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ORIGINAL ARTICLE

Emulsification/internal gelation as a method for preparation of diclofenac sodium–sodium alginate microparticles

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KEYWORDS

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Abstract Emulsification/internal gelation has been suggested as an alternative to extrusion/external gelation in the encapsulation of several compounds including non-steroidal anti-inflammatory drugs such as diclofenac sodium. The objective of the present study was a trial to formulate diclofenac sodium as controlled release microparticles that might be administered once or twice daily. This could be achieved via emulsification/internal gelation technique applying Box-Behnken design to choose these formulae. Box-Behnken design determined fifteen formulae containing specified amounts of the independent variables, which included stirring speed in rpm (*X*1), drug:polymer ratio (*X*2) and the surfactant span 80% (*X*3). The dependent variables studied were cumulative percent release after two hours (*Y*1), four hours (*Y*2) and eight hours (*Y*3). The prepared microparticles were characterized for their production yield, sizes, shapes and morphology, entrapment efficiency and Diclofenac sodium in vitro release as well. The results showed that the production yield of the prepared diclofenac sodium microparticles was found to be between 79.55% and 97.41%. The formulated microparticles exhibited acceptable drug content values that lie in the range 66.20–96.36%. Also, the data obtained revealed that increasing the mixing speed (*X*1) generally resulted in decreased microparticle size. In addition, scanning electron microscope images of the microparticles illustrated that the formula contains lower span concentration (1%) in combination with lower stir-

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ring speed (200 rpm) which showed wrinkled, but smooth surfaces. However, by increasing surfactant concentration, microspheres' surfaces become smoother and slightly porous. Kinetic treatment of the in vitro release from drug-loaded microparticles indicated that the zero order is the drug release mechanism for the most formulae.

1. Introduction

Emulsification/internal gelation has been suggested as an alternative to extrusion/external gelation in the encapsulation of several compounds including sensitive biologicals such as protein drugs. An emulsification/internal gelation method is proposed for producing small diameter alginate microspheres in large quantity. The difficulty in using dispersion/external gelation techniques with ionic polysaccharide is that the calcium source ($CaCl_2$) is insoluble in the oil phase. As an alternative, internal gelation of the dispersed alginate droplets may be initiated by releasing Ca^{2+} from an insoluble complex (calcium salt) through pH reduction (Friese et al., 2000; Gref et al., 2001). Diclofenac sodium has analgesic, antipyretic and anti-inflammatory properties. It is an inhibitor of prostaglandin synthetase. It is used for the relief of pain and inflammation in conditions such as rheumatoid arthritis, osteoarthritis, acute gout and following some surgical procedures. The usual dose by mouth is 75–150 mg daily in divided doses (Sanchez et al., 2003). By controlling the conditions under which the water-in oil dispersion is produced, the bead size can be controlled from a few microns to millimeters in diameter. Biodegradable polymeric particles, especially microparticles and nanoparticles, have attracted considerable attention as potential drug controlled delivery devices (Catarina et al., 2006; Qiu and Park, 2001; Silva et al., 2006). Alginates, which are naturally occurring substances found in brown seaweed and algae have received much attention in pharmaceutical dosage forms, particularly as a vehicle for controlled drug delivery (Chen and Subirade, 2007). Alginates can be considered as block polymers, which mainly consist of mannuronic acid (M), guluronic acid (G) and mannuronic–guluronic (MG) blocks. One of the methods under consideration for the production of these drug delivery systems is emulsification/internal gelation. In this context, the use of alginate microcapsules as oral delivery system for NSAIDs seems very attractive. First, the alginate matrix could protect the drug from hostile environments (Chan et al., 2002). Second, alginate possesses mucoadhesive properties which could increase the contact time between microcapsules and absorptive sites, and therefore could enhance the uptake of encapsulated drug (Guan et al., 2001). Third, biodegradable alginate microcapsules may show variable release kinetics (Perumal, 2001). Fourth, the low toxicity and low immunogenicity of alginate make this polymer a safe matrix (Chan et al., 2002). Fifth, alginate is readily available and inexpensive. ~~Therefore, the goal of this study was to prepare and fully characterize diclofenac sodium loaded alginate microcapsules. The effect of some factors, such as drug:polymer ratio, concentration of span 80 and the stirring speed on the mean particle size, microcapsule yield, drug release and drug entrapment efficiency of the resulting diclofenac–alginate microcapsules was investigated.~~

~~The purpose of this research is to outline use of the emulsification/internal gelation for microencapsulation of diclofenac sodium, with particular reference to the use of alginate as the polymer matrix.~~

Therefore, the goal of this study was to prepare, optimize and fully characterize diclofenac sodium loaded alginate microcapsules. The effect of some factors, such as drug:polymer ratio, concentration of span 80 and the stirring speed on the mean particle size, microparticles yield, drug release and drug entrapment efficiency of the resulting diclofenac–alginate microparticles was investigated. In addition, the study aims to outline the use of the emulsification/internal gelation for microencapsulation of diclofenac sodium, with particular reference to the use of alginate as the polymer matrix.

2. Experimental materials

Diclofenac sodium and Sodium alginate (MWt 216) were purchased from Sigma chemical Co. (NJ, USA). Span 80 was obtained from Fluka Chemica (Buch, Switzerland). Paraffin oil (heavy) was obtained from El-Nasr Co. (Abu-Zabal, Cairo Egypt). Polysorbates 80 (Tween 80) was purchased from BDH chemical Ltd. Co. (Poole, England). Other materials and solvents are of reagent or analytical grade, and they were used without further purification.

2.1. Design of the experiment

A Box-Behnken design was selected for formulating diclofenac sodium microparticles with the following independent variables: stirring speed, rpm, ($X1$), drug:polymer ratio ($X2$) and span 80% ($X3$). Three levels (−1, 0 and + 1) of each independent variable were used for the above design. The values of the corresponding variables were 200, 400 and 600 rpm for the machine stirring speed; 1:1, 1:2 and 1:3 for drug–polymer ratio and 1%, 1.5% and 2% for span 80%. The effect of these three factors; namely, drug to polymer ratio, span 80 concentration and the speed of stirring on the microparticles attributes was studied.

2.2. Preparation of alginate coated microparticles

Composition of different suggested formulae of diclofenac sodium microparticles is listed in Table 1. A basal encapsulation protocol was used to prepare microparticles (Silva et al., 2006). In brief, different concentrations of sodium alginate solution were prepared by dissolving the specified amount of the polymer (0.5–1.5 gm) in 30 ml hot water and then diclofenac sodium was dispersed in this solution using a magnetic stirrer (Stuart SM27, Dublin, Ireland) for 10 min. The concentrations of diclofenac sodium to sodium alginate were prepared in different drug:polymer ratios 1:1, 1:2 and 1:3. A suspension of $CaCO_3$ at 5% (w/v) was added to the alginate-diclofenac sodium solution,

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Table 1 Composition of different suggested formulae of diclofenac sodium microparticles using sodium alginate according to pharmaceutical point of view.

Formula No.	Drug (gm)	Sodium alginate (gm)	Calcium carbonate (gm)	Liquid paraffin (ml)	Span 80 (ml)	Speed (rpm)	Total weight (gm)
F1	0.5	0.5	0.375	100	1.5	200	1.375
F2	0.5	0.5	0.375	100	1.0	400	1.375
F3	0.5	0.5	0.375	100	2	400	1.375
F4	0.5	0.5	0.375	100	1.5	600	1.375
F5	0.5	1	0.375	100	1.0	200	1.875
F6	0.5	1	0.375	100	2	200	1.875
F7	0.5	1	0.375	100	1.5	400	1.875
F8	0.5	1	0.375	100	1.5	400	1.875
F9	0.5	1	0.375	100	1.5	400	1.875
F10	0.5	1	0.375	100	1.0	600	1.875
F11	0.5	1	0.375	100	2	600	1.875
F12	0.5	1.5	0.375	100	1.5	200	2.375
F13	0.5	1.5	0.375	100	1.0	400	2.375
F14	0.5	1.5	0.375	100	2	400	2.375
F15	0.5	1.5	0.375	100	1.5	600	2.375

Speed	+1 = 600	0 = 400	−1 = 200
Drug: polymer ratio	+1 = 1:3	0 = 1:2	−1 = 1:1
Span 80%	+1 = 2%	0 = 1.5%	−1 = 1%

after homogenization (Mechanika Precyzyjna-MPW-309, Poland), the mixture was dispersed into paraffin oil (30% internal phase ratio, v/v) containing different concentrations of span 80 as emulsifying agent and was emulsified by stirring at different speeds. After emulsification for 15 min, 20 ml of paraffin oil containing 0.2 ml glacial acetic acid (acid/Ca molar ratio of 3.5) was added to the w/o emulsion and stirring was continued to permit calcium carbonate solubilization (Chen and Subirade, 2007). A solution of $CaCl_2$ (0.05 M) containing 1% Tween 80 was added to the partition to recover the gelled microspheres from oily phase by decantation. Microparticles were washed with 0.05 M $CaCl_2$ containing 1% Tween 80 to remove residual oil. Microparticles were recovered from oily phase by using an acetate buffer at pH 4.5 and successively washed with this buffer until no more oil was detected by optical microscope observation. A sample of the prepared microparticles for all formulae is examined under optical microscope to detect the presence of oil droplets. Furthermore a sample of the prepared microparticles is pressed between two filter papers to detect the presence of any oily droplets. Microparticles were dried for 48 h at room temperature and stored in a dessicator until starting experiment. The experiment was repeated three times for each formula.

2.3. Production yield determination

The yield of the microparticles was determined in triplicate by dividing the weight of the prepared microparticles by the original amount of the polymer and drug used and the results were expressed as a percentage according to the equation (Jelvehgari et al., 2010):

$$\%\,\text{Yield} = (\text{Actual weight of product}/\text{Total weight of excipient and drug}) \times 100$$

2.4. Particle size determination

The dried microparticles were weighed and sized using USP standard sieve set, (Rx-86-1, Cole-Parmer Instrument Co., USA). The fraction of microparticles remaining on each sieve was collected and the mean particle size of the microparticles was assigned as the percentage of microparticles retained at each sieve multiplied by the average particle size of the sieve used (Choi et al., 2002). Each experiment was carried out in triplicate.

2.5. Determination of drug content

The drug content of the prepared diclofenac sodium microparticles was determined by the digestion method (Perumal, 2001; Jelvehgari et al., 2010) and the experiments were carried out in triplicate. One hundred micrograms of diclofenac sodium microparticles was crushed carefully in a glass mortar and a definite weight was transferred to a 100 ml volumetric flask using phosphate buffer pH 7.4. The volumetric flask was completed to the volume with phosphate buffer pH 7.4 then agitated for 5 min each hour for 5 h. The sample was filtered and the drug concentration was determined spectrophotometerically at 277 nm (Spectrophotometer UV. 1601, Shimadzu Co., Japan). The same procedure was applied for the plain formula, which was used as a blank.

2.6. Microparticles morphology by scanning electron microscopy

The morphology of the microparticles surfaces was investigated using scanning electron microscopy. Microspheres were spread on a carbon double-adhesive layer on a metal holder and gold-coated using Ion-Sputtering device (Jeol Fine-Coat JFC 1100E, Jeol Ltd., Tokyo, Japan). The microparticles were scanned by Scanning Electron Microscope (SEM) (Jeol JSM-5400 LV, Jeol Ltd., Tokyo, Japan).

2.7. In vitro release of diclofenac sodium microparticles

Dissolution testing of the prepared microparticles equivalent to 100 mg of diclofenac sodium was performed with the rotating basket apparatus according to USP 24 apparatus 1 (SR11 6 Flask, Hanson Co., USA). Hard gelatin capsules No. 2 filled with known amount of microparticles were used for dissolu-

Table 2 Production yield and percentage recovery (drug content) of diclofenac sodium–sodium alginate microparticles.

Formula No.	Drug–polymer ratio	Production yield%	Theoretical drug content (gm)	Actual drug content (gm)	Drug content%
F1	1:1	93.70 ± 3.67	50.00	46.49 ± 1.96	92.98 ± 3.92
F2	1:1	92.90 ± 4.26	50.00	45.90 ± 2.22	91.80 ± 4.44
F3	1:1	83.93 ± 3.78	50.00	33.51 ± 3.45	66.20 ± 6.90
F4	1:1	81.82 ± 5.01	50.00	37.83 ± 3.34	75.66 ± 6.68
F5	1:2	96.75 ± 4.21	33.33	32.11 ± 2.01	96.36 ± 4.02
F6	1:2	85.02 ± 2.98	33.33	23.40 ± 1.97	70.21 ± 3.94
F7	1:2	88.39 ± 3.65	33.33	25.23 ± 2.46	81.69 ± 4.92
F8	1:2	90.25 ± 4.46	33.33	29.55 ± 3.12	88.65 ± 6.24
F9	1:2	88.54 ± 3.98	33.33	29.05 ± 2.46	87.15 ± 4.92
F10	1:2	86.90 ± 4.34	33.33	24.51 ± 2.34	73.53 ± 4.68
F11	1:2	79.55 ± 3.87	33.33	26.12 ± 2.18	78.36 ± 4.36
F12	1:3	89.07 ± 4.22	25	23.82 ± 1.24	95.28 ± 2.48
F13	1:3	97.41 ± 4.78	25	22.26 ± 1.66	89.04 ± 3.32
F14	1:3	82.29 ± 3.66	25	19.71 ± 2.08	78.84 ± 4.16
F15	1:3	85.30 ± 4.44	25	21.17 ± 1.68	84.68 ± 3.36

Table 3 Fraction percent of weight distribution of different formulae of diclofenac sodium–sodium alginate microparticles.

Formula No.	Fraction percent of weight distribution in:					
	890–630 μm	630–400 μm	400–315 μm	315–200 μm	200–160 μm	160–100 μm
F1	27.72	29.85	16.33	19.76	3.38	2.963
F2	14.68	29.59	29.35	14.88	9.0	2.5
F3	17.29	33.513	24.97	12.865	5.941	5.421
F4	8.04	29.78	18.67	29.33	11.29	2.56
F5	32.68	25.58	15.55	20.27	5.53	0.39
F6	29.73	28.59	14.58	16.57	5.75	4.78
F7	16.42	27.24	26.05	20.74	4.68	4.87
F8	15.24	27.08	23.12	25.67	5.24	3.65
F9	13.32	30.01	12.70	28.31	9.77	5.89
F10	5.25	22.37	32.65	19.76	14.29	5.68
F11	6.66	22.62	19.73	27.65	15.89	7.45
F12	22.26	31.05	16.21	22.25	5.01	3.22
F13	13.55	19.46	18.05	32.54	7.54	8.86
F14	11.60	21.50	16.54	35.86	7.45	7.05
F15	9.79	27.80	15.69	31.13	11.34	4.24

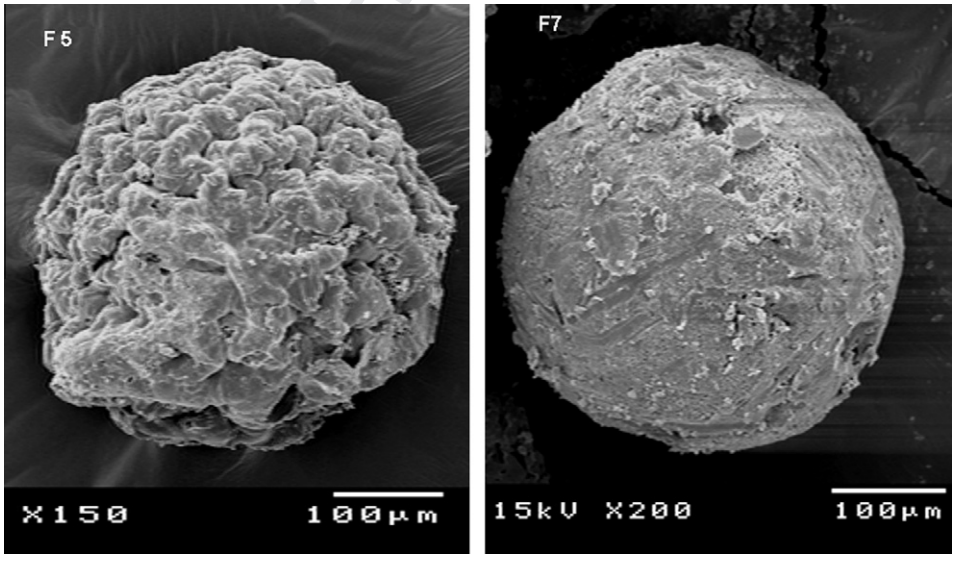

Figure 1 Scanning electron micrograph of diclofenac sodium–sodium alginate microparticles, F5, F7.

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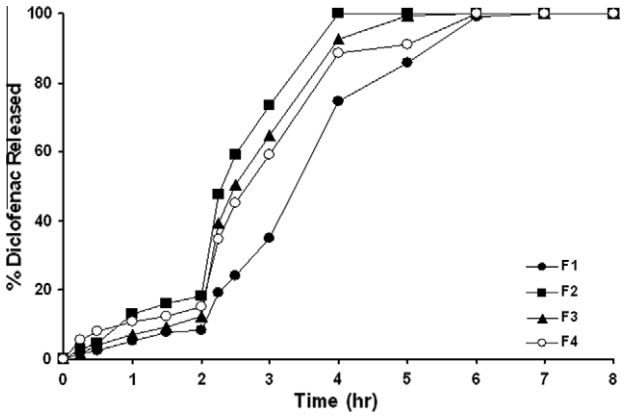

Figure 2 In vitro release of diclofenac sodium–sodium alginate capsules containing drug:polymer ratio 1:1.

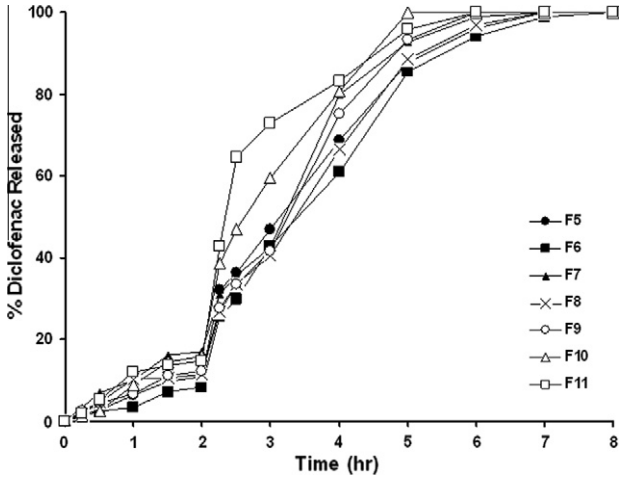

Figure 3 In vitro release of diclofenac sodium–sodium alginate capsules containing drug:polymer ratio 1:2.

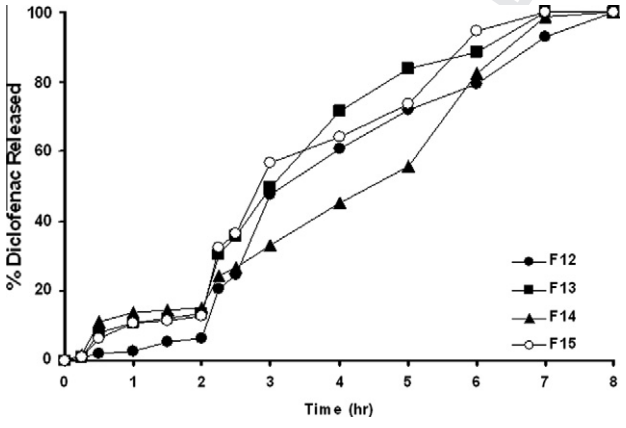

Figure 4 In vitro release of diclofenac sodium–sodium alginate capsules containing drug:polymer ratio 1:3.

tion testing using basket speed of 50 rpm and a temperature of 37 °C ± 0.5. Regarding the dissolution medium, the pH shift method (Mahrous et al., 2010) was used. First, 500 ml of 0.1 N HCl pH 1.2, was used as the release medium for two hours, followed by addition of (14.25) milliliters of 7 M potassium dihydrogen orthophosphate containing 16.75% (w/v) NaOH in order to change the pH of the medium to 7.4 and the experiment was continued for another six hours. Three milliliters of each sample were removed at specific intervals throughout the whole 8 h (0.25, 0.5, 1, 1.5, 2, 2.25, 2.5, 3, 4, 5, 6, 7 and 8 h). The samples were diluted appropriately with the release medium and absorbance was measured at the predetermined λ_{max} of each medium against a blank of this medium. The withdrawn samples were replaced with equal volumes of the release medium. It is worthy to mention that the experiments were carried out in triplicate.

2.8. Kinetics of the in vitro release of diclofenac sodium capsules

The kinetic parameters for the in vitro release of diclofenac sodium were determined and then analyzed in order to find the proper order of the drug release using a specific computer program (Stategraph plus). Zero and first order kinetics, as well as controlled diffusion or Higuchi diffusion model (Higuchi et al., 1963), in addition to Hixson-Crowell cube root law (Hixon and Crowel, 1977) and Baker-Lonsdale equation (Baker and Lonsdal, 1974) were investigated.

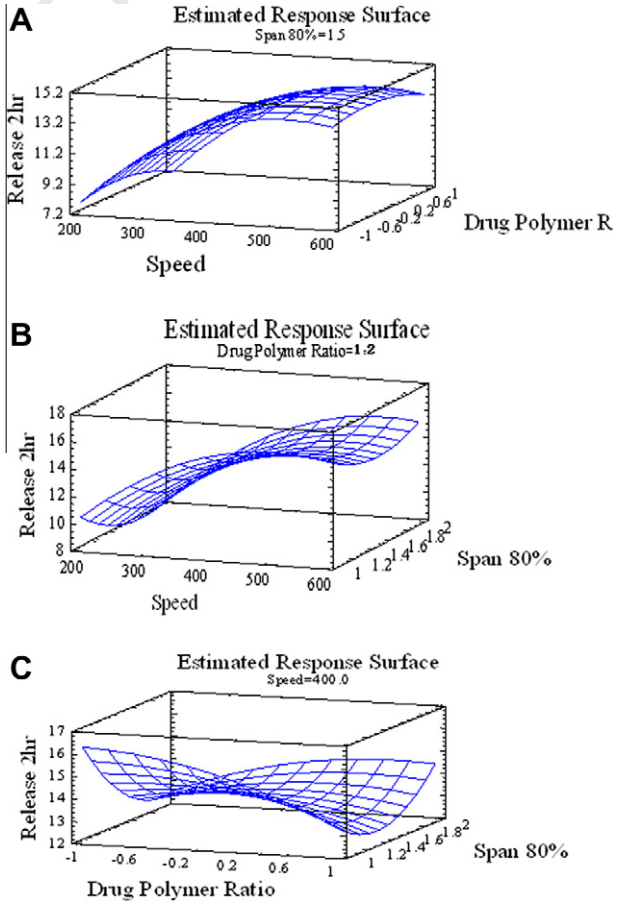

Figure 5 Three dimensional contour plots for the effect of speed ($X1$), drug–polymer ratio ($X2$) and Span 80% ($X3$) on the cumulative percent release after two hours ($Y1$).

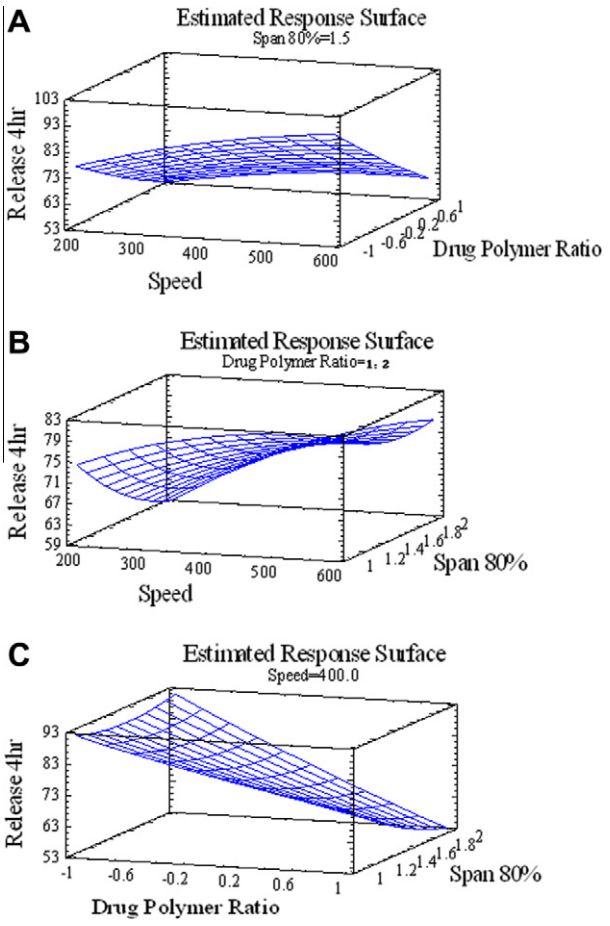

Figure 6 Three dimensional contour plots for the effect of speed ($X1$), drug–polymer ratio ($X2$) and Span 80% ($X3$)) on the cumulative percent release after four hours ($Y1$).

3. Results and discussion

3.1. Experimental design

Box-Behnken design, as shown in Table 1, was used for formulating diclofenac sodium microparticles (Kramar et al., 2003) deals with optimization of formulation variables to improve the in vitro release of dosage forms. The three independent variables are stirring speed ($X1$), drug:polymer ratio ($X2$) and span 80% ($X3$). According to Box-Behnken design, 15 formulae of Diclofenac sodium-loaded microparticles were prepared.

Three levels of the speed were used 200, 400 and 600 rpm denoted the values −1, 0 and +1 in the above design, respectively. Drug:polymer ratio was varied to be 1:1, 1:2 and 1:3, also denoted the values −1, 0 and +1, respectively. Moreover, span 80% was chosen to be 1%, 1.5% and 2%, denoted −1, 0 and +1 value, respectively. The chosen dependent variables to be tested for the prepared microparticles were the in vitro release of the drug capsules after 2 h ($Y1$), 4 h ($Y2$) and 8 h ($Y3$).

3.2. Production yield determination

The range of the production yield of the prepared diclofenac sodium was found to be between 79.55% and 97.41% as shown in Table 2. The highest microparticles crop was obtained in case of formula 13 (97.41%), in which stirring speed was intermediate value (400 rpm) in combination with lower span concentration (1%) and increasing the polymer weight ratio as well. By applying the highest stirring speed (600 rpm) in combination with the highest span concentration (2%), a lowest microparticles yield was obtained as the case of formula 11 (79.55%).

3.3. Microparticles drug content

The drug content determination measures the actual loaded weight of diclofenac sodium inside the microparticles. Microparticles formulated by using slow stirring rate (200) in combination with lower or intermediate span concentrations were found to have higher drug contents (as the case of formulations F5, F12, F1), Table 2. On the other hand, microparticle formulations prepared by using higher stirring speeds and/or higher span concentrations exhibited lower drug contents, as the case of F3 (66.2%). Alipour et al. (2010) showed that microencapsulation by the emulsification/gelation method involves two major steps, the formation of stable droplets of the polymer solution with drug incorporated in as an emulsified system and the subsequent solidification of the droplets. These two steps have a significant effect on size and encapsulation efficiency of microparticles.

3.4. Particle size distribution

The fraction percent of weight distribution of different formulae of diclofenac sodium–sodium alginate microparticles determined by sieve analysis is illustrated in Table 3. The range of sieve employed ranged from 890 to 100 μm. The narrowest distribution patterns were observed in an ascending order for formulae F11, F10, F14, F13 and F15, in which the microparticle sizes lay in the range 315–200 μm. An intermediate distribution profile was recorded with F10 (400–315 μm). In addition, the fraction percent of the fines (160–100 μm) was found to increase by increasing span concentration and stirring speed (F7), Table 3. Moreover, slight increases in the microparticle sizes were detected in formulae F4, F9, F8, F7, F2, F3, F12, and F1, in which particle sizes in the range of 630–400 μm were exhibited. Furthermore, the microparticles sizes of formulae F5 and F6 were found to be the largest (890–630 μm). Stirring speed is the most important parameter for controlling the drug/matrix dispersion's droplet size in the continuous phase. It was shown that increasing the stirring speed generally results in decreased microparticle size, as it produces smaller emulsion droplets through stronger shear forces and increased turbulence (Perumal, 2001).

In this study, the high stirring speed (600 rpm) produced microparticles with small particle size while the lower stirring speed (200 rpm) produced large sized microparticles.

3.5. Microparticles' shapes and surfaces (SEM)

Scanning electron microscopy was used to characterize the shapes and the surfaces of the prepared diclofenac sodium microparticles. Fig. 1 displays the SEM images of the formulations F5 and F7 as representatives of all microparticles formulae. For comparison, F–5 (1% span and 200 rpm stirring) microparticles showed rough and irregular surfaces and no aggregation was observed. Upon increasing the span concentration and stirring speed, as the case of F7 (1.5% span and

400 rpm stirring), microparticles' surfaces become more smooth and slightly porous.

3.6. *In vitro* release of diclofenac sodium microparticles

The in vitro release of diclofenac sodium from its-loaded alginate microparticles was evaluated by measuring the cumulative percent release. The results showed that at pH 1.2, all the microparticles were retained intact nearly without swelling. This behavior depends on the nature of the used polymer.

Fig. 2 shows the in vitro release of diclofenac sodium from its-loaded microparticles containing formulae (F1–F4) using constant drug:polymer ratio 1:1 (*X*2) with variable span 80, 1% for F2; 1.5% for F1 and F4; 2% for F3 (*X*3), and the variable speeds; 200 rpm for F1; 400 rpm for F2 and F3; 600 rpm for F4 (*X*1). The results showed that the in vitro drug release from these formulae is biphasic under the control of dissolution medium pH. In the acidic region, no swelling could be observed for microparticles formulations, which slowed the drug release rate (not more than 16% of the loaded drug was released). In addition, the very poor solubility of diclofenac sodium plays an important role in retarding its release from microparticles in the acidic medium (Higuchi et al., 1963). In contrast, upon shifting the release medium to the alkaline region, a pronounced enhancement was detected in the drug release rate, as a result of microparticles swelling and increased drug solubility. The maximum and minimum percent released were observed to be 15.43% and 8.46% at the end of two hours for formulae F14 and F2, respectively (*Y*1). After eight hours of dissolution (*Y*3), 100% was released for the aforementioned two formulae. It has been reported that the swelling can be enhanced in the presence of phosphate ions which act as calcium sequestrates. The exchange of the divalent calcium involved in electrostatic links between various carboxylate moieties of the alginate chain, with the monovalent sodium leads to an increased osmotic pressure inside the gel, causing it to swell. The swelling of the alginate impregnated in the microparticles increased their porosity, thereby allowing the quick release (Al-Kassas et al., 2007).

The in vitro release of diclofenac sodium from its microparticles containing formulae F5-F11 is illustrated in Fig. 3. Different formulation variables were studied in these formulae including drug:polymer ratio (*X*2), span 80 concentration (*X*3), and stirring speed (*X*1). The maximum and minimum percent released were observed to be 16.99% and 8.40% released at the end of two hours (*Y*1), while 83.34% and 60.76% release values were recorded after four hours for F6 and F11, respectively. After eight hours of dissolution (*Y*3), 100% and 94.22% were released for the aforementioned two formulae.

Moreover, Fig. 4 illustrates the in vitro release of diclofenac sodium from its-loaded microparticles made of a higher sodium alginate concentration (1.5%), i.e., formulae F12-F15, using constant drug:polymer ratio (1:3) (*X*2) with varying both span 80 weight ratio (*X*3) and stirring speed (*X*1). Combination of higher span concentration with lower and medium stirring speed resulted in slower in vitro release rates in the alkaline pH (F14 and F12). In contrast, fast release rates were observed with the formulae prepared by using lower and medium span 80 concentrations in combination of higher speed values (F15 and F13).

Silva et al. (2006) noted that increasing alginate concentration caused a slightly higher retention of insulin at pH 1.2. They also observed that insulin release in acidic medium decreased ~~from~~ when alginate concentration was increased.

From Table 4 and Figs. 5-7, it could be concluded that by increasing *X*2 and decreasing *X*1, the drug release (*Y*3) decreased at fixed *X*3 levels. This indicates a negative correlation between *Y*3 and *X*2, Figs. 5-7(B). In addition, at lower and medium *X*3 levels and at all *X*2 levels, the increase in *X*1 level did not prevail ~~in~~ an observable change in the in vitro release rate (*Y*3). However, the effect of increasing *X*1 level on the in vitro release rate is only pronounced at medium and higher *X*2 and *X*3 levels indicating a positive correlation between *Y*3 and *X*1. For example, when *X*2 was fixed at medium level (1:2) and *X*3 at high level (2%), *Y*3 increased from 85.44% to 95.82% by increasing *X*1 from low level (200 rpm) to high level (600 rpm).

Moreover, the effect of increasing *X*2 level on the in vitro release rate (*Y*3) could be noticeable only at a higher *X*1 level in combination with medium and higher *X*2 levels. For example, when *X*2 at high level (1:3) was used, *Y*3 decreased from 83.65% to 55.63% when *X*3 increased from low level (1%) to high level (2%). Also, at fixed higher *X*1 level (600 rpm high level) and at medium *X*2 level (1:2), *Y*3 decreased from 100% to 95.82% when *X*3 increased from low level (1%) to high level (2%).

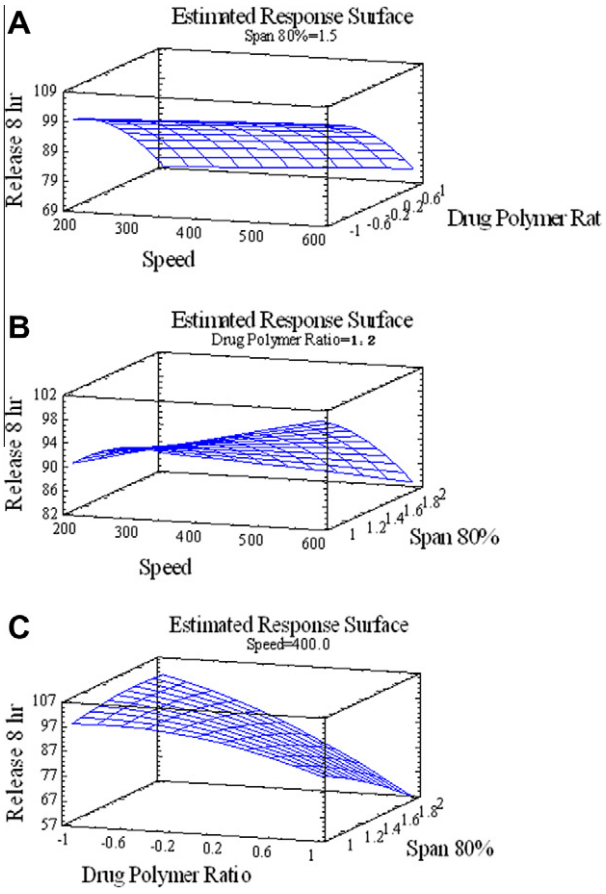

Figure 7 Three dimensional contour plots for the effect of speed (*X*1), drug–polymer ratio (*X*2) and Span 80% (*X*3) on the cumulative percent release after eight hours.

Table 4 Observed values of responses for the Box-Behnken design of diclofenac sodium–sodium alginate capsules.

Formula	Variable level in coded form			Cumulative percent release		
	*X*1	*X*2	*X*3	*Y*1 (2 h)	*Y*2 (4 h)	*Y*3 (8 h)
F1	−1	−1	0	8.46	74.56	74.56
F2	0	−1	−1	15.43	100	100
F3	0	−1	+1	12.34	92.70	92.70
F4	+1	−1	0	14.56	88.65	88.65
F5	−1	0	−1	10.77	68.65	100
F6	−1	0	+1	8.40	60.76	94.22
F7	0	0	0	16.99	79.76	100
F8	0	0	0	11.50	66.41	97.64
F9	0	0	0	12.22	75.21	100
F10	+1	0	−1	15.87	80.76	100
F11	+1	0	+1	14.88	83.34	100
F12	−1	+1	0	6.54	60.76	94.43
F13	0	+1	−1	13.57	71.54	100
F14	0	+1	+1	15.32	45.26	100
F15	+1	+1	0	12.76	64.34	100

Table 5 The calculated correlation coefficient values and zero order kinetic parameters for the in vitro release of diclofenac sodium–sodium alginate capsules employing different kinetic orders or systems.

Formula	Zero order	First order	Higuchi	Hixon-Crowel	B-L	($t_{1/2}$) h	Kinetic rate constant
F1	0.942	0.905	0.879	0.919	0.953	2.854	17.51
F2	0.953	0.923	0.916	0.942	0.898	2.187	22.85
F3	0.909	0.901	0.833	0.132	0.201	2.64	1.081
F4	0.955	0.922	0.910	0.939	0.894	2.42	20.57
F5	0.973	0.902	0.912	0.934	0.814	2.72	18.36
F6	0.974	0.913	0.915	0.942	0.878	2.80	17.80
F7	0.967	0.859	0.902	0.904	0.811	2.77	17.98
F8	0.975	0.892	0.919	0.935	0.879	2.79	17.90
F9	0.959	0.868	0.888	0.907	0.825	2.60	19.21
F10	0.974	0.943	0.928	0.956	0.873	2.55	19.59
F11	0.958	0.912	0.934	0.950	0.897	2.36	21.15
F12	0.976	0.957	0.939	0.972	0.924	3.49	14.30
F13	0.986	0.933	0.961	0.972	0.927	3.34	14.95
F14	0.973	0.884	0.920	0.924	0.884	4.04	12.76
F15	0.982	0.940	0.950	0.965	0.898	3.27	15.28

The results obtained indicated the insignificant effect of span 80% (*X*3), significant effect of speed and drug–polymer ratio, so speed must be in low level (200 rpm) while drug–polymer ratio must be in high level (1:3).

From the above discussed results, the best value having the minimum drug release after 4 h (*Y*2) appears in formula F14 (45.26%) when *X*1 is at medium speed (400 rpm), *X*2 at high level (1:3) and *X*3 at high level (2%).

3.7. Kinetics of in vitro release of diclofenac sodium microparticles

The kinetic treatment was done by plotting the time in hours versus the cumulative percent released of diclofenac sodium for zero, first, Hixson-Crowell cube root low and Baker-Lonsdale equation. The kinetic treatment for Higuchi diffusion model was calculated by plotting the square root of time in hours versus the cumulative percent of diclofenac sodium release. The calculated correlation coefficient values for the in vitro release of the drug from its-loaded microparticles indicate that the zero order is the drug release mechanism, Table 5. An exception was observed in case of F3, in which the release mechanism was found to follow first-order with $t_{1/2}$ of 2.64 h, Table 5. The $t_{1/2}$ values for formulations F1, F2, F4, F5, F6, F7, F8, F9, F10, F11, F12, F13, F14 and F15 were found to be 2.854, 2.187, 2.42, 2.72, 2.8, 2.77, 2.79, 2.6, 2.55, 2.36, 3.49, 3.34, 4.04 and 3.27 h, respectively.

4. Conclusion

Diclofenac sodium-loaded alginate microparticles were successfully obtained by emulsification/internal gelation, which is a simple and economic method for microencapsulation. Stirring speed is the most effective parameter for controlling the drug/matrix dispersion's droplet size in the continuous phase, so it must be in low level (200 rpm). In addition, drug:polymer ratio must be at high level (1:3), while span 80 has no significant effect. The drug release from the most prepared sodium alginate microparticles was found to follow zero order kinetics, which is optimum for the controlled drug delivery.

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