*Original Article*

# Optimization and characterization of diclofenac sodium microspheres prepared by a modified coacervation method

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**ABSTRACT: A modified coacervation method for preparing diclofenac sodium loaded chitosan (DFS-C) microspheres, using sodium citrate as cross-linking agent was optimized. A full $2^3$ factorial design was used to evaluate the effect of chitosan (CS) concentration, cross-linking agent concentration, and cross-linking time on the properties of the prepared microspheres. The modified coacervation method resulted in higher yield of spherical microspheres even with a lower concentration of CS (0.3%, w/v). The morphology of the microspheres was found to be dependent on the formulation and process parameters. The cross-linking agent concentration had the largest impact on swelling, mucoadhesion, and drug release. Kinetic analysis of the release data revealed a quasi-Fickian diffusion mechanism.**

***Keywords:*** Diclofenac sodium, chitosan microspheres, modified coacervation, factorial design

## 1. Introduction

Diclofenac sodium (DFS) is an inhibitor to prostaglandin synthetase. It is used to relieve pain and inflammation in conditions such as rheumatoid arthritis, osteoarthritis, ankylosing spondylitis, and acute gout. Diclofenac sodium is rapidly absorbed from the gastrointestinal tract with the plasma peak level reached in 1-2 h (*1*). It has a very short plasma half-life and circulates in a bound-to-free equilibrium with plasma proteins. It is hydroxylated in the liver and undergoes enterohepatic recirculation (*2*). The most common side effects in long term therapy involve gastrointestinal side effects including gastritis, peptic ulcers, and bleeding (*3*). Although several approaches, such as enteric-coating and sustained release have been studied, none of them have yet solved the drug problems completely (*4-7*).

Carrier technology has offered an intelligent approach to modulate the release and absorption characteristics of drugs by coupling them to polymeric carriers (such as microspheres, nanoparticles, and liposomes). In general, microspheres have the potential to be used to target and control release of drugs. However, coupling a bioadhesive polymer like chitosan (CS) to microspheres provides an additional advantage of efficient drug absorption and bioavailability due to a high surface to volume ratio and a much more intimate contact with mucus (*8-10*).

Chitosan microspheres were used to improve the bioavailability of degradable substances such as protein, as well as to enhance the uptake of hydrophilic substances across epithelial membranes (*11*). Various methods of microsphere preparations were reported including ionotropic gelation (*12*), complex-coacervation (*13*), modified emulsification (*14*), spray drying (*15*), and precipitation by either salting out (*16*) or chemical cross-linking (*17*).

The present study aimed to optimize a modified coacervation method for the preparation of diclofenac sodium loaded chitosan (DFS-C) microspheres, using sodium citrate as cross-linking agent with no need for a complex apparatus and/or special precautions. A $2^3$ full factorial design was used to predict an optimum formula for the microspheres that would prolong drug release and minimize its side effects.

## 2. Materials and Methods

### 2.1. *Chemicals*

Diclofenac sodium was kindly donated from Novartis Pharmaceutical Co., Cairo, Egypt. Chitosan with 85% degree of deacetylation was obtained from Sigma Chemical Co., St. Louis, MO, USA. Analytical grades of acetic acid (96%) and sodium citrate were obtained from El-Nasr Pharmaceutical Chemical Co., Cairo, Egypt.

### 2.2. *Preparation of chitosan microspheres*

Diclofenac sodium loaded CS microspheres were

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prepared by a modified method of the coacervation process previously described by Berthold *et al*. (*16*). A new spraying technique was adopted based on spraying a solution of the drug and cross-linking agent over the polymeric solution. This method was found to overcome the difficulties encountered in preparation of microspheres by the capillary extrusion method. The technique developed allowed the use of DFS in its soluble form instead of its dispersion in an acidic solution of CS. It also permitted the use of high concentrations of the polymer solution without the possibility of plugging the spray nozzle with the viscous polymer solution.

Diclofenac sodium (1%, w/v) was added to 20 mL of aqueous solutions of 5, 7.5, or 10% (w/v) sodium citrate. After the drug was thoroughly dissolved, the solution was sprayed into a magnetically stirred CS solution (0.3, 0.4, or 0.5%, w/v) in 20 mL acetic acid (2%, v/v) using an atomizer with 0.7 mm inner diameter. The microspheres were collected by filtration and washed thoroughly with distilled water, then allowed to dry at 40°C.

### 2.3. *Factorial design and statistical analysis*

An experimental design ($2^3$ full factorial design with one central point) was used to evaluate the effect of formulation variables on the physicochemical properties of the DFS-C microspheres (Table 1). Statistical analysis of the experimental data was performed by an ANOVA test using JMP software version 4.0.4 (SAS Institute, Cary, NC, USA).

### 2.4. *Particle size and morphological characterization*

The mean particle size (MPS) of microspheres was determined using a particle size analyzer (Microvision image analysis system APSI stage micrometer scale, London). The analysis was performed using samples of microspheres uniformly dispersed in purified water. The surface morphology was examined using scanning electron microscopy (SEM). The microspheres were vacuum dried, coated with gold palladium and examined microscopically (JEOl, JXA 840A electron probe microanalyzer, Japan).

### 2.5. *Percent yield, drug loading and encapsulation efficiency*

The percentage yield of the microspheres was determined with respect to the drug and polymer weight. Total drug content was determined according to the method described by Acikgoz *et al*. (*18*). Ten milligrams of DFS-C microspheres were dispersed in 10 mL hydrochloric acid solution (1%, v/v) and magnetically stirred for 2 h. This mixture was allowed to stand at 50°C to allow for polymer dissolution and complete drug dispersion. The mixture was then centrifuged and the supernatant was decanted. The residue was dried, dissolved in methanol and the absorbance was measured after appropriate dilution using a UV spectrophotometer (Lambda EZ 201, PerkinElmer, USA) at 276 nm. The drug encapsulation efficiency (%EE) of the microspheres was calculated as a percentage according to the following equation:

**Table 1. The independent formulation variables of a $2^3$ full factorial design with one central point***

| Independent variables | Levels | |
|---|---|---|
| Sodium citrate concentration (%, w/v) | 5 | 10 |
| Cross-linking time (h) | 1 | 3 |
| Chitosan concentration (%, w/v) | 0.3 | 0.5 |

* Central points of the variables were 7.5% (w/v), 2 h, and 0.4% (w/v) for the sodium citrate, cross-linking time and chitosan concentration, respectively.

$$\%EE = (\text{practical drug loading}/\text{theoretical drug loading}) \times 100 \quad \text{---- Eq. 1}$$

Theoretical drug loading was based on the assumption that the entire drug present in the chitosan solution was entrapped in the microspheres and no loss occurred at any stage of preparation.

### 2.6. *Swelling behavior*

Water-absorbing capacity of the microspheres was determined by a gravimetric method (*19*). On a previously weighed stainless steel support one hundred milligrams of microspheres were accurately weighed ($W_o$) before soaking the support together with the microspheres in water at room temperature. At predetermined time intervals, the stainless steel support and the swelled microspheres were removed from the medium, blotted carefully with cleansing tissues and immediately weighed to determine the weight of swelled microspheres (W). Measurements were made in triplicate and the percent degree of swelling (%DS) was calculated as follows:

$$\%DS = (W_t - W_o)/W_o \times 100 \quad \text{---- Eq. 2}$$

where $W_t$ and $W_o$ were the weights of the microspheres after soaking for time t and in the dry state, respectively.

### 2.7. *In vitro mucoadhesive studies*

The bioadhesive properties of the microspheres were determined according to the everted sac method (*20*). Non-fasting male rats 300-400 g were sacrificed and their intestinal tissue was excised and flushed with 10 mL of ice-cold phosphate buffered saline (pH 7.4) containing 200 mg/dL glucose (PBSG). Segments of the jejunum (6 cm) were everted using a stainless steel

rod and washed with PBSG to remove the contents. The segment sacs were filled with 1-1.5 mL of PBSG and ligatures were placed at both ends. 60 mg of microspheres were suspended in test tubes, each containing 5 mL saline and one intestinal segment. The sets were shaken at 37°C in a water bath shaker. After 30 min, the sacs were removed and the remaining microsphere dispersion was centrifuged for 20 min at 3,000 rpm. The supernatant fluid was discarded and the residual microspheres were allowed to dry in an incubator (FTC 90-E, Refrigerated incubator, made in Europe) at 40°C. The weight of bound microspheres was determined by subtraction of the weight of the dried residual microspheres from their initial weight and was recorded as percent mucoadhesion.

2.8. *In vitro drug release studies*

The release of DFS from microspheres was carried out using a USP type II dissolution apparatus (Hanson Research Dissolution tester, Chatsworth, LA, USA). A total volume of 100 mL phosphate buffer (pH 7.4) was kept at 37°C and rotated at 100 rpm. Twenty five mg of the microspheres containing a known amount of DFS were placed in an open-end glass tube with one side wrapped with a cellophane membrane (Spectrapor membrane tubing No. 2, Spectrum Medical Industries, Houston, TX, USA) and the other was attached to the shaft of the apparatus. At predetermined intervals, 1 mL of the dissolution medium was taken and replaced with an equal volume of fresh dissolution medium. The sample was diluted to 5 mL with phosphate buffer and analyzed for drug concentration using a UV spectrophotometer at 276 nm.

2.9. *Kinetic analysis of the drug release data*

To examine the kinetics and mechanism of drug release, the release data were fitted to models representing zero-order, first-order and Higuchi's square root of time. The correlation coefficients were determined from regression plots of m *vs*. t, log ($m_o$ – m) *vs*. t and m *vs*. $t^{1/2}$, for zero-order, first-order and Higuchi's model, respectively. In these plots, m presented the percent of drug released at time t, and $m_o$ – m was the percent of drug remaining after time t. To understand the mechanism of DFS diffusion from CS microspheres, the results were further analyzed according to the Korsmeyer-Peppas equation (Eq. 3):

$$m_t/m_\infty = k \bullet t^n \quad \text{---- Eq. 3}$$

where $m_t/m_\infty$ was the fraction of the drug released after time t and n was a characteristic exponent for the release mechanism. Based on the Korsmeyer-Peppas equation, values of the n exponent equal to or less than 0.5 were characteristic of Fickian or quasi-Fickian diffusion, whereas values in the range of 0.5 to 1 were an indication of an anomalous mechanism for drug release. On the other hand, a unity value for n would be expected for zero-order release.

## 3. Results

3.1. *Particle size and morphology*

The MPS of all the microspheres batches ranged between 13-25 µm depending on the composition of each formula (Table 2). The results revealed that sodium citrate concentration was the major factor affecting the MPS of the microspheres whereas, increasing its concentration from 5 to 10% (w/v) resulted in a decrease in the MPS from about 24 µm to 14 µm. Changing the cross-linking time and/or the CS concentration had no effect on the microsphere MPS (22-25 µm).

Figures 1a and 1b represent the SEM photographs of the microspheres containing 1% (w/v) DFS, and 0.3% (w/v) chitosan that were cross-linked for 1 h with 5% and 10% (w/v) sodium citrate, respectively. The photographs revealed the formation of solid dense microspheres with CS solutions less than 1% (w/v). This result was contrary to the results of Shu and Zhu (*21*), who reported that 1% (w/v) CS solution was the minimum requirement to obtain microspheres.

Generally, the microspheres were not completely spherical in shape and had a rough surface. The microspheres prepared with 5% sodium citrate (Figure 1a)

**Table 2. Properties of the DFS-C microspheres prepared according to $2^3$ full factorial design**

| Formula code | Sodium citrate concentration (%, w/v) | Cross-linking time (h) | Chitosan concentration (%, w/v) | Mean particle size (µm) | %Yield | %Encapsulation efficiency | %Degree of swelling | %Mucoadhesion |
|---|---|---|---|---|---|---|---|---|
| F1 | 5 | 1 | 0.3 | 23 | 93 | 90 | 133 | 51 |
| F2 | 10 | 1 | 0.3 | 16 | 46 | 24 | 193 | 40 |
| F3 | 5 | 3 | 0.3 | 25 | 96 | 100 | 110 | 50 |
| F4 | 10 | 3 | 0.3 | 18 | 69 | 32 | 183 | 37 |
| F5 | 5 | 1 | 0.5 | 24 | 100 | 91 | 119 | 50 |
| F6 | 10 | 1 | 0.5 | 13 | 60 | 61 | 188 | 39 |
| F7 | 10 | 3 | 0.5 | 14 | 76 | 66 | 183 | 50 |
| F8 | 5 | 3 | 0.5 | 22 | 93 | 100 | 106 | 54 |
| F9 | 7.5 | 2 | 0.4 | 23 | 94 | 90 | 165 | 44 |

were relatively denser, more spherical and smoother than those prepared with 10% sodium citrate which seemed to be more porous in structure (Figure 1b).

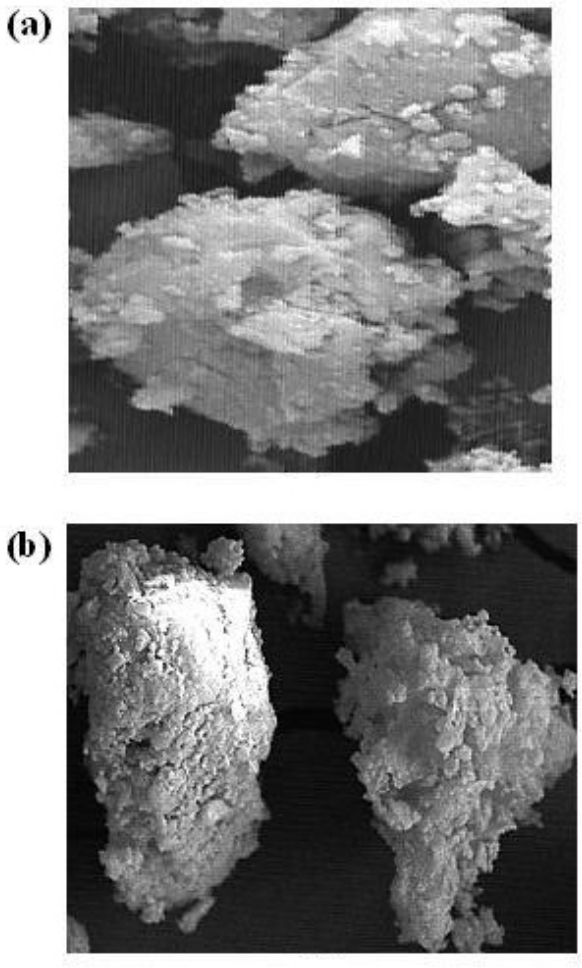


**Figure 1. SEM photographs of DFS-C microspheres prepared with 0.3% (w/v) chitosan and cross-linked for 1 h with 5% (w/v) (a) and 10% (b) sodium citrate.**

3.2. *Percentage yield and encapsulation efficiency*

The %yield and encapsulation efficiency of the microspheres were taken as an indication of the reproducibility and efficiency of the processing technique. The greater %yield and %EE of the prepared formulas (Table 2) were statistically significant with the low level of sodium citrate (5%, w/v) compared to the high level (10%, w/v) (Table 3). The estimated negative coefficient for sodium citrate concentration verified the decrease in the %yield and %EE with the increase in sodium citrate concentration. The ANOVA results showed that the cross-linking time had a significant influence on the %yield and %EE ($F < 0.0001$) (Table 3). On the contrary, the chitosan concentration had only a significant effect on the %EE which had a positive coefficient.

Three-dimensional response surface plots were drawn to show the effects of the interaction of the sodium citrate concentration and cross-linking time on the %yield as well as the interaction of the sodium citrate concentration and CS concentration on the %EE (Figures 2 and 3, respectively). The two figures showed no obvious effect from increasing the cross-linking time or CS concentration on %yield and %EE, respectively, at the low level of the cross-linking agent (5%, w/v sodium citrate). However, a pronounced effect was observed at the high level of the cross-linking concentration significantly increased the %yield and %EE, respectively, at the high level of sodium citrate (positive coefficients).

3.3. *Swelling behavior*

All the formulas prepared at the low level of cross-linking agent (5%, w/v sodium citrate) had %DS in the range of 106-133 (Table 2). On the other hand,

**Table 3. Statistical analysis for the effect of independent variables and their interactions on the microspheres characteristics**

| | | | %Yield | %Encapsulation | %Swelling | %Mucoadhesion | %Release after 6 h |
|---|---|---|---|---|---|---|---|
| Variables | Sodium citrate concentration | *F*-value | < 0.0001* | < 0.0001* | < 0.0001* | < 0.0001* | < 0.0001* |
| | | Estimate coefficient | – 16.54 | – 24.50 | 35.29 | – 6.26 | 13.75 |
| | Cross-linking time | *F*-value | 0.0021* | < 0.0001* | < 0.0001* | 0.19 | < 0.0001* |
| | | Estimate coefficient | 4.54 | 9.13 | – 7.16 | – 1.16 | – 2.9 |
| | Chitosan concentration | *F*-value | 0.033 | < 0.0001* | 0.0352 | 0.75 | 0.016 |
| | | Estimate coefficient | 2.95 | 4.34 | – 2.92 | – 0.27 | 1.37 |
| Variables interaction | Sodium citrate concentration *vs*. Cross-linking time | *F*-value | 0.0004* | 0.0154 | 0.0015* | 0.303 | 0.004* |
| | | Estimate coefficient | 5.54 | – 0.81 | 4.1 | 0.91 | 1.68 |
| | Sodium citrate concentration *vs*. Chitosan concentration | *F*-value | 0.1446 | < 0.0001* | 0.236 | 0.89 | < 0.0001* |
| | | Estimate coefficient | 1.96 | 8.59 | 1.5 | 0.115 | – 5.98 |
| | Chitosan concentration *vs*. Cross-linking time | *F*-value | 0.1446 | 0.084 | 0.362 | 0.67 | 0.98 |
| | | Estimate coefficient | 1.96 | – 0.56 | 1.23 | 0.36 | 0.0104 |

* Significant at $p < 0.01$.

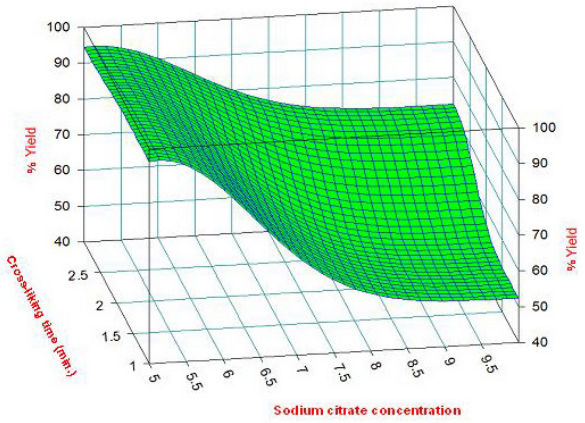


**Figure 2. Effect of the interaction between sodium citrate concentration and cross-linking time on %yield of DFS-C microspheres.**

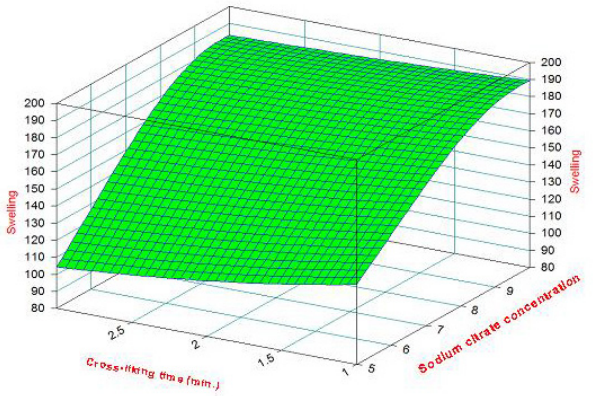


**Figure 4. Effect of the interaction between sodium citrate concentration and cross-linking time on %degree of swelling of DFS-C microspheres.**

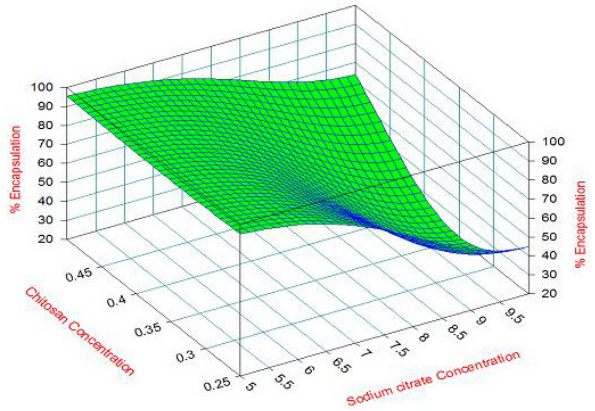


**Figure 3. Effect of the interaction between sodium citrate concentration and chitosan concentration on %encapsulation efficiency of DFS-C microspheres.**

the microspheres prepared at the high level of sodium citrate (10%, w/v) showed an increase in %DS values to the 183-193 range. The results also showed that cross-linking time and CS concentration had a lesser effect on %DS. The ANOVA results (Table 3) revealed linking time and CS concentration had a lesser effect on %DS. The ANOVA results (Table 3) revealed a significant effect of both the cross-linking agent concentration and time on the %DS but showed no significant effect for the CS concentration. The interaction between sodium citrate concentration and cross-linking time had the only significant effect on the swelling behavior (*F* value = 0.0015). This interaction presented in Figure 4, revealed that increased sodium citrate concentration increased the %DS at the second level of cross-linking time.

### 3.4. *Mucoadhesive studies*

The percentage mucoadhesion for all the microspheres formulas ranged between 39 and 54% (w/w) (Table 2). The ANOVA results (Table 3) demonstrated a significant correlation between microsphere mucoadhesiveness and the sodium citrate concentration ($F < 0.0001$). The negative estimated coefficient observed for the effect of sodium citrate on the %mucoadhesion indicated that the higher the sodium citrate concentration the lower the mucoadhesive property of the microspheres. However, CS concentration and cross-linking time had no effect on the mucoadhesive properties of the microspheres.

### 3.5. *In vitro release studies*

The *in vitro* release profiles of DFS from different batches of DFS-C microspheres are shown in Figure 5. The profiles of all prepared microspheres exhibited a biphasic pattern of drug release. An initial burst effect was shown during the first hour that may be related to immediate release of the surface associated drug, followed by a slow release phase of the entrapped drug.

As expected, the sodium citrate concentration had a strong influence on the drug release behavior (Figure 5a). Increasing sodium citrate concentration from 5% (F5) to 10% (w/v) (F6) resulted in an increase in the percentage of drug released after 6 h from 69% to 97%. Figures 5b and 5c showed a decrease in the initial burst release within the first hour by increasing either the cross-linking time from 1 h (F1) to 3 h (F3) or the CS concentration from 0.3% (w/v) (F1) to 0.4% and 0.5% (w/v) (F9 and F7, respectively). After the first hour, all the profiles showed a continuous slow release of the drug entrapped within the microsphere matrix.

ANOVA results (Table 3) showed a significant effect of all variables on the percentage drug released. The significance of these factors was in order; concentration of sodium citrate > the cross-linking time > the CS concentration ($p < 0.01$). A significant interaction was also recorded for the cross-linking

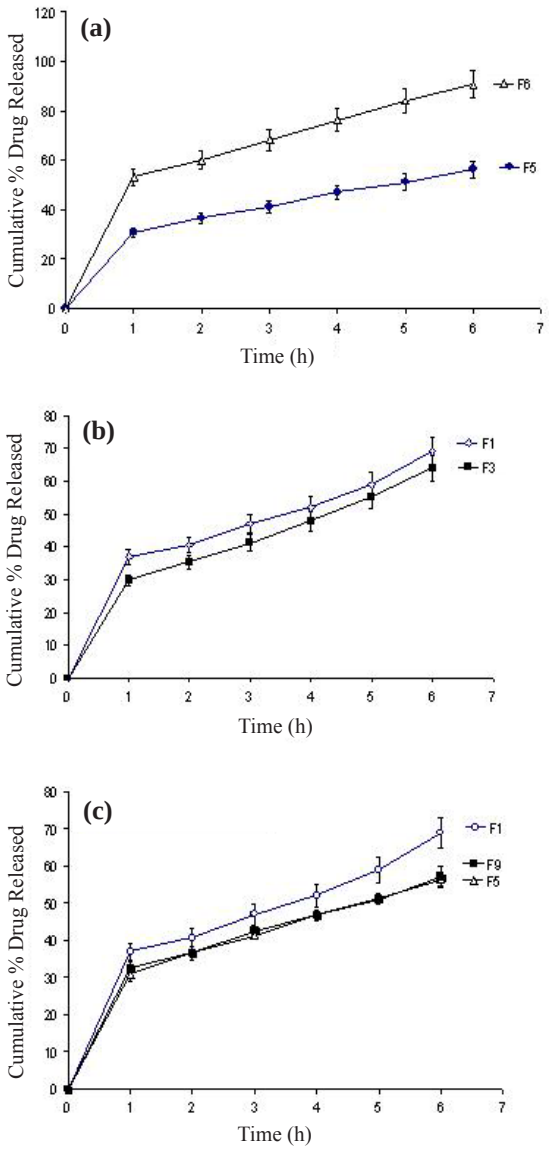


**Figure 5. *In vitro* release profiles of diclofenac sodium from DFS-C microspheres under various conditions.** A, sodium citrate concentration; B, cross-linking time; C, chitosan concentration.

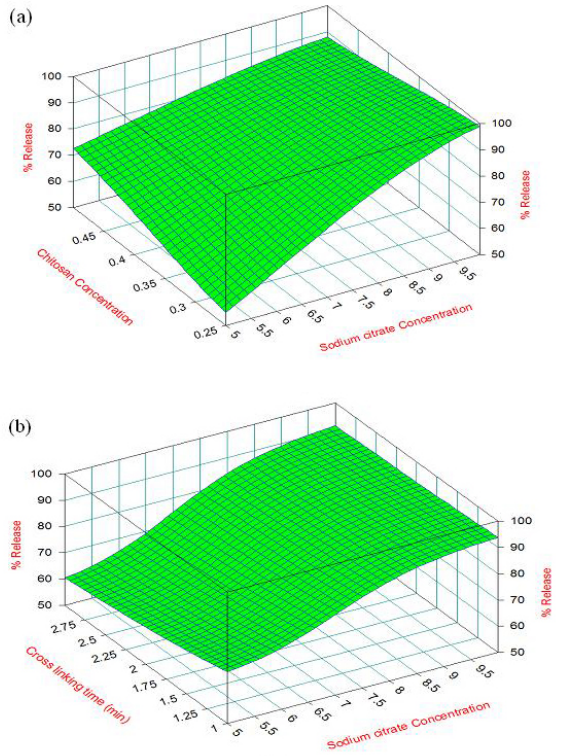


**Figure 6. Effect of the interaction of sodium citrate concentration with (a) chitosan concentration and (b) cross-linking time on the percent of drug released after 6 h.**

agent concentration with either the cross-linking time or the CS concentration ($F$ = 0.004 and < 0.0001, respectively). The three dimensional profiles showed that increasing sodium citrate concentration greatly increased the percentage of drug released which was more obvious at the lower level of the CS concentration and cross-linking time (Figure 6).

### 3.6. *Kinetic analysis of release data*

The coefficients of the drug release kinetics ($r^2$) and the exponent "n" for equation 3 are presented in Table 4. It could be seen that the $r^2$ values for zero-order and Higuchi kinetics were much closer to 1 than the first-order. The release data were also fitted to the Korsmeyer-model where the exponent "n" values for all formulas were found to be in the range of 0.26-0.48.

## 4. Discussion

The use of factorial design allows for testing a number of factors simultaneously and precludes the use of a huge number of independent runs when the traditional step by step approach is used. The effect of three independent variables namely, the cross-linking agent concentration, the cross-linking time and CS concentration were studied at two levels. The irregularity of the microsphere shape and the roughness of their surface might be due to the deposition of drug microparticles on the microsphere outer surface. On the other hand, the observed porous structure of microspheres prepared with 10% (w/v) sodium citrate was related to the effect of increasing sodium citrate concentration on the matrix cross-linking. The difference in the physical characteristics of the microspheres was related to the effect of pH on the degree of CS ionization and its cross-linking properties. Doubling the concentration of sodium citrate from 5% to 10% (w/v) increased the pH of the cross-linking solutions from pH 5 to 6.5, respectively. At pH 5, the amino groups of CS had a higher degree of ionization that would provide a better condition for electrostatic interaction between the protonated amine groups of CS and the negatively charged carboxylate groups of the citrate anions. Thus, more cross-linked polyelectrolyte complexes were formed at 5% sodium

**Table 4. Kinetic analysis for the percentage drug released from DFS-C microspheres***

| Formulas | $r^2$ | | | | 'n' ** |
|---|---|---|---|---|---|
| | Zero-order ($m_o - m = k \cdot t$) | First-order ($\ln [m] = \ln [m_o - k \cdot t]$) | Higuchi-model ($m_t/m_\infty = k \cdot t^{1/2}$) | Korsmeyer-model ($m_t/m_\infty = k \cdot t^n$) | |
| F1 | 0.999 | 0.995 | 0.996 | 0.995 | 0.41 |
| F2 | 0.985 | 0.959 | 0.996 | 0.996 | 0.34 |
| F3 | 0.994 | 0.996 | 0.999 | 0.997 | 0.37 |
| F4 | 0.994 | 0.902 | 0.994 | 0.990 | 0.26 |
| F5 | 0.999 | 0.990 | 0.995 | 0.993 | 0.41 |
| F6 | 0.999 | 0.970 | 0.997 | 0.995 | 0.37 |
| F7 | 0.996 | 0.984 | 0.994 | 0.998 | 0.39 |
| F8 | 0.999 | 0.995 | 0.998 | 0.994 | 0.48 |
| F9 | 0.981 | 0.974 | 0.996 | 0.950 | 0.38 |

* Analyzed by the regression coefficient method; $r^2$: Coefficient of determination; ** Diffusional exponent Korsmeyer-model indicative of the mechanism of drug release.

citrate leading to a relatively spherical, smoother surface microsphere.

The relationship between the morphological characteristics of ionically cross-linked microspheres and the pH of the medium was explained by Shu *et al.* (*22*) and Lee *et al*. (*23*). They reported that as pH of the CS (weak polybasic) solution decreased the ionization of amine groups increased. Ko *et al*. (*24*) also showed that microparticles prepared with tri-polyphosphate solution at a low pH value (pH 2.5) had a more spherical shape and smoother surface than those prepared at a high pH of 8.6. They related such results to the tendency of the former solution to form a high density cross-linked matrix. Accordingly, at pH 6.5 (10%, w/v sodium citrate) the CS polymer had a lower degree of ionization, cross-linking sites and cross-linking density to constrain the polymer matrix (*i.e.,* low density structure). This led to a decrease in the %yield and %EE along with an increase in the %DS.

The significant effect of sodium citrate concentration and cross-linking time on the swelling behavior of the microspheres (Figure 4) clearly revealed that the concentration of sodium citrate (cross-linking agent) was the most prominent effective variable on the %DS of the microspheres. The results also confirmed that the effect of increasing the curing time was not remarkable as the pH of the medium shifted from pH 5 to 6.5.

Moreover, the effect of the sodium citrate concentration on the pH of the medium and %DS was found to influence the mucoadhesive behavior of the chitosan microspheres. Thus, the lower the cross-linking agent concentration used the higher was the degree of CS ionization and the mucoadhesion properties of the microspheres. A similar result was reported by Dhawan *et al*. (*25*) who found that the amount of chitosan adsorbed on the tissue increased with a decrease in the cross-linking level. Mortazavi and Smart (*26*) also reported that a certain degree of swelling was essential for microsphere adhesion to mucin, whereas, over swelling led to slipping of the microspheres over the tissue surface.

The biphasic release pattern was related to the release of the drug particles adsorbed on the microsphere surfaces during the first hour, followed by slow release of the drug from the matrix. The increase in the percentage drug released with increasing the sodium citrate concentration was attributed to the increase in pH of the medium and the consequent decrease in the polymer cross-linking. Shu *et al*. (*22*) showed that chitosan films cross-linked with sodium citrate possessed pH-sensitive swelling and drug release properties. They also reported that preparation of CS microparticles in an acidic pH led to a dense matrix with complete ionic cross-linking that enabled a controlled drug release. Ozbas-Turan *et al*. (*27*) recorded a fast release pattern for interleukin-2 from chitosan microspheres when a larger volume of sodium sulfate (ionic cross-linking agent) was used during preparation.

The noticeable decrease in the percent drug released with an increase of cross-linking time might be attributed to an increase in the time allowed for polymer matrix to constrain. Ko *et al*. (*24*) reported the impact of curing time on the formation of a tripolyphosphate-chitosan matrix and the release of the drug. The results were also in accordance with Acikgoz *et al*. (*18*) who reported the formation of a denser matrix by increasing the curing time. This was thought to be associated with a decrease in the release of drug.

Moreover, the decrease in the percent drug released by increasing the CS concentration was attributed to the increase in the viscosity of the swollen microspheres accompanied by an increase in the diffusion path length traversed by the drug molecules. Ko *et al*. (*24*) illustrated the relationship between the drug release behavior and the viscosity of the chitosan solution. The increase in the viscosity of the solution led to the formation of relatively strong-walled microparticles. Lim *et al*. (*28*) reported the

formation of weak microspheres with the use of a low concentration of chitosan solution.

For the kinetics analysis data, although the correlation coefficients ($r^2$) of the release data closely fitted zero-order and Higuchi kinetics models, the low values of n (< 0.5) indicated that the mechanism of drug release from all the formulas could be described as a quasi-Fickian diffusion mechanism.

## 5. Conclusion

The addition of diclofenac sodium and cross-linking agent onto the chitosan solution in the form of spray droplets was shown to be a simple efficient technique for preparing microspheres. The microspheres were characterized by good percent yield, encapsulation efficiency, swelling and mucoadhesion properties along with controlled drug release. The concentration of the cross-linking agent and the pH of its solution must be taken into consideration as the most effective variables influencing the properties of the microspheres.

From the results it can be concluded that formula F8 with the composition of 1% DFS, 0.5% CS, 5% sodium citrate and cross-linked for 3 h, fulfilled the requisites for an optimum formulation.

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