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Glutaraldehyde cross-linked chitosan microspheres for controlled release of centchroman

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Abstract—Glutaraldehyde cross-linked chitosan microspheres were prepared for controlled release of centchroman, a nonsteroidal contraceptive. The cross-linked microspheres with low-molecular-weight (LMW) chitosan (260 kg mol^{-1}) have shown maximum degree of swelling (287 wt %) but were found to be poor in loading and release behavior for centchroman. The microspheres with medium-molecular-weight (MMW) chitosan (1134 kg mol^{-1}) have shown 250 wt % degree of swelling and 37.5 wt % loading of centchroman, but microspheres with high-molecular-weight (HMW) chitosan (2224 kg mol^{-1}) have shown a low degree of swelling (150 wt %) and centchroman loading (30 wt %). The microspheres with MMW chitosan have released 82 wt % of loaded centchroman in a controlled release manner within a period of 70 h in comparison to low- (260 kg mol^{-1}) and high-MW (2224 kg mol^{-1}) chitosan microspheres. The chitosan microspheres with 62 wt % degree of deacetylation (DDA) were more efficient in the controlled release of centchroman in comparison to chitosan microspheres with low (48 wt %) and high-DDA (75 wt %). The fractional release of centchroman (M_t/M_∞) from chitosan microspheres was used to predict the mechanism of drug release and to determine the diffusion constant (D) of centchroman.

Keywords: Chitosan; Deacetylation; Centchroman; Glutaraldehyde; Microspheres

1. Introduction

Chitosan is a naturally occurring biodegradable polymer and found to be useful in various applications. It is a copolymer of glucosamine and *N*-acetylglucosamine repeat units. The repeat units in chitosan depend on the degree of deacetylation in chitin obtained from crustacean shells. The mucoadhesivity of chitosan[1,2] is responsible for its applications to develop controlled delivery systems.[3–6] Chitosan (**1**) is biocompatible and a non-irritant; hence, it is found to be suitable for applications in living systems. The solubility of chitosan in acidic media has been found to be useful to avoid toxic organic solvents for its processing. The properties of chitosan ultimately depend on the degree of deacetylation (DDA) and molecular weight; hence, investigations have been carried out using chitosan with different degrees of deacetylation and molecular weights.[7] Derivatives of chitosan have also been used in gene delivery systems.[8] To develop microspheres, various techniques have been used, but the coacervation/precipitation technique has been found to be more useful to prepare controlled release systems.[9] The response of microspheres for the release of drugs depends on the type and degree of cross-linking, which has been carried out either chemically[10,11] or physically.[6,12–16] Although controlled delivery systems for various drugs[17–20] have been developed, a controlled delivery system for centchroman is limited to a single article.[21] Centchroman (**2**) is a nonsteroidal contraceptive that has shown estrogenic and anti-estherogenic properties without affecting the hypothalamic pituitary ovarian axis. It has also shown anti-breast cancer activity in addition to its contraceptive properties; hence, in these investigations, efforts were made to prepare a controlled delivery system for centchroman using chitosan of different molecular weights and degrees of

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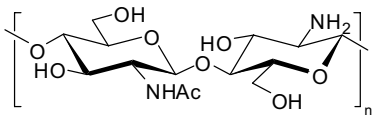
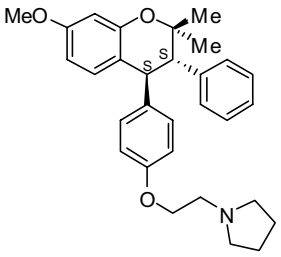
deacetylation with glutaraldehyde as the chemical cross-linker. The degree of swelling and control characteristics of cross-linked microspheres were evaluated as a function of the molecular weight and degree of deacetylation (DDA) of chitosan.



Chitosan (**1**)



Centchroman (**2**)

2. Experimental

2.1. Materials

Chitosan was purchased from Sigma–Aldrich Chemical Company (USA) and purified further by dissolving in HOAc (2.0 wt %) and passing the solution through a filter. The filtrate was precipitated in a solution of sodium hydroxide (1.0 M), and the resultant chitosan was dried at 20 °C in a vacuum oven after washing with deionized water. Glutaraldehyde was supplied by Loba Chemie, Mumbai, India and used without further purification. Centchroman was a gift from Torrin Pharmaceuticals Ltd, Ahmedabad, India, and it is used after recrystallization from EtOH.

2.2. Measurement of molecular weight of chitosan

The molecular weight of samples of chitosan was determined viscometrically using Eq. 1

$$[\eta]_{25\ ^\circ C} = 1.81 \times 10^{-3}\ \mathrm{cm^3\ g^{-1}\ M^{0.93}}\ (CH_3COONa) \quad (1)$$

2.3. Measurement of the degree of deacetylation (DDA) in chitosan

The degree of deacetylation (DDA) in samples of chitosan was determined potentiometerically[21] by dissolving 0.025 g chitosan in excess HCl (25 mL of 1.75×10^{-3} N HCl) and back titrating with NaOH (1.0×10^{-2} N). By this method the moles of amino groups

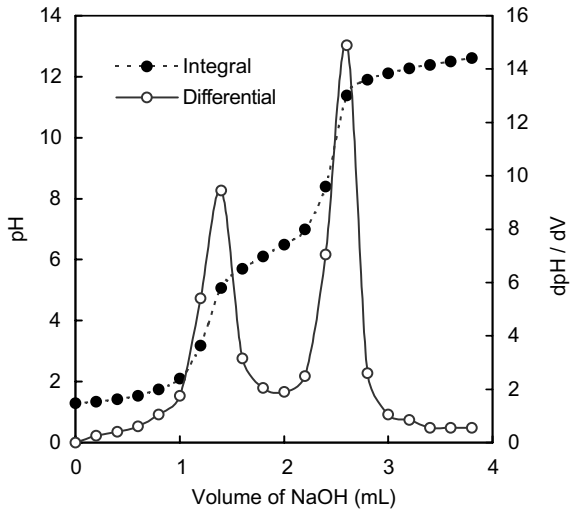


Figure 1. Potentiometric determination of the degree of deacetylation (DDA) in chitosan. [Chitosan] = 0.025 g, [HCl] = 1.75×10^{-3} mol dm^{-3} (25 mL). [NaOH] = 1.75×10^{-2} mol dm^{-3}.

corresponding to the DDA in chitosan were determined. A differential curve was drawn between $\Delta pH/\Delta V$ versus volume of NaOH (Fig. 1), and the differential volume of NaOH (ΔV) between the first and second peaks was used to calculate the DDA in chitosan using Eq. 2

$$\mathrm{DDA}\ (\%) = \frac{203Q}{1 + 42Q} \times 100 \quad (2)$$

$$\text{where } Q = \frac{N\Delta V}{w}$$

w is the weight of chitosan, and N and ΔV are, respectively, the normality and volume of NaOH used in the titration.

2.4. Preparation of cross-linked chitosan microspheres

The glutaraldehyde cross-linked chitosan microspheres were prepared by dissolving purified chitosan (500 mg) in 200 mL HOAc (2.0 wt %) under vigorous stirring for about 3 h at room temperature. The chitosan solution was subsequently blown through a nozzle into 100 mL of a methanolic solution of NaOH (1.0 M) to form chitosan microspheres. The microspheres were coacervated and settled in the container with methanolic solution of alkali. Finally the microspheres were centrifuged and washed with deionized water. They were then transferred to a solution of glutaraldehyde (25 mL, 6.0 wt %) for cross-linking. After 6 h, the microspheres were removed and washed with hot and cold water and vacuum dried at 30 °C. The procedure was repeated to prepare microspheres with chitosan of different molecular weight (MW) and degree of deacetylation (DDA).

2.5. Degree of swelling in cross-linked chitosan microspheres

The degree of swelling (S_w) in microspheres was determined by keeping 100 mg of the microspheres in 25 mL of a solution of phosphate buffer (pH 7). The increase in weight ($W_t - W_0$) of microspheres at different time intervals in comparison to initial weight (W_0) of microspheres was used to calculate the degree of swelling (S_w) using Eq. 3

$$S_w(\%) = \frac{(W_t - W_0)}{W_0} \times 100 \quad (3)$$

where W_0 and W_t are the initial and final weights of the microspheres.

2.6. Measurements of size, shape, and morphology of chitosan microspheres

The size, shape and morphology of the chitosan microspheres were determined using micrographs recorded with a scanning electron microscope (Leo-435 VP England). To record micrographs, the microspheres were mounted on metal studs with double adhesive tape and vacuum coated with gold. The shape factor (S) of microspheres was determined using average perimeter (L) and area (A) of about 20 microspheres and using Eq. 4

$$S = \frac{L^2}{4\pi A} \quad (4)$$

where L and A are the average perimeter and area of the selected surface on microspheres. The deviation from the spherical shape in microspheres was predicted on the basis of shape factor (S).

2.7. Loading of centchroman on cross-linked chitosan microspheres

The loading of centchroman on chitosan microspheres was carried out by keeping 20 mg of the microspheres in 100 mL of a phosphate buffered solution (pH 5) of centchroman for about 48 h under gentle stirring. The amount of centchroman loaded on the microspheres was determined with the absorbance of the remaining solution ($\lambda_{max} = 275$ nm) determined by a Shimandzu UV-VIS-1601 PC spectrophotometer. The loading of centchroman on the microspheres was also carried out at different initial concentrations of centchroman in the solution. The loading of centchroman in the microspheres was calculated as percent loading (PL) and as efficiency of loading (EL_{max}) using Eqs. 5 and 6

$$\text{PL}\ (\%) = \frac{\text{Weight of centchroman loaded (mg)}}{\text{Weight of microspheres taken for loading (100 mg)}} \times 100 \quad (5)$$

$$\text{EL}_{max}\ (\%) = \frac{\text{Weight of centchroman loaded (mg)}}{\text{Weight of centchroman taken for loading}} \times 100 \quad (6)$$

2.8. Release of centchroman from cross-linked chitosan microspheres

The release characteristics of chitosan microspheres were determined by keeping 100 mg of centchroman loaded chitosan microspheres in 20 mL of phosphate buffered (pH 7) solution for different time intervals. The amount of centchroman released in the media was determined by recording the absorbance ($\lambda_{max} = 275$ nm) with replacement of the solution withdrawn for testing. The experiments were also repeated with microspheres of different molecular weights and DDAs. The release of centchroman from microspheres was presented as efficiency of controlled release (ECR_{max}) and efficiency of burst release (EBR_{max}) using Eqs. 7 and 8

$$\text{ECR}_{max}\ (\%) = \frac{\text{Total weight of centchroman released in controlled manner (mg)}}{\text{Weight of centchroman (mg) loaded per 100 mg of microspheres}} \times 100 \quad (7)$$

$$\text{EBR}_{max}\ (\%) = \frac{\text{Total weight of centchroman released in burst manner (mg)}}{\text{Weight of centchroman (mg) loaded per 100 mg of microspheres}} \times 100 \quad (8)$$

The centchroman released in a controlled manner was taken as the sum of constant fractional releases $(\sum W_t/W_0)_{constant}$ of centchroman for fixed time interval of 10 h, and centchroman released in a burst release manner was taken as the sum of variable fractional releases $(\sum W_t/W_0)_{variable}$ of centchroman for fixed time interval of 10 h.

2.9. Mechanism of drug release and diffusion constant

The diffusion constant (D) and release behavior of centchroman from microspheres was determined using frac-

tional release of centchroman (M_t/M_∞) as a function of release time (t) using Eq. 9

$$\frac{M_t}{M_\infty} = \sqrt{\frac{16Dt}{\Pi r^2}} \quad (9)$$

where r is the average radius of cross-linked chitosan microspheres.

3. Results and discussion

The degree of swelling, efficiency and release profile of the drug in chitosan microspheres have shown dependence on both the molecular weight[22–24] and degree of deacelylation[22] in chitosan; hence, chitosan samples were analyzed for their molecular weight using a viscometric method. The results were 260 kg mol^{-1} for the LMW fraction, 1134 kg mol^{-1} for the MMW fraction and 2224 kg mol^{-1} for the HMW fraction. The degree of deacetylation (DDA) in chitosan samples (**1**) was determined by a potentiometric method, and depending on the degree of deacetylation (DDA), the chitosan samples of medium-molecular-weight (1134 kg mol^{-1}) were categorized as low (48 wt %, LDDA), medium (62 wt %, MDDA), and high (75 wt %, HDDA) deacetylated chitosan.

The low-molecular-weight chitosan samples (260 kg mol^{-1}) were soluble in mineral acids and acetic acid (2.0 wt %) within 2 h, but chitosan samples with high-molecular-weights (1134 kg mol^{-1} and 2224 kg mol^{-1}) were soluble only after 24 h. The solubility of chitosan in acetic acid varied with the degree of deacetylation. The chitosan samples with a high degree of deacetylation (75 wt %, DDA) were more soluble in acetic acid in comparison to chitosan samples with a low degree of deacetylation (48 wt %, DDA). These observations clearly indicate that the molecular weight (MW) and degree of deacetylation (DDA) in chitosan play a significant role in controlling the chitosan–solvent interactions. To study the effect of molecular weight and degree of deacetylation of chitosan on the release characteristics of centchroman, microspheres prepared with chitosan of different molecular weights and degree of deacetylation at a fixed concentration of glutaraldehyde (6.0 wt %) were used to study the release behavior of centchroman. The cross-linking with glutaraldehyde has shown variations in the structures and properties of chitosan microspheres. The pure chitosan and glutaraldehyde cross-linked chitosan microspheres have shown variations in their size from 119 μm to 39.78 μm as clear from their SEM micrographs (Fig. 2a and c). In addition to size variations, the glutaraldehyde cross-linking in chitosan microspheres has also shown a significant effect on surface morphology of the microspheres as is clearly revealed by their SEM micrographs (Fig. 2b and d). The microspheres on cross-linking with glutaraldehyde have shown smooth surface morphology (Fig. 2d) in comparison to uncross-linked chitosan microspheres (Fig. 2b). The molecular weight

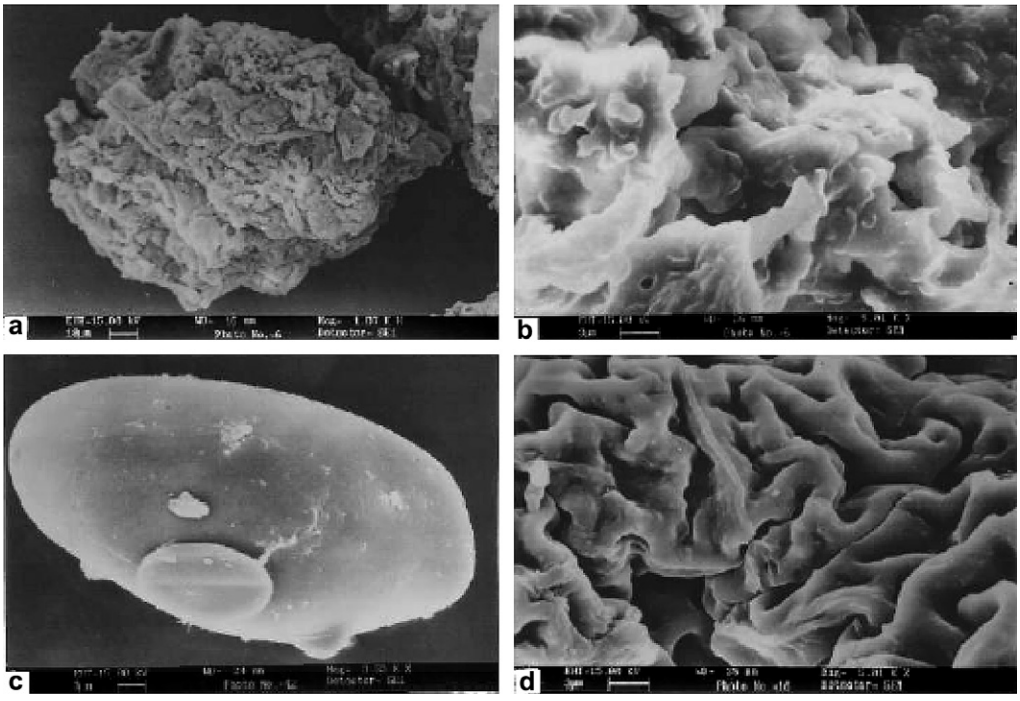


Figure 2. SEM micrographs of chitosan glutaraldehyde cross-linked microspheres.

Table 1. Physical and release characteristics of microspheres prepared from chitosan with different molecular weight (MWs) and constant degree of deacetylation (62 wt %) at constant concentration of glutaraldehyde (6 wt %)

Type of microspheres	Size of microspheres (μm)	Shape factor (S)	S_w (wt %)	Centchroman loading (PL) (wt %)	EL_{max} (wt %)	ECR_{max} (wt %)	D (10^{-12} cm^2 s^{-1})
260 kg mol^{-1} (LMW)	62.20	0.664	287	33.5	67.0	75.0	2.90
1134 kg mol^{-1} (MMW)	39.78	0.832	250	37.5	75.0	82.0	0.24
2224 kg mol^{-1} (HMW)	22.60	0.864	150	37.0	50.0	48.0	0.05

Table 2. Physical and release characteristics of microspheres prepared from chitosan with different degree of deacetylation (DDAs) and constant molecular weight (1134 kg mol^{-1}) at constant concentration of glutaraldehyde (6 wt %)

Type of microspheres	Size of microspheres (μm)	Shape factor (S)	S_w (wt %)	Centchroman loading (PL) (wt %)	EL_{max} (wt %)	ECR_{max} (wt %)	D (10^{-12} cm^2 s^{-1})
48 wt % (LDDA)	76.15	0.682	282	27.0	67.50	55.0	3.34
62 wt % (MDDA)	39.78	0.832	250	37.5	75.00	82.0	0.24
75 wt % (HDDA)	18.75	0.867	213	32.0	53.33	65.0	0.03

of chitosan has also influenced the size and shape as is clearly indicated by the value of shape factor (S) determined for these microspheres (Table 1). The microspheres with low-molecular-weight chitosan were less spherical in comparison to microspheres with medium- and high-molecular-weight chitosan as indicated by the variations in the value of the shape factor (S) from spherical geometry (Table 1). The microspheres with low-molecular-weight of chitosan were rough, non-spherical and larger in size (62.20 μm) due to poor molecular packing and cross-linking in comparison to microspheres obtained from medium- and high-molecular-weight chitosan (Table 1). The chitosan microspheres with different degrees of deacetylation have shown variations in their size on cross-linking with glutaraldehyde. The microspheres with a low degree of deacetylation (48 wt %) were larger in size (76.15 μm) in comparison to microspheres prepared from those with a medium (62 wt %) and high (75 wt %) degree of deacetylation (Table 2). The microspheres with low DDA chitosan (48 wt %) were less spherical, larger in size and with rough surface morphology. The value of the shape factor (S) for these microspheres was different (0.682) than the value of the shape factor (>0.8) found for spherical microspheres. The microspheres from medium-DDA (62 wt %) and high-DDA (75 wt %) batches were more spherical as the value of the shape factor (S) for these microspheres was close to the value of spherical symmetry (Table 2). The variation in the size and morphology of the chitosan microspheres with degree of deacetylation was due to the variation in degree of cross-linking with glutaraldehyde. The microspheres with a high degree of deacetylation were highly cross-linked; hence, they were smooth and more spherical in shape (Table 2). These investigations have clearly indicated that the size, shape, and surface morphology varies with the degree of deacetylation and molecular weight of chitosan.

3.1. Degree of swelling (S_w) in chitosan microspheres

In order to determine the experimental conditions for optimum loading and release of centchroman from chitosan microspheres, the swelling behavior of chitosan microspheres was studied at different time intervals in phosphate buffer solution (pH 7). Microspheres with low-molecular-weight chitosan (260 kg mol^{-1}) showed a maximum degree of swelling (287 wt %) within the first 20 h (Table 1 and Fig. 3), whereas microspheres with medium- (1134 kg mol^{-1}) and high-molecular-weight chitosan (2224 kg mol^{-1}) showed a maximum degree of swelling of 250 wt % and 150 wt % at 40 h and 70 h, respectively (Table 1 and Fig. 3). The microspheres with high-molecular-weight chitosan (2224 kg mol^{-1}) have shown a linear increase in the degree of swelling up to 70 h, after which time, the degree of swelling became almost constant (Fig. 3). The microspheres prepared

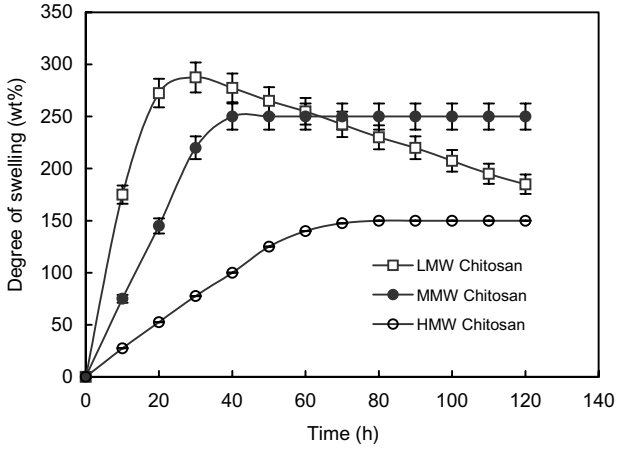


Figure 3. Effect of chitosan MW on the degree of swelling. [Glutaraldehyde] = 6 wt %, swelling media = 20 mL buffered solution (pH 7), microspheres = 100 mg, T = 37 °C.

with low-molecular-weight chitosan (260 kg mol^{-1}) have shown 287 wt % degree of swelling but eroded after 20 h of swelling due to the decrease in inter molecular interactions; hence, the overall swelling in these microspheres was decreased (Fig. 3). Thus microspheres with low-molecular-weight chitosan (260 kg mol^{-1}) were less useful for sustained delivery of centchroman than microspheres with medium-molecular-weight chitosan (1134 kg mol^{-1}). The microspheres with high-molecular-weight chitosan (2224 kg mol^{-1}) have shown a low degree of swelling (150 wt %) due to strong intermolecular interactions and a high degree of cross-linking (Table 1). These microspheres were compact; hence, they were less useful in formulation of controlled delivery systems. These studies have clearly indicated that microspheres with medium-molecular-weight chitosan (1134 kg mol^{-1}) were more useful to prepare controlled delivery systems. The degree of swelling in chitosan microspheres with different degrees of deacetylation was also studied (Table 2 and Fig. 4), which clearly indicates that microspheres with a low degree of deacetylation (48 wt %) were less compact and showed 282 wt % degree of swelling within a swelling period of 40 h, after which time, the degree of swelling in these microspheres was decreased (Table 2 and Fig. 4). The microspheres with a high degree of deacetylation (75 wt %) were more compact and showed the least degree of swelling (Table 2 and Fig. 4), but microspheres with a medium degree of deacetylation (62 wt %) showed a 250 wt % degree of swelling within a period of 40 h (Table 2 and Fig. 4). At the end of this time, the degree of swelling was almost constant. The studies have clearly indicated that microspheres with a 62 wt % degree of deacetylation were suitable for the loading and release of centchroman.

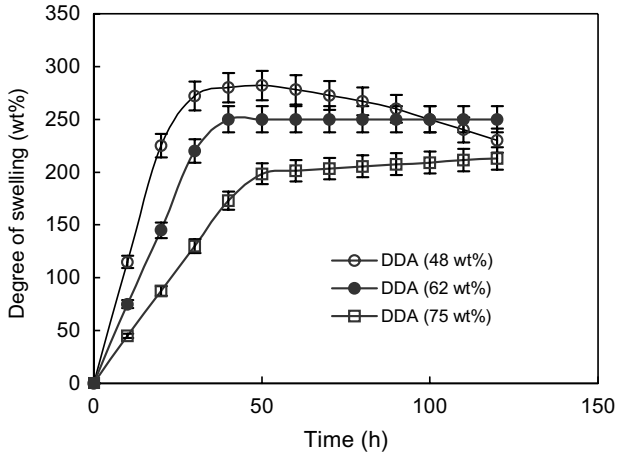


Figure 4. Effect of DDA of chitosan on the degree of swelling, [glutaraldehyde] = 6 wt %, swelling media = 20 mL buffered solution (pH 7), microspheres = 100 mg, T = 37 °C.

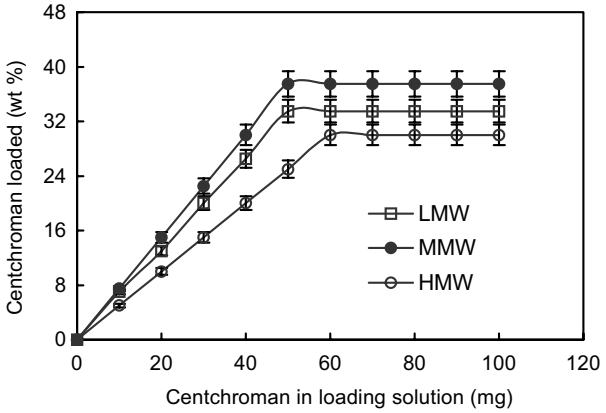


Figure 5. Effect of MW of chitosan on centchroman loading. [Glutaraldehyde] = 6 wt %, loading media = 20 mL buffered solution of centchroman (pH 5), Microspheres = 100 mg, T = 37 °C.

3.2. Loading of centchroman on cross-linked chitosan microspheres

The microspheres prepared using chitosan with different molecular weights and degree of deacetylation were also evaluated for the loading of centchroman (**2**) using Eqs. 5 and 6. The trend of centchroman loading on microspheres with different molecular weights of chitosan initially increased, and after a certain concentration of centchroman, the loading of centchroman on the microspheres essentially stopped (Fig. 5). The microspheres with high-molecular-weight chitosan (2224 kg mol^{-1}) showed a maximum efficiency of loading (EL_{max}) of 50 wt % at 60 mg/20 mL concentration of centchroman in the loading solution (Table 1). The microspheres with low- (260 kg mol^{-1}) and medium-molecular-weight chitosan (1134 kg mol^{-1}) have shown a continuous increase in centchroman loading up to 50 mg/20 mL concentration of centchroman in the loading media. These microspheres have shown a maximum efficiency of loading (EL_{max}) of 67 wt % and 75 wt %, respectively (Table 1 and Fig. 5).

The trend of centchroman loading in microspheres with high-molecular-weight chitosan is apparently due to molecular compaction and a high degree of cross-linking, which decreases the maximum loading efficiency to 50 wt %. Microspheres with low-molecular-weight chitosan (260 kg mol^{-1}) have shown improvement in the efficiency of loading to 67 wt % in comparison to the high-molecular-weight chitosan microspheres because these microspheres are less compact and have a low retention for centchroman in comparison to medium- (1134 kg mol^{-1}) and high-molecular-weight chitosan (2224 kg mol^{-1}) microspheres (Table 1 and Fig. 5).

The poor retention of centchroman in microspheres with low-molecular-weight chitosan is due to a high degree of swelling (287 wt %) and a fragile network structure. The microspheres with medium-molecular-weight

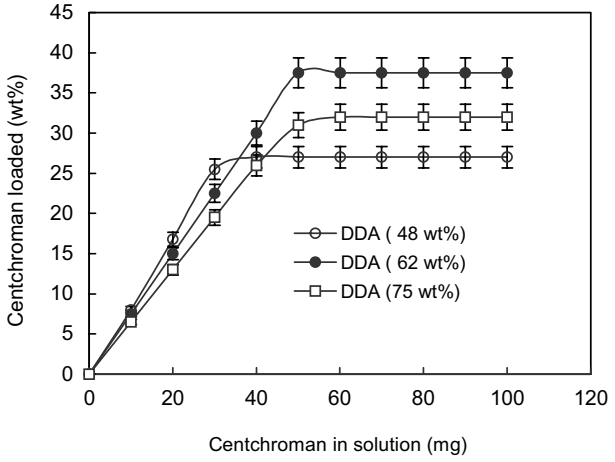


Figure 6. Effect of DDA of chitosan on centchroman loading. [Glutaraldehyde] = 6 wt %, loading media = 20 mL buffered solution of centchroman (pH 5), microspheres = 100 mg, $T = 37$ °C.

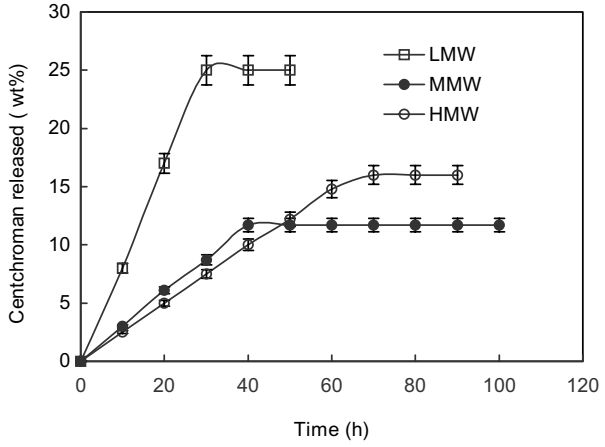


Figure 7. Effect of MW of chitosan on release of centchroman. [Glutaraldehyde] = 6 wt %, release media = 20 mL buffered solution (pH 7), microspheres = 100 mg, $T = 37$ °C.

chitosan (1134 kg mol^{-1}) showed a maximum efficiency of loading of 75 wt % at 50 mg/20 mL concentration of centchroman (Table 1 and Fig. 5), which is apparently due to the optimum degree of swelling (250 wt %) and cross-linking in these microspheres.

The loading of centchroman in chitosan microspheres with different degrees of deacetylation was also evaluated as shown in Table 2 and Figure 6. These microspheres have initially shown increasing trends for centchroman loading (Fig. 6). The microspheres with 62 wt % DDA were initially less efficient in loading of centchroman in comparison to microspheres with 48 wt % DDA, but the overall centchroman loading in these microspheres was around 75 wt % (Table 2 and Fig. 6). The variation in centchroman loading in microspheres with different degrees of deacetylation is apparently due to the variation in the degree of cross-linking, which controls the size and degree of swelling in these microspheres. Although microspheres with 48 wt % DDA showed high degree of swelling (287 wt %), the centchroman loading in these microspheres was low (67.5 wt %) due to a low degree of cross-linking and high degree of porosity (Table 2). These studies clearly indicate that the degree of deacetylation in chitosan controls the degree of swelling through cross-linking. The microspheres with 62 wt % DDA have the optimum degree of swelling and pore size; hence, these microspheres showed a maximum efficiency (75 wt %) for centchroman loading.

3.3. Drug release from cross-linked chitosan microspheres

To determine the effect of molecular weight[24] and degree of deacetylation of chitosan on in vitro release of centchroman from chitosan microspheres, the fractional release of centchroman (wt %) from microspheres at fixed time intervals (10 h) was determined (Figs. 7 and 8). The sum of the constant fractional release $(\sum W_t/W_0)_{\text{constant}}$ of centchroman from microspheres was expressed as the efficiency of controlled release (ECR_{max}) (Tables 1 and 2), whereas the sum of the variable fractional release $(\sum W_t/W_0)_{\text{variable}}$ of centchroman from microspheres was expressed as the burst release (EBR_{max}). From the drug release trends of the microspheres (Figs. 7 and 8), the release time, when microspheres started releasing centchroman in a controlled manner and the total time of controlled release of centchroman from microspheres, was determined. The release profiles of microspheres have clearly indicated that the molecular weight (Fig. 7) and degree of deacetylation (Fig. 8) of chitosan have significantly influenced the release of centchroman from chitosan microspheres. The microspheres have initially shown a burst release of

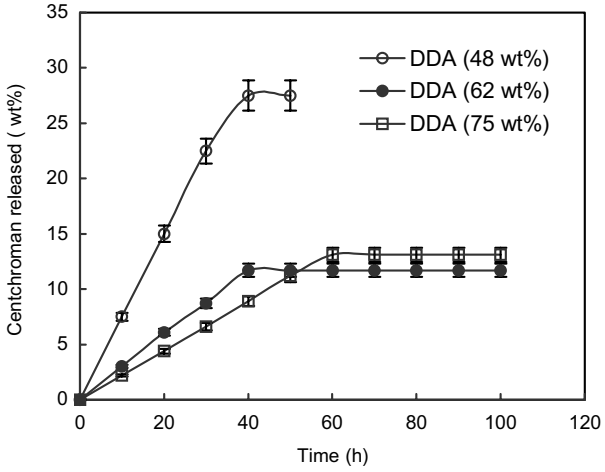


Figure 8. Effect of DDA of chitosan on release of centchroman. Release media = 20 mL buffered solution (pH 7), microspheres = 100 mg, $T = 37$ °C.

centchroman, and after a certain time interval, the microspheres started releasing centchroman in a controlled manner. The efficiency of controlled release (ECR_{max}) and the period for controlled drug release has shown significant dependence on the molecular weight (Fig. 7) and the degree of deacetylation in chitosan microspheres (Fig. 8). The microspheres prepared with medium-molecular-weight chitosan (1134 kg mol^{-1}) released 82 wt % loaded centchroman in controlled manner (ECR_{max}) within a period of 70 h (Fig. 7 and Table 1). In comparison, microspheres prepared with low- (260 kg mol^{-1}) and high-molecular-weight (2224 kg mol^{-1}) chitosan, released 75 wt % and 48 wt % loaded centchroman in controlled manner (ECR_{max}) within a period of 30 h (Table 1 and Fig. 7), and 25 wt % and 52 wt % of loaded centchroman was released in burst release manner (EBR_{max}) within a period of 20 h and 5 h, respectively (Fig. 7). These observations clearly indicate that the microspheres with medium-molecular-weight chitosan (1134 kg mol^{-1}) are more efficient in releasing the centchroman in a controlled manner (82 wt %) within a period of 70 h (Table 1 and Fig. 7) in comparison to low- and high-molecular-weight chitosan microspheres. Similarly, the chitosan microspheres with 62 wt % DDA were more efficient (82 wt %) in releasing the centchroman in a controlled manner (Fig. 8) within a period of 70 h, in comparison to microspheres with 48 wt % and 75 wt % DDA (Table 2 and Fig. 8), in which the sustained release of centchroman was 55 wt % and 65 wt %, respectively, within a period of 20 h and 50 h, respectively. These observations clearly indicate that the optimum degree of swelling and cross-linking in chitosan microspheres with 62 wt % DDA is responsible for the optimum loading (75 wt %) and controlled release (82 wt %) of centchroman within a period of 70 h (Table 2 and Fig. 8). The initial release of centchroman from microspheres with different molecular weights (Fig. 7) and degrees of deacetylation (Fig. 8) has followed first-order kinetics, and zero-order kinetics was followed in the controlled step of centchroman release from chitosan microspheres.[25]

To ascertain the mechanism of drug release from chitosan microspheres, the fractional release profile of centchroman (M_t/M_∞) from microspheres was analyzed as shown in Figures 9 and 10.

The initial burst release of centchroman from microspheres with different molecular weights (Fig. 9) and different degrees of deacetylation of chitosan (Fig. 10) has varied linearly with the square root of the release time ($\sqrt{t}$), indicating a diffusion-controlled release of centchroman from these microspheres ($n = 0.5$). However, the release of centchroman in the controlled stage of drug release was anomalous.[26] The initial slope of these curves (Figs. 9 and 10) was used to calculate the diffusion coefficient (D) for centchroman from chitosan microspheres using Eq. 8 as given in Tables 1 and 2.

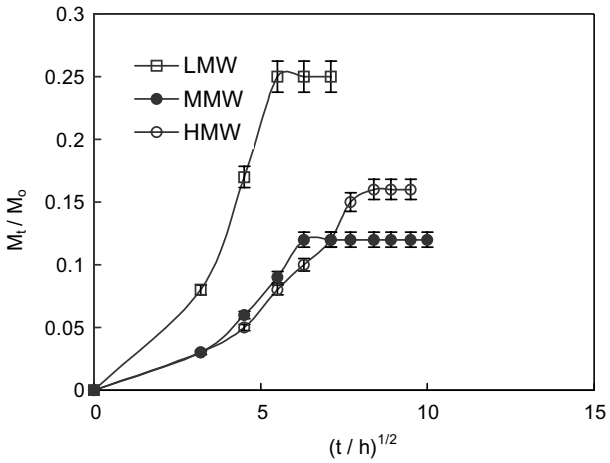


Figure 9. Effect of MW of chitosan on fractional release of centchroman. [Glutaraldehyde] = 6 wt %, release media = 20 mL buffered solution (pH 7), microspheres = 100 mg, $T = 37$ °C.

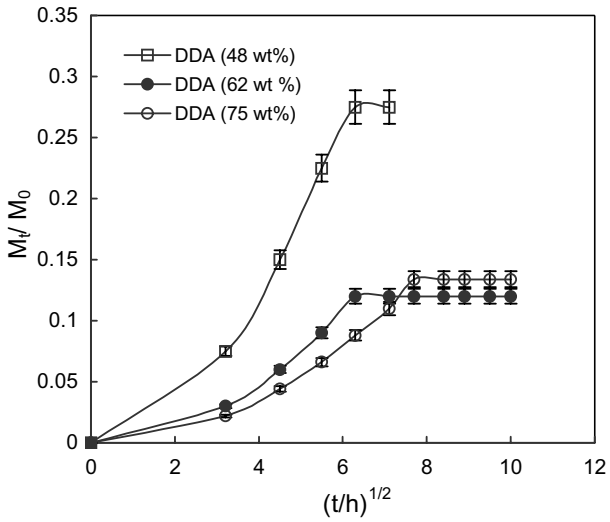


Figure 10. Effect of DDA of chitosan on fractional release of centchroman. Release media = 20 mL buffered solution (pH 7), microspheres = 100 mg, $T = 37$ °C.

The diffusion coefficient (D) for centchroman varied with molecular weight and degree of deacetylation in chitosan. The diffusion coefficient (D) for centchroman from microspheres with low-molecular-weight chitosan and low degree of deacetylation was found to be 2.9×10^{-12} cm^2 s^{-1} and 3.34×10^{-12} cm^2 s^{-1}, respectively (Tables 1 and 2). The value of the diffusion constant decreased to 0.05×10^{-12} cm^2 s^{-1} on increasing the molecular weight of chitosan, and it decreased to 0.03×10^{-12} cm^2 s^{-1} on increasing the DDA in chitosan (Table 2), which clearly indicates that the release of centchroman from these microspheres is diffusion controlled and the variation in diffusion coefficient (D) of centchroman on varying the molecular weight and degree of deacetylation in chitosan is due to the variations in the structure of microspheres.

4. Conclusions

The analysis of swelling, loading, and release trends of centchroman from microspheres with chitosan of different molecular weights and degrees of deacetylation, clearly indicates that the molecular weight and degree of deacetylation of chitosan play a significant role in controlling the release characteristics of microspheres for centchroman. The microspheres prepared with medium-molecular-weight (1134 kg mol^{-1}) and medium-DDA (62 wt %) were more efficient in the controlled release of centchroman (82 wt %) than other microspheres. The glutaraldehyde cross-linked (6 wt %) microspheres with medium-molecular-weight chitosan (1334 kg mol^{-1}) and 62 wt % degree of deacetylation have shown 50 wt % degradation within three weeks, but microspheres with high-molecular-weight chitosan (2224 kg mol^{-1})/high degree of deacetylation (>62 wt %)/with high degree of cross-linking (>6 wt %) have shown a degradation of 20 wt % within a period of three months. The cross-linked microspheres in the concentration range of glutaraldehyde and DDA studied were nontoxic and biodegradable; hence, these microspheres may be considered as suitable candidate for the oral delivery of centchroman.

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