figure 6a), showed that the absorption band of the OH moiety has shifted to 3466.23 cm^{-1} and the broadening of the OH stretching band was an indication of the increasing of intermolecular hydrogen bonding (Benfattoum et al., 2018). We could suggest that the hydroxyl

groups of SA polysaccharide, as H-bond acceptors, may undergo dimerization by hydrogen bonding between the hydroxyl groups of D polysaccharide, as proton donors ($\text{O-H}\cdots\text{O-H}$). Additionally, the $-\text{COO}$ stretching bands present in SA, shifted to higher wave number to 1643.63 cm^{-1} . This indicates that the formation of ion-pair electrostatic interactions between Ca^{2+} and carboxylate anion ($-\text{COO}-\text{Ca}-\text{OOC}-$) with sufficient coordination by other electronegative oxygen atoms (Malakar, Nayak et al., 2012). The SD drug molecule shown in figure 6b, displayed a significant peak at 1290.78 cm^{-1} resulted from C-N stretching, whereas peak at 1569.90 cm^{-1} occurred from carboxyl group. Other many peaks were observed

Figure 6. FTIR spectra of (a) excipients, blank D-CA particle beads, (b) pure SD, blank and SD loaded D-CA particle and (c) scheme of the eventual chemical interactions in the optimum formula

between 1500 and 700 cm^{-1} due to the aromatic ring present in the SD structure. These observations are similar in the literature (Piyakulawat et al., 2007) confirming the identity and purity of the SD drug molecule. The significant peaks found in the FTIR spectra of SD discovered to be retained in case of the optimum formula containing SD without an important shift in peak positions. This suggests that there were no significant drug-excipients interactions or process incompatibilities. It also indicates that the drug had retained its initial anhydrous form after the formulation process (Nayak and Pal 2011). The possible interactions between SA, D, SD and Ca^{2+} ions are presented in the figure 6c.

The figure 7, showed the TGA/DTA thermograms of SD drug molecule, excipients, and the optimum bead formula. By comparing the thermograms of SA and D polysaccharide, it could be concluded that upon cross-linking with calcium, the thermal stability of the natural polymer increased slightly as the percent weight loss was reduced and the degradation plot shifted

to a higher temperature. The TGA thermograms of the formulation showed a multi-step weight loss as marked in table 4 due to the progressive degradation of individual components by increasing temperature. The first stage of weight loss from 150 to 235 $^{\circ}\text{C}$ (28 %) corresponds to the deterioration of a part of dextran polysaccharide. The gradual degradation of SD, the other part of dextran polysaccharide, as well as the SA polysaccharide, contributed to the second phase weight loss from 235 to 340 $^{\circ}\text{C}$ (43 %). Characteristics of every component of the formulation were found in table 4. Comparing with blank D-CA particle beads, SD loaded D-CA particle beads showed almost similar total weight loss within this temperature range. From this; it could be inferred that there was no interaction between any of the components and no change has happened to the physical or chemical nature of the drug or excipients. This is in agreement with the results of FTIR study.

Figure 7. (a) The TGA curves, and (b) The P-XRD patterns of SD drug molecule, excipients and optimum formula

Table 4. Thermo-gravimetric parameters of pure biopolymers, pure SD and D-CA beads

Parameters	T_{10} ($^{\circ}\text{C}$) ^a	T_{50} ($^{\circ}\text{C}$) ^b	T_d ($^{\circ}\text{C}$) ^c		Char Yield (%) ^d
			Stage 1	Stage 2	
SA	135.58	288.54	233.66	-	38.45
D	219.81	342.35	201.05	302.11	29.96
SD	289.62	+ 550	296.05	-	58.93
O-D Blank	187.86	399.25	194.96	294.62	45.06
O-D SD	179.36	528.58	187.46	291.57	49.63

^{a,b}Temperature at which 5% or 10% weight loss; ^c Main stage degradation temperature; ^d Weight percentage of material left after TGA analysis at maximum temperature 550 $^{\circ}\text{C}$.

In the third step, to explore the physical nature of the drug molecule in the particle bead formulations, the P-XPD patterns of SD, physical mixture SD, excipient, and optimum formula were collected and are presented in figure 7b. The SD drug molecule showed significant characteristic peaks at different scattering angle ranges from 5° to 50° with various signal intensities. Regarding peak positions, the P-XRD pattern of SD is matched with their anhydrous form reported in the literature (Sinha, Ubaidulla et al., 2015), which confirmed the anhydrous and crystalline nature of the SD. All the central characteristic crystalline peaks produced by the SD drug molecule were observed in the physical mixture and the optimum formula, but with reduced peak intensities (Guru et al., 2013). This is due to the reduction of the weight percentage of SD drug molecule in the presence of excipients. Therefore, it can be concluded that SD was stable and retained its crystalline structure in the particle bead as well.

Swelling degree

Three significant elements control the swelling process of hydrogel particle beads: the cross-link content, the ionic content and the hydrophilic content (Omidian et al., 2008). In brief, the concentration of the cross-linking, CaCl_2 , and the percentage of D biopolymer introduced during the formulation are affected the cross-linked content. Therefore, the swelling degree of SD loaded D-CA particle bead formulations in acidic or basic medium (showed in figure 8a-b) is variable by making all the prepared formulate. It was found lower in acidic gastric pH (1.2) compared with alkaline intestinal pH (7.4). This is due to a hydrophilic nature of alginate and his water-soluble (Choi et al., 1999). However, it is insoluble in acidic condition. At low pH, the quantity of positively charged ions is high, and they decrease the electrical repulsion between negatively charged alginate

molecules. This results showed the protonation of alginate into an insoluble form of alginic acid. Therefore, for acidic pH, the penetration of dissolution fluid through the polymer is slowed down. Moreover, the introduction of other polysaccharides into alginate matrices increases the bead viscosity and allows the synergistic interaction, which enhances the stability of the beads in low pH solution. In alkaline intestinal pH, maximum swelling of the particle beads was noticed at 2.5–3.5 h and after which, erosion and dissolution took place. The swelling behavior of these particle beads in alkaline pH could be explained by the ion exchanging between Ca^{2+} ions of the ionotropically cross-linked beads and the Na^+ ions present in phosphate buffer with the influence of Ca^{2+} -retaining phosphate ions (Nayak et al., 2011). This could result in a disaggregation in the matrix structure leading to matrix erosion, and a dissolution, which can vary slightly in the stomach and begin to swell more when these beads subsequently move to the upper intestine, where the SD is absorbed.

In vitro drug release

The drug release profile of the various newly developed SD loaded D-CA particle beads is shown in figure 9. In the acidic gastric pH, the release amount of SD was minor (less than 5% after 2 h). This could be due to the shrinkage of alginate gel at an acidic pH. The trace amount of SD release could probably be due to the presence of drug crystals into particle bead surface (Nayak and Pal, 2011). Once the particle beads introduced in the alkaline intestinal pH after 2 h, the SD release gradually increases. This behavior is due to the deprotonation of alginic acid that occurs at higher pH. It will draw fluid into the particle

Figure 8. The swelling behavior of various SD loaded D-CA particle bead formulations (a) in acidic medium, pH 1.2, and (b) in phosphate buffer, pH 7.4

Table 5. Results of curve fitting of the *in vitro* SD release data from various SD loaded D-CA particle bead formulations

Formulation code	Zero-order	First- order	Hixson-Crowell	Higuchi Model	Korsmeyer-Peppas	N
D1	0.970	0.946	0.982	0.878	0.962	2.238
D2	0.971	0.955	0.983	0.879	0.967	1.981
D3	0.974	0.951	0.982	0.884	0.969	1.954
D4	0.964	0.956	0.984	0.868	0.950	2.132
D5	0.964	0.957	0.985	0.867	0.949	2.111
D6	0.966	0.960	0.987	0.870	0.963	2.060
D7	0.961	0.958	0.986	0.861	0.959	2.295
D8	0.964	0.922	0.988	0.867	0.991	2.730
D9	0.965	0.957	0.988	0.868	0.972	2.187
O-D	0.965	0.956	0.985	0.870	0.961	2.190

Figure 9. The *In vitro* drug release of various SD loaded D-CA beads formulations at different pH for first 2 h and then, in alkaline intestinal pH for next 6 h (mean $\pm$ S.D, n = 3)

beads, which led to swelling and disintegration. Consequently, the SD release from the beads occurred rapidly. The pattern of SD release indicates that the SD can be continuously released from the acidic pH to nearly neutral condition in which the release amount and speed of freedom were much higher and faster than those in acidic medium. The cumulative percentage of SD released from particle bead formulations after 8 h was within the range of 65.14 ± 3.14 to 78.30 ± 2.34 %. Nevertheless, it was also found to be delayed with the decreasing of polymers blend ratio, so, the increasing of polymer amounts in the formulation matrix. In the case of particle beads containing higher polymer contents, the more hydrophilic property of the polymers could probably bind better with water to form a viscous gel structure, which might blockade the pores on the surface of beads and could be delayed the drug release from these particles formulated beads. Another reasonable explanation of the delayed drug release can be attributed to increasing CaCl_2 concentration employed in the formulation

(Nayak et al., 2011).

The *in vitro* drug release data were evaluated kinetically using various mathematical models like zero-order, first-order, Hixson-Crowell, Higuchi, and Korsmeyer-Peppas models. The result of the curve fitting into different mathematical models is given in table 5. When respective correlation coefficients of these particle beads were compared, the SD release from these beads was found to follow Hixson-Crowell model ($R^2 = 0.982-0.988$) over a period of 8 h. However, Zero-Order ($R^2 = 0.961-0.961$) and Korsmeyer-Peppas model ($R^2 = 0.949-0.991$) were found to be closer to the best-fit Hixson-Crowell model. From this result, it can be concluded that the model indicates the drug release from these particle beads followed a controlled-release pattern. The values of the diffusional exponent (n) determined from Korsmeyer-Peppas model ranged from 1.954 to 2.730, indicating that the drug release from these the particle beads followed the super case-II transport mechanism controlled by swelling and relaxation of D-CA particle bead formulation. This could be attributed due to polymer dissolution and enlargement or relaxation of the polymeric chain (Benfattoum et al., 2018).

Stability studies

The stability studies of the optimized formula under storage conditions for 6 months did not show any physical change and did not expose any degradation of the drug. It showed that there is no significant reduction in the percentage drug retained in the particle bead formulations and also there was no significant difference in the drug release profile for the samples.

Conclusions

The current study was carried out to evaluate a new potentially reinforced carrier for oral drug delivery

system. In this goal, the SD drug molecule was actively loaded into the particle bead carriers during crosslinking process. The SD stacked dextran-calcium alginate particle beads carries as core accomplished designed using a green ionotropic gelation technique were evaluated and optimized by using a 3^2 factorial design. The variation in polymer ratio and cross-linker agent can result in wide range of physico-chemical properties that should provide different drug release patterns. The optimum formula produced has an excellent combination of high drug encapsulation efficiency and suitable controlled drug release pattern over a prolonged period of 8 h, which could be gainful regarding to advanced patient compliance with reduced dosing interval. The in vitro drug release of the SD loaded D-CA bead particles also exhibited pH-dependent swelling that could be advantageous for intestinal drug delivery.

References

- Abdellatif A, El Hamd M, Saleh K. 2016. A Formulation, Optimization and Evaluation of Controlled Released Alginate Beads Loaded-Flurbiprofen. *Journal of Nanomedicine and Nanotechnology*, 7(357):2.
- Agarwal T, Narayana SGH, Pal K, Pramanik K, Giri S, Banerjee I. 2015. Calcium alginate-carboxymethyl cellulose beads for colon-targeted drug delivery. *International journal of biological macromolecules*, 75:409-417.
- Baş D, Boyacı IH. 2007. Modeling and optimization I: Usability of response surface methodology. *Journal of food engineering*, 78(3):836-845.
- Benfattoum K, Haddadine N, Bouslah N, Benaboura A, Maincent P, Barillé R, Sapin-Minet A, El-Shall MS. 2018. Formulation characterization and in vitro evaluation of acacia gum-calcium alginate beads for oral drug delivery systems. *Polymers for Advanced Technologies*, 29(2):884-895.
- Chadha R, Bhandari S. 2014. Drug-excipient compatibility screening—role of thermoanalytical and spectroscopic techniques. *Journal of pharmaceutical and biomedical analysis*, 87:82-97.
- Choi YS, Hong SR, Lee YM, Song KW, Park H, Nam YS. 1999. Study on gelatin-containing artificial skin: I. Preparation and characteristics of novel gelatin-alginate sponge. *Biomaterials*, 20(5):409-417.
- Chowdary K, Mohapatra P, Krishna. 2006. Evaluation of olibanum and its resin as rate controlling matrix for controlled M M. Release of diclofenac. *Indian journal of pharmaceutical sciences*, 68(4).
- Cortesi R, Esposito E, Osti M, Menegatti E, Squarzone G, Davis SS, Nastruzzi C. 1999. Dextran cross-linked gelatin microspheres as a drug delivery system. *European journal of pharmaceuticals and biopharmaceuticals*, 47(2):153-160.
- Das B, Dutta S, Nayak AK, Nanda U. 2014. Zinc alginate-carboxymethyl cashew gum microbeads for prolonged drug release: development and optimization. *International journal of biological macromolecules*, 70:506-515.
- George M, Abraham TE. 2006. Polyionic hydrocolloids for the intestinal delivery of protein drugs: alginate and chitosan—a review. *Journal of controlled release*, 114(1):1-14.
- Ghosh A, Chakraborty P. 2013. Formulation and mathematical optimization of controlled release calcium alginate micro pellets of frusemide. *BioMed Research International* 2013.
- Goyal S, Vashist H, Gupta A, Jindal S, Goyal A. 2011. Development of alginate gel beads-entrapped liposome for colon specific drug delivery of Prednisolone. *Der Pharmacia Sinica*, 2:31-38.
- Guru PR, Nayak AK, Sahu RK. 2013. Oil-entrapped sterculia gum-alginate buoyant systems of aceclofenac: development and in vitro evaluation. *Colloids and Surfaces B: Biointerfaces*, 104:268-275.
- Hasnain MS, Nayak AK, Singh M, Tabish M, Ansari MT, Ara TJ. 2016. Alginate-based bipolymeric-nanobioceramic composite matrices for sustained drug release. *International journal of biological macromolecules*, 83:71-77.
- Heyn AN. 1974. The infrared absorption spectrum of dextran and its bound water. *Biopolymers: Original Research on Biomolecules*, 13(3):475-506.
- Kato y, Hosokawa T, Hayakawa E, Ito K. 1993. Influence of liposomes on tryptic digestion of insulin. *Biological and Pharmaceutical Bulletin*, 16(5):457-461.
- Kim CK, Lee EJ. 1992. The controlled release of blue dextran from alginate beads. *International Journal of pharmaceuticals*, 79(1-3):11-19.
- Laroui H, Dalmasso G, Nguyen HTT, Yan Y, Sitaraman SV, Merlin D. 2010. Drug-loaded nanoparticles targeted to the colon with polysaccharide hydrogel reduce colitis in a mouse model. *Gastroenterology*, 138(3):843-853. e842.
- Malakar J, Nayak AK, Pal D. 2012. Development of cloxacillin loaded multiple-unit alginate-based floating system by emulsion-gelation method. *International journal of biological macromolecules*, 50(1):138-147.
- Matricardi P, Di Meo C, Coviello T, Hennink WE, Alhaique F. 2013. Interpenetrating polymer networks

- polysaccharide hydrogels for drug delivery and tissue engineering. *Advanced Drug Delivery Reviews*, 65(9):1172-1187.
- Mokhtarzadeh A, Alibakhshi A, Yaghoobi H, Hashemi M, Hejazi M, Ramezani M. 2016. Recent advances on biocompatible and biodegradable nanoparticles as gene carriers. *Expert opinion on biological therapy*, 16(6):771-785.
- Morales E, Rubilar M, Burgos-Díaz C, Acevedo F, Penning M, Shene C. 2017. Alginate/Shellac beads developed by external gelation as a highly efficient model system for oil encapsulation with intestinal delivery. *Food Hydrocolloids*, 70:321-328.
- Nayak AK, Pal D. 2011. Development of pH-sensitive tamarind seed polysaccharide–alginate composite beads for controlled diclofenac sodium delivery using response surface methodology. *International journal of biological macromolecules*, 49(4):784-793.
- Nayak AK, Pal D, Santra K. 2014. Tamarind seed polysaccharide–gellan mucoadhesive beads for controlled release of metformin HCl. *Carbohydrate polymers*, 103:154-163.
- Omidian H, Park K. 2008. Swelling agents and devices in oral drug delivery. *Journal of Drug Delivery Science and Technology*, 18(2):83-93.
- Palanisamy P, Jayakar B, Chandira RM, Venkateshwarlu B, Pasupathi A. 2013. Formulation, Evaluation and Development of Immediate Release Film Coated Tablets of Atorvastatin and Sustained Release Film Coated Tablets of Ezetimibe in Capsules Form Usp. *International Journal of Medicine and Pharmacy*, 1(1):33-58.
- Petruzzelli M, Vacca M, Moschetta A, Sasso RC, Palasciano G, van Erpecum KJ, Portincasa P. 2007. Intestinal mucosal damage caused by non-steroidal anti-inflammatory drugs: role of bile salts. *Clinical biochemistry*, 40(8):503-510.
- Piyakulawat P, Praphairaksit N, Chantarasiri N, Muangsin N. 2007. Preparation and evaluation of chitosan/carrageenan beads for controlled release of sodium diclofenac. *Aaps PharmSciTech*, 8(4):120.
- Sanchez A, Tobío MA, González L, Fabra A, Alonso M a J. 2003. Biodegradable micro-and nanoparticles as long-term delivery vehicles for interferon-alpha. *European journal of pharmaceutical sciences*, 18(3-4):221-229.
- Shiraishi S, IMAI T, OTAGIRI M. 1993. Controlled-release preparation of indomethacin using calcium alginate gel. *Biological and Pharmaceutical Bulletin*, 16(11):1164-1168.
- Siddiqui NN, Aman A, Silipo A, Qader SAU, Molinaro A. 2014. Structural analysis and characterization of dextran produced by wild and mutant strains of *Leuconostoc mesenteroides*. *Carbohydrate polymers*, 99:331-338.
- Singh B, Sharma D, Gupta A. 2009. A study towards release dynamics of thiram fungicide from starch–alginate beads to control environmental and health hazards. *Journal of hazardous materials*, 161(1):208-216.
- Sinha P, Ubaidulla U, Hasnain MS, Nayak AK, Rama B. 2015. Alginate-okra gum blend beads of diclofenac sodium from aqueous template using ZnSO₄ as a cross-linker. *International journal of biological macromolecules*, 79:555-563.
- Sun G, Mao JJ. 2012. Engineering dextran-based scaffolds for drug delivery and tissue repair. *Nanomedicine*, 7(11):1771-1784.
- Tønnesen HH, Karlsen J. 2002. Alginate in drug delivery systems. *Drug development and industrial pharmacy*, 28(6):621-630.
- Volodkin DV, Petrov AI, Prevot M, Sukhorukov GB. 2004. Matrix polyelectrolyte microcapsules: new system for macromolecule encapsulation. *Langmuir*, 20(8):3398-3406.
- Vu B, Chen M, Crawford RJ, Ivanova EP. 2009. Bacterial extracellular polysaccharides involved in biofilm formation. *Molecules*, 14(7):2535-2554.
- Yotsuyanagi T, Ohkubo T, Ohhashi T, Ikeda K. 1987. Calcium-induced gelation of alginic acid and pH-sensitive reswelling of dried gels. *Chemical and Pharmaceutical Bulletin*, 35(4):1555-1563.