Drug Development and Industrial Pharmacy, 35:379–386, 2009

ISSN: 0363-9045 print / 1520-5762 online
DOI: 10.1080/03639040802395185

informa
healthcare

The Effect of Chitosan Molecular Weight on the Characteristics of Spray-Dried Methotrexate-Loaded Chitosan Microspheres for Nasal Administration

Yu Sun, Fude Cui, Kai Shi, Jiamiao Wang, Mengmeng Niu, and Ruijing Ma
Department of Pharmaceutics, School of Pharmacy, Shenyang Pharmaceutical University, Shenyang, China

In this article, the effect of the chitosan molecular weight (MW) on the characteristics of methotrexate (MTX)-encapsulated non-cross-linked chitosan microspheres was studied. Microspheres composed of low-molecular-weight (LMW, 40,000 Da), medium-molecular-weight (MMW, 480,000 Da) and high-molecular-weight (HMW, 850,000 Da) chitosan with the same degree of deacetylation (96%) were obtained by a simple spray-drying method. The MW of chitosan had a noticeable influence on the size distribution, encapsulation efficiency, micromeritic properties (angle of repose and bulk density), controlled release behavior, and mucoadhesive properties. The entrapment efficiencies were in the range of 90–99%. Spray-dried microspheres had a D_{50} value of 3.3–4.9 μm, which was suitable for nasal insufflations. The microspheres with LMW chitosan have the best flowability and highest bulk density but were found to be poor in terms of adhesion and in controlling the release behavior of MTX. The MMW chitosan microspheres exhibited the strongest adhesion to the mucosal surface, and the angle of repose values were between 34 and 47 degrees. They could control the release rate by modifying the drug/polymer ratios. Microspheres with HMW chitosan exhibited a lower adhesion than MMW chitosan and a lower release rate of MTX. The physical state of MTX in the chitosan matrix was studied by differential scanning calorimetry, which indicated the presence of a solid dispersion of the amorphous drug in the chitosan matrix. Nasal ciliotoxity showed only minor cilia irritation due to the microspheres, and consequently, they are suitable for nasal drug delivery.

Keywords nasal; chitosan; methotrexate; microspheres; mucoadhesive

INTRODUCTION

Recently, the nasal route has attracted increasing attention as an alternative route for systemic drug delivery and brain targeting. However, one of the problems for nasal administration is the rapid mucociliary clearance that limits the time available for drug absorption from the nasal mucosa. A drug solution can be cleared by the mucociliary process from the cavity into the nasopharynx with a half-life of 15 min (Mao et al., 2004). To overcome these difficulties, recently, bioadhesive microparticulate delivery systems prepared from some high molecular materials such as starch (Illum et al., 2001), gelatin (Morimoto et al., 2001), chitosan (Alexander et al., 2006), chitin (Zhang et al., 2005), and dextran (Pereswetoff & Edman, 1995) have been widely used for nasal administration. In particular, chitosan is a positive linear natural polysaccharide. The biodegradability and bioadhesivity of chitosan are useful properties in formulations for nasal drug delivery, which need prolonged retention in the nasal cavity. The main parameters that control the physicochemical and biological properties of chitosan are the molecular weight (MW) (Seyfarth et al., 2008) and the degree of deacetylation (Ventura et al., 2008; Xu & Du, 2003). Commercial chitosan is characterized by a degree of deacetylation between 70 and 95% and a MW between 50,000 and 2,000,000 Da. In our study, three different chitosans with a MW in the range of 40–850 kDa were used to determine the effect of the chitosan MW on the properties of the microspheres prepared. Methotrexate (MTX), used as a model drug in our study, is a folic acid antagonist, which is also the basic chemotherapy agent used for the treatment of brain tumors, especially primary central nervous system lymphoma and leptomeningeal metastatic cancer. Hence, investigations on a mucoadhesive drug delivery system for MTX via the nose to brain pathway are considered worthwhile. As reported by Singh and Udupa (1998), MTX chitosan microspheres were prepared by an emulsion cross-linked method, which involved two or more steps and always involved some cross-linkers and organic solvents. Spray drying was a mild, simple process, especially without any organic solvents. As confirmed by Genta et al. (1998), the bioadhesive properties of microparticulate drug delivery system were reduced for glutaraldehyde cross-linked chitosan microspheres. Therefore, in our study, chitosan microspheres containing MTX without any cross-linkers were prepared by a spray-drying method.

Address correspondence to Fude Cui, Department of Pharmaceutics, School of Pharmacy, Shenyang Pharmaceutical University, No. 103, Wenhua Road, Shenyang 110016, China. E-mail: cuifude@163.com

The aim of our study was to assess the effect of the MW of chitosan on the preparation and characteristics of chitosan microspheres loaded with MTX and also to choose an appropriate formulation for our next investigations. The micromeritic properties, adhesive studies, swelling properties, and control characteristics of non-cross-linked microspheres were evaluated as a function of the MW of chitosan.

MATERIALS AND METHODS

Materials

Chitosan was purchased from Yuhuan Ocean Biochemical Co., Ltd. (Hangzhou, Zhejiang Province, China) with different MWs (40, 480, and 850 kDa) and the same degree of deacetylation (96%). MTX was obtained from Suzhou Sunray Pharmaceutical Co., Ltd., Suzhou, Jiangsu Province, China. All solvents were of reagent grade available commercially.

Preparations of Chitosan Microspheres

The microspheres were produced by the spray-drying method. Briefly, aqueous solutions containing different concentrations of polymer were prepared by dissolving chitosan and MTX in 0.1 N HCl solution, followed by gentle agitation at room temperature for 1–2 h. The microspheres were obtained by spraying the solutions through the nozzle (0.7 mm diameter) of a spray dryer (Spray Dryer SD-1000, EYELA, Tokyo, Japan). The conditions of the spray-drying process were set as follows: inlet air temperature 120°C, outlet air temperature 88°C, air flow rate 0.7 m^3/min, air pressure 180 kPa, and spray rate of feed about 5 mL/min. The microspheres were then harvested from the collector. The volumes of the feed for the preparation were 200 mL.

The factors tested included the MWs (40, 480, and 850 kDa) and concentrations (0.2, 0.4, and 0.5 mg/mL) of chitosan (see Tables 1 and 2 for details). Each batch was examined in triplicate.

Determination of MTX Content of Microspheres

Drug content and encapsulation efficiency (EE) were evaluated. Ten milligrams of microspheres was dispersed in 0.04 mol/L NaOH (100 mL), and the drug was extracted from the microspheres for 24 h under magnetic stirring. Then the dispersion was passed through 13-mm polytetrafluoroethylene (PTFE) syringe filters (0.45-μm porosity), and the MTX concentration was determined by UV analysis at 302 nm. The EE was calculated from the ratio of the actual MTX content (AMC) to the theoretical MTX content (TMC) and expressed as a percentage.

TABLE 1
Characteristics of Chitosan Used in this Study

	$\eta_{mpa.s}$[a]	MW (kDa)	DD (%)
LMW	—	40	96
MMW	75	480	96
HMW	135	850	96

MW, molecular weight; DD, degree of deacetylation; LMW, low molecular weight; MHW, medium molecular weight; HMW, high molecular weight.

[a]Dynamic viscosity.

Size Distribution and Morphology

Particle size analysis was performed by laser diffraction using a Coulter LS 230 instrument (Beckman-Coulter Co., Ltd., Fullerton, USA). The diameters were calculated using the volume-based distribution. The morphology was observed through an optical microscope (Nikon Fx-35A, Nikon, Tokyo, Japan) and scanning electron microscopy (SEM) (Hitachi S-5200, Hitachi, Tokyo, Japan).

Measurement of Micromeritic Properties of Microspheres

Bulk density was measured by placing the microspheres in a 10 mL measuring cylinder with a funnel, and angle of repose was measured according to the fixed funnel method (Allen, Popovich, & Ansel, 2005). A funnel was fixed perpendicularly on a steel shelf above a round-shaped disk. Then the microspheres were carefully poured through the funnel until the microspheres were able to flow through the rim of the conical pile. The height of the conical pile (H) and the diameter of the disk ($2R$) were determined. The tangent of the angle of repose was given by the equation

$$\tan \alpha = \frac{H}{R} \tag{1}$$

where α is the repose angle.

Differential Scanning Calorimetry

Differential scanning calorimetry (DSC) patterns of the samples were obtained using a differential scanning calorimeter (DSC-60, Japan Shimadzu Co., Kyoto, Japan). Each sample was heated from 30 to 300°C at a scanning rate of 10°C/min. DSC analyses were carried out on blank (MMW chitosan) and drug-loaded microparticles (CM1). A physical mixture of the blank microspheres and the pure drug was used as a control.

In Vitro Release Studies

In vitro release tests were performed in 250 mL phosphate buffer (pH 7.4) at 37°C and 100 rpm. At fixed time intervals (5, 15, 25, 35, 45, 60, 120, and 185 min), 5 mL samples were withdrawn and replaced with the same volume of dissolution medium. The MTX content in the dissolution samples was measured by UV spectrophotometric analysis at 302 nm. The dissolved amount of drug at each time was expressed as a percentage of the dose. The dissolution rate of the MTX as a raw

TABLE 2
Formulas and Characteristics of Spray-Dried Chitosan Microspheres Loaded with MTX (Mean ± *SD*, *n* = 3)

		CSC	TMC	AMC	EE	PY	D_{50} (μm)	Angle of Repose (Degrees)	Bulk Density (g/mL)
LMW	CL1	0.2	23.08	21.00 ± 0.05	90.99 ± 0.34	59.57 ± 3.78	3.3 ± 0.16	30.2 ± 2.2	0.142 ± 0.012
	CL2	0.4	13.01	11.97 ± 0.04	92.08 ± 0.56	50.78 ± 5.87	3.8 ± 0.23	40.0 ± 1.7	0.154 ± 0.015
	CL3	0.5	10.70	10.01 ± 0.07	93.55 ± 0.47	56.34 ± 6.90	3.6 ± 0.43	41.6 ± 2.3	0.153 ± 0.006
MMW	CM1	0.2	23.08	21.08 ± 0.01	91.36 ± 0.05	53.6 ± 4.23	4.2 ± 0.69	34.5 ± 1.8	0.126 ± 0.008
	CM2	0.4	13.01	11.76 ± 0.03	90.48 ± 0.30	59.1 ± 3.89	4.8 ± 0.17	41.6 ± 1.9	0.113 ± 0.004
	CM3	0.5	10.70	10.04 ± 0.03	93.84 ± 0.29	53.5 ± 4.97	4.2 ± 0.98	47.3 ± 2.4	0.114 ± 0.015
HMW	CH1	0.2	23.08	21.03 ± 0.07	91.16 ± 0.33	46.7 ± 5.85	4.5 ± 0.54	44.6 ± 1.5	0.115 ± 0.017
	CH2	0.4	13.01	12.17 ± 0.05	93.61 ± 0.38	51.6 ± 3.42	5.0 ± 0.37	51.8 ± 1.4	0.091 ± 0.004
	CH3	0.5	10.70	10.55 ± 0.06	98.56 ± 0.53	50.6 ± 3.37	4.9 ± 0.73	57.0 ± 1.6	0.097 ± 0.001

CSC, chitosan concentration (%, wt/vol); TMC, theoretical methotrexate content (%, wt/wt); AMC, actual methotrexate content (%, wt/wt); EE, drug encapsulation efficiency (%); PY, production yield (%).

material was determined under the same conditions as reported above.

The procedure was repeated to prepare microspheres with chitosan samples of different MWs. Each experiment was performed in triplicate.

In Vitro Mucoadhesion Studies

The bioadhesive properties of microspheres were determined by an adapted method described by Hascicek, Gönül, and Erk (2003). The principle of this test is based on simulating a biological flow by washing a mucous membrane covered with the product to be tested. A freshly cut 5-cm-long piece of rat intestine was obtained and cleaned by washing with isotonic saline solution. Accurately weighed 50-mg samples of microspheres were placed on the mucosal surface, which was fixed over a slide support and then maintained at 75% relative humidity for 15 min in a desiccator. After that, the intestine was thoroughly washed with phosphate buffer solution (pH 7.4) at the rate of 15 mL/min using a peristaltic pump. The concentration of the drug in the collected perfusate was determined by UV spectrophotometry at 302 nm. The amount of microspheres corresponding to the amount of drug in the perfusate was calculated. The amount of adherent microspheres was estimated from the difference between the amount of applied microspheres and the amount of flowing microspheres. The ratio of the adhered microspheres to the applied microspheres was computed as the percentage mucoadhesion.

Nasal Ciliotoxicity

Nasal ciliotoxicity studies were performed using the in situ toad palate model (Jiang, Cui, Fang, Wei, & Xi, 1995). In brief, the upper palate of a toad (30–40 g, Experimental Animal Center of Shenyang Pharmaceutical University, Shenyang, China) was exposed and treated with 50 mg samples for 1 h, and then rinsed with saline. The palate was cut up carefully with scissors and tweezers, avoiding any destruction of the mucosa, and then spread on a glass slide and examined under an optical microscope (Nikon Fx-35A, Nikon). Saline and sodium deoxycholate solution (a very toxic nasal mucociliary agent, 1% [wt/vol] solution) were used as a negative and positive control, respectively.

Statistical Analysis

Statistical analysis was performed with the Student's unpaired *t* test. A difference was considered to be statically significant when the *p* value was less than .05.

RESULTS AND DISCUSSION

Characterization of the Microspheres

The characteristics of the chitosan used in our study are listed in Table 1. To study the effect of the MW and concentration of chitosan on the characteristics of mucoadhesive microspheres, chitosan microspheres were obtained at a fixed MTX concentration of 0.6 mg/mL for all the formulations. To determine the drug content in the microspheres, 0.04 mol/L NaOH solution was used here for drug extraction rather than an acidic solution, because the solubility of MTX was much higher in 0.04 mol/L NaOH than in acidic solutions. From Table 2, it can be seen that the EE of all three kinds of chitosan microspheres was above 90%. An increase in the concentration of chitosan solution resulted in an increase in the drug encapsulation efficiency (EE), giving an EE value up to 98.56% with 0.5% chitosan (CH3). These results were apparently due to the greater amount of polymer being able to entrap more drug. The production yields of all microspheres produced were between 50 and 60%. The relatively lower yields were due to both the low quantity of the feed used and the fact that the structure of

the apparatus made it impossible to collect the smallest and lightest particles, as reported by Giunchedi et al. (2000).

Size Distribution and Morphology

As shown in Table 2, all the microspheres exhibited normal size distributions, and the D_{50} values were in the range 3.3–4.9 μm, which is suitable for nasal delivery (Behl et al., 1998). The size of drug-loaded microspheres formed was increased with increasing chitosan MW at the same polymer concentration. As the MW increases, the viscosity of the chitosan solution rises, and under the same preparation conditions, the droplets formed from the higher viscosity chitosan solution are larger in size, resulting in a larger microspheres being formed; this is in agreement with an earlier report (Ping et al., 1999). The morphology of the chitosan microspheres was examined by light microscopy as well as SEM (Figure 1). The SEM of the pure drug was characterized by particles of acicular shape and heterogeneous particle size. Chitosan microspheres loaded with MTX exhibited a smooth surface, with an irregularly depressed shape and a hollow inside. There was no free drug present on the surface, which, as expected, indicated that the microspheres formed were amorphous in nature, because powders generated through spray drying are known to be predominately amorphous in nature (Tristan et al., 2008).

The reason that the particles were not perfectly spherical was probably attributed to the rapid drying process used, leading to fast evaporation on the surface of a droplet and subsequently the inner side, giving rise to numerous invaginations. According to previous reports (Liu, Chen, Zeng, & Liu, 2005; Ventura et al., 2008), the mechanisms could be as shown below (Figure 2).

Measurement of Micromeritic Properties of Microspheres

From Table 2, we can see that the MW of chitosan played an important role in the micromeritic properties. On increasing the MW of chitosan, the angle of repose increases, whereas the bulk density decreases. LMW chitosan (40 kDa) microspheres exhibited the best flow properties, with the angle of repose values varying between 30 and 41 depending on the chitosan concentration. However, as the MW rose to 850 kDa, the microspheres obtained exhibited poor flowability, easily aggregating. The decreasing bulk density can be explained by the fact that small spherical spray-dried particles would be expected to pack together closely leading to a lower volume to mass ratio, and, therefore, higher bulk density. Conversely, the large particles would be expected to exhibit poor packing characteristics, resulting in a lower bulk density. For HMW chitosan microspheres, poor flowability can also prevent the particles from packing tightly, leading to a lower bulk density.

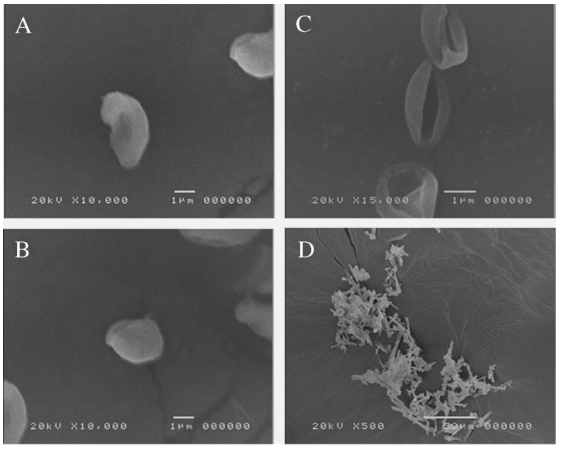

FIGURE 1. The SEM photographs of MTX microspheres CM1 with different shapes (A, B, C) and raw material methotrexate (D).

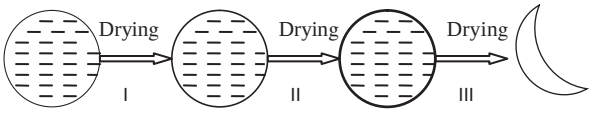

FIGURE 2. Formation mechanism of particles with hollow shape.

DSC

Three endotherms were found in the DSC thermograms of MTX (Figure 3A). The first two endotherms located at 62.86°C

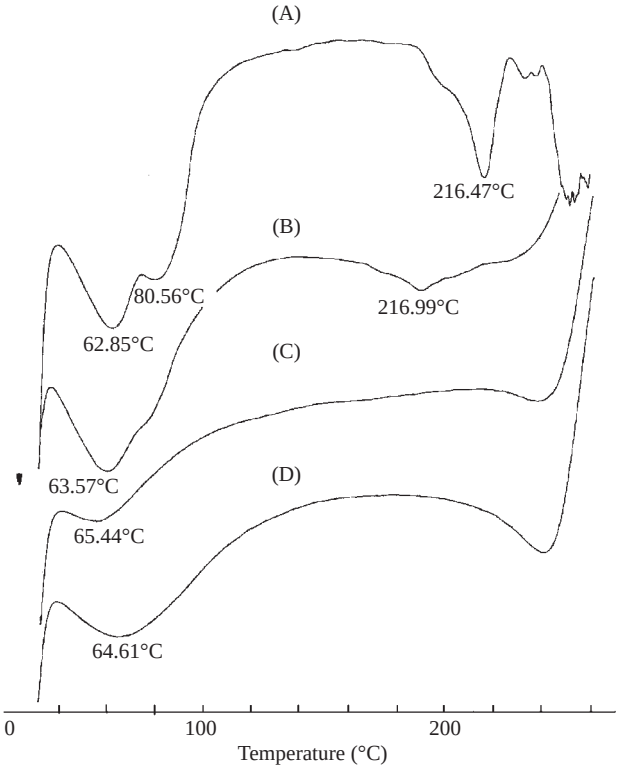

FIGURE 3. DSC analysis of MTX (a), physical mixture of blank microspheres and MTX (b), blank microspheres (c), chitosan microspheres loaded with the drug (d).

and 80.56°C were associated with loss of free and bound water, respectively. The third endotherm at 216.47°C corresponded to the melting peak of MTX (Dhanikula & Hildgen, 2007). The blank microspheres (Figure 3C) showed a wide peak at about 65.44°C due to the loss of water linked to the amine or hydroxyl groups of chitosan. In addition, no endothermic peaks were found near the melting peak of MTX. As seen in Figure 3B, the thermal behavior of both MTX and blank microspheres was not changed in the case of the DSC thermograms of their physical mixtures, whereas their thermal peaks were retained. The melting peak of MTX disappeared completely in the calorimetric curve of MTX-loaded chitosan microspheres (Figure 3D), which indicated the presence of a solid dispersion of the amorphous drug into the chitosan matrix.

In Vitro Release Studies

The in vitro drug release profiles of MTX-loaded microspheres with different formulations are shown in Figure 4. As expected, the pure drug underwent very rapid dissolution, with 100% MTX being released within 5 min. The chitosan microspheres demonstrated delayed release characteristics; an increase in the MW of the chitosan was associated with a more sustained release profile. For instance, the LMW chitosan microspheres were unable to control the release rate of the MTX, and released 100% of the MTX after 10 min; hence, we only give CL1 release profiles, whereas 3–4 h were necessary for HMW chitosan microspheres to release all the drug. The MMW chitosan displayed a relatively higher release rate than HMW chitosan, and total drug release was obtained in less than 3 h. The release behavior of MMW chitosan microspheres was mainly driven by the drug concentration gradient, which meant that a higher level of drug loading led to a higher diffusion rate. In the case of CM1 and CM2 (with 21.08 and 11.76% loading capacity, respectively), the different release rates were attributed to the MTX concentration gradient. However, for CM3, with a 10.7% loading capacity, this exhibited a higher release rate than CM2. One possible explanation is that the higher concentration of chitosan provides a more viscous medium. During the spray-drying process, the viscous medium is unable to allow the formation of compact microspheres, which are porous with many invaginations, resulting in a higher release rate than CM2.

According to the release curve in pH 7.4 medium, MTX is released more slowly from the microspheres having HMW chitosan than from those with MMW chitosan during the same release period. This can be attributed to the higher density of the shell around the drug particles resulting in a lower degree of swelling, and thus, a decrease in the release profiles occurred (Gupta & Jabrail, 2007; Peng, Zhang, & Kennedy, 2006).

To explore further the difference between MMW and HMW chitosan microspheres, the Ritger–Peppas equation (Ritger & Peppas, 1987) was introduced here to investigate their release mechanisms. The logarithm of the accumulated release was plotted as a function of the logarithm of the time (Figure 5). In each case, a linear relationship was observed; the mechanism parameter n value, the correlation coefficients (r values), rate of drug release (k values, obtained from the intercept on the y-axis), and the time taken for 50% drug release are all presented in Table 3. Different function models were applied to describe the release profiles by previous investigators (Peng et al., 2006; Zambito & Colo, 2003). Here, on the basis of our previous analyses, the Ritger–Peppas equation was the most suitable one to determine the release mechanism for MTX microspheres.

As reported by Ritger and Peppas (1987), the n value is an empirical parameter characterizing the release mechanism. On the basis of the diffusion exponent, an n value below 0.45 indicates that the drug release mechanism approaches that of Fickian diffusion-controlled release, whereas an n value above

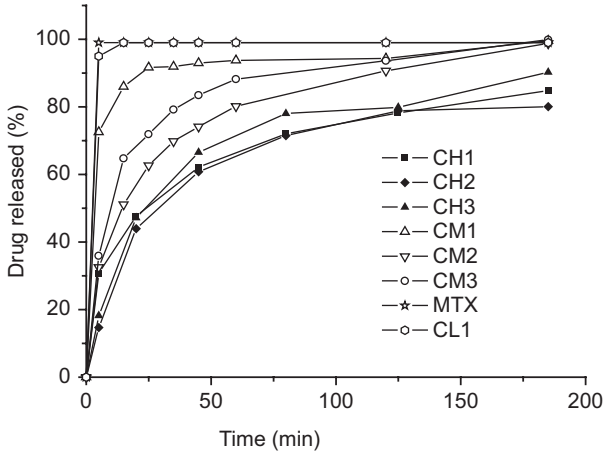

FIGURE 4. In vitro drug release tests of microspheres carried out in phosphate buffer (pH 7.4). Data were average values (n = 3).

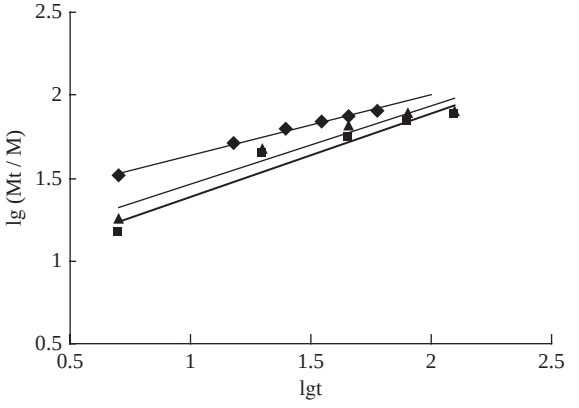

FIGURE 5. Plots of release data lg (Mt/M) versus lgt for chitosan microspheres. ♦, CM2; ■, CH2; ▲, CH3.

TABLE 3
The Kinetic Constants (k), Diffusional Exponents (n), Correlative Coefficients (r), and the Time Taken for 50% Drug Release Following Linear Regression of Release Data of CM2, CH2, and CH3 Microspheres

	r	K	n	$t_{50\%}$ (min)
CM2	.9958	18.49694	0.3678	14.9
CH2	.9694	7.6243	0.5055	41.3
CH3	.9696	9.788136	0.4702	32

0.89 indicates that the drug release mechanism approaches zero-order release. An n value of 0.45–0.89 represents a drug release mechanism for non-Fickian diffusion or chain relaxation controlled release.

From Table 3, we can see that the n value was in the range 0.3678–0.5055 and increased with increasing chitosan MW. For CM2, the n value was 0.3678, which indicated that the release mechanism of MMW chitosan microspheres followed Fickian diffusion-controlled release, attributable to the thinner shell around the drug particles, and a concentration-gradient-controlled release occurred. In the case of HMW microspheres, the MTX release mechanism was non-Fickian diffusion and was controlled by the swollen microspheres. However, an interesting phenomenon occurred in the case of CH3, similar to CM3 (Figure 4), where the kinetic constant value (9.788) was higher than that of CH2 (7.624) due to the porous structure caused by the relatively higher viscous medium.

Although the in vitro release tests here cannot simulate the physiological environment in the nasal cavity where there is a low quantity of nasal secretions, mucociliary movement, and certain nasal enzymes, these studies can provide us with comparative information about the rate of drug release from different batches and further allow us to choose appropriate formulations for in vivo use (Tristan et al., 2008). The aim of our present study was to prepare chitosan mucoadhesive microspheres for nasal administration. In view of the mucociliary clearance as the main limit of nasal drug delivery, and some reports demonstrating that the mucoadhesive microspheres can prolong the contact time with the mucosa and be retained in the nasal cavity for 1–2 h (Mao et al., 2004), we expected that the drug could exhibit sustained released for 2 h. From the results given above, we can conclude that due to the poor controlling characteristics, the LMW chitosan is not suitable for delivering MTX. The MMW and HMW chitosan will be more useful for modifying the release behavior of MTX.

In Vitro Mucoadhesive Studies

Differences in mucoadhesive characteristics were expected by changing the chitosan concentrations and MW. From Figure 6, significant differences were seen between LMW and MMW ($p < .01$) and between LMW and HMW ($p < .01$).

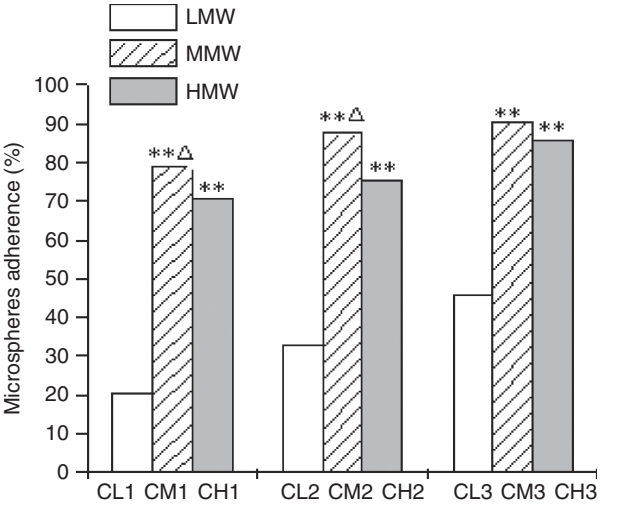

FIGURE 6. Influence of chitosan molecular weight on mucoadhesive properties of spray-dried microspheres. The asterisks represent significant difference from LMW chitosan. $**p < .01$ and △, significant difference from HMW chitosan ($p < .05$).

LMW microspheres showed a significantly lower binding to mucin than the other types of chitosan. A statistically higher adhesion of MMW microspheres was observed compared with HMW microspheres studied at the same concentration ($p < .05$). Other authors had already pointed out the existence of a direct relationship between the chitosan MW and its bioadhesiveness because of its greater ability to penetrate within the mucus chains (Bravo-Osuna et al., 2007; Takeuchi et al., 2005). The mucoadhesive process is rather complicated, and there are several theories to explain the mucoadhesion of polymeric materials (Campion, 1975; Deryaguin, Toporow, & Mueler, 1997; Helfand & Tagami, 1972; Kaelble, 1977). Diffusion is one of the main theories proposed to describe mucoadhesion which includes the action of polymer-chain entanglement (Thongborisute & Takeuchi, 2008). The diffusion theory states that inter-penetration of the chains of polymer and mucus may lead to sustained mucoadhesion and mechanical interlocking between mucin and mucoadhesive. So, we can conclude that as the MW of MMW is nearly 10 times greater than that of LMW, the contribution of physical entanglement to the adhesion phenomenon between MMW and the surface of mucin particles was clearly much stronger than for LMW under our experimental conditions. However, considering HMW microspheres, the adhesive properties were unexpectedly lower than those of MMW microspheres. The reason was that HMV chitosan with relatively longer chains can bend and become entangled leading to relative less amino groups being available, thus providing a lower positive charge to interact with the mucosa. The results also indicated that higher chitosan concentrations produced more mucoadhesion, which was due to the presence of a greater amount of polymer in the same volume of liquid droplets, and thus provided a greater positive charge to interact

with the negative mucin surface. From these results, we can conclude that microspheres with LMV chitosan are less useful for preparing mucoadhesive microspheres and MMW and HMW chitosan microspheres have excellent mucoadhesive properties. Consequently, we can expect that these microspheres loaded with MTX will reduce the mucocilia clearance and prolong the contact time with the nasal mucosal surface.

Nasal Ciliotoxicity

As the nasal ciliary movement contributes to the nonspecific defensive mechanism of the body, the nasal ciliotoxicity study is one of the most important methods to evaluate any nasal drug delivery system (Hermens & Merkus, 1987). Hence, one of the requirements for the microspheres prepared is that they do not produce any nasal irritation.

The optical microscopic study (40 × 10) (seen in Figure 7A) indicated that the cilia movement was intact, dense, and beating actively after 1 h of treatment with saline. When treated with 1% sodium deoxycholate solution (Figure 7B), the entire cilia became detached and no movement was observed. A minor effect of the microspheres on the cilia movement was observed, and the cilia displayed an irregular and fast beat after a 1-h treatment with MTX chitosan microspheres (Figure 7C). Hence, the observed results indicated that the microspheres produced a minor degree of nasal ciliotoxicity.

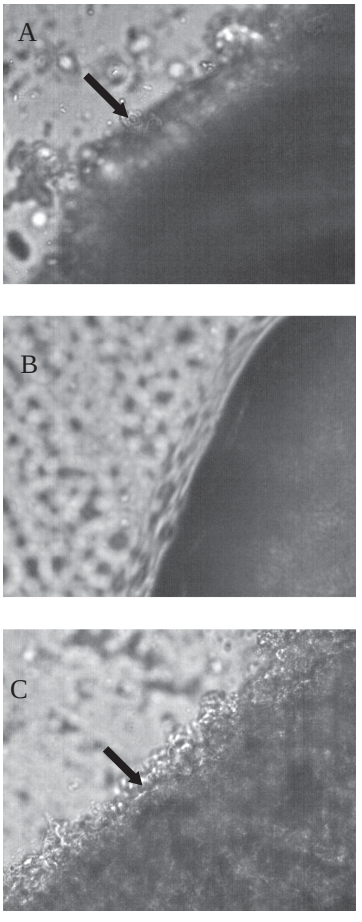

FIGURE 7. Optical microscopic images of (A) negative control (saline), (B) positive control (1% sodium deoxycholate solution), and (C) MTX microspheres. Cilia are indicated by arrow (10 × 40 magnification, $n = 3$).

CONCLUSIONS

The effect of the chitosan concentration and MW on the characteristics of MTX microspheres was studied, and it was found that chitosan with MMW (480 kDa) was useful to produce mucoadhesive microspheres with better flowability and mucoadhesion properties. When the MW of chitosan was around 40 kDa, the microspheres displayed favorable micromeritic properties but poor controlled drug release and mucoadhesive properties. However, when the MW of chitosan was over 850 kDa, the microspheres obtained were easy to aggregate and did not fit the pharmaceutical requirements. Therefore, chitosan with a MW around 450 kDa exhibited the most suitable characteristics for nasal MTX administration. The in vitro evaluation studies showed that spray drying was an ideal method to prepare chitosan microspheres with MMW chitosan exhibiting better mucoadhesion and minor nasal ciliotoxicity, which can be used as a candidate material in our further investigations.

REFERENCES

Alexander, H. K., Davide, G. I., & Andreas, B. S. (2006). Thiolated chitosan microparticles: A vehicle for nasal peptide drug delivery. *Int. J. Pharm., 307*, 270–277.

Allen, L. V., Jr., Popovich, N. G., & Ansel, H. C. (2005). *Ansel's pharmaceutical dosage forms and drug delivery systems* (8th ed., p. 190). Baltimore, MD: Lippincott Williams and Wilkins.

Behl, C. R., Pimplaskar, H. K., Sileno, A. P., deMeireles, J., & Romeo, V. D. (1998). Effects of physicochemical properties and other factors on systemic nasal drug delivery. *Adv. Drug Deliv. Rev., 29*, 89–116.

Bravo-Osuna, I., Vauthier, C., Farabollini, A., Palmierib, G. F., & Ponchel, G. (2007). Mucoadhesion mechanism of chitosan and thiolated chitosan-poly (isobutyl cyanoacrylate) core-shell nanoparticles. *Biomaterials, 28*, 2233–2243.

Campion, R. P. (1975). The influence of structure on autohesion (self-tack) and other forms of diffusion into polymers. *J. Adhesion, 7*, 1–23.

Deryaguin, B. V., Toporow, Y. P., & Mueler, V. M. (1997). On the relationship between the electrostatic and molecular component of the adhesion of elastic particles to a solid surface. *J. Colloid Interface. Sci., 58*, 528–533.

Dhanikula, R. S., & Hildgen, P. (2007). Influence of molecular architecture of polyether–co–polyester dendrimers on the encapsulation and release of methotrexate. *Biomaterials, 28*, 3140–3152.

Genta, I., Costantini, M., Asti, A., Conti, B., & Montanari, L. (1998). Influence of glutaraldehyde on drug release and mucoadhesive properties of chitosan microspheres. *Carbohydr. Polym., 36*, 81–88.

Giunchedi, P., Gavini, E., Bonacucina, G., & Palmieri, G. F. (2000). Tabletted polylactide microspheres prepared by a w/o emulsion-spray drying method. *J. Microencapsul., 17*, 711–720.

Gupta, K. C., & Jabrail, F. H. (2007). Glutaraldehyde cross-linked chitosan microspheres for controlled release of centchroman. *Carbohydr. Res., 342*, 2244–2252.

Hascicek, C., Gönül, N., & Erk, N. (2003). Mucoadhesive microspheres containing gentamicin sulfate for nasal administration: Preparation and in vitro characterization. *Farmaco, 58*, 11–16.

Helfand, E., & Tagami, Y. (1972). Theory of the interface between immiscible polymers. *J. Chem. Phys., 57*, 1821–1823.

Hermens, W. A. J. J., & Merkus, F. W. H. M. (1987). The influence of drugs on nasal ciliary movement. *Pharm. Res., 4*, 445–449.

Illum, L., Fisher, A. N., Jabbal-Gill, I., & Davis, S. S. (2001). Bioadhesive starch microspheres and absorption enhancing agents act synergistically to enhance the nasal absorption of polypeptides. *Int. J. Pharm., 222*, 109–119.

Jiang, X. G., Cui, J. B., Fang, X. L., Wei, Y., & Xi, N. Z. (1995). Toxicity of drugs on nasal mucocilia and the method of its evaluation. *Acta Pharm. Sin., 30*, 848–853.

Kaelble, D. H. (1977). A surface energy analysis of bioadhesion. *Polymer, 18*, 475–482.

Liu, H. D., Chen, Y. F., Zeng, D. D., & Liu, J. (2005). Preparation of porous chitosan particles for pulmonary drug delivery. *J. Funct. Mater., 36*, 616–618.

Mao, S. R., Chen, J. M., Wei, Z. P., Liu, H., & Bi, D. Z. (2004). Intranasal administration of melatonin starch microspheres. *Int. J. Pharm., 272*, 37–43.

Morimoto, K., Katsumata, H., Yabuta, T., Iwanaga, K., Kakemi, M., Tabata, Y., & Ikada, Y. (2001). Evaluation of gelatin microspheres for nasal and intramuscular administrations of salmon calcitonin. *Eur. J. Pharm. Sci., 13*, 179–185.

Peng, X. H., Zhang, L. N., & Kennedy, J. F. (2006). Release behavior of microspheres from cross-linked N-methylated chitosan encapsulated ofloxacin. *Carbohydr. Polym., 65*, 288–295.

Pereswetoff, M. L., & Edman, P. (1995). Dextran microspheres as a potential nasal drug delivery system for insulin-in vitro and in vivo properties. *Int. J. Pharm., 124*, 37–44.

Ping, H. A., Stanley, S., Davis, A., & Lisbeth, I. (1999). Chitosan microspheres prepared by spray drying. *Int. J. Pharm., 187*, 53–65.

Ritger, P. L., & Peppas, N. A. (1987). A simple equation for description of solute release. II. Fickian and anomalous release from swellable devices. *J. Control. Release, 5*, 37–42.

Seyfarth, F., Schliemann, S., Elsner, P., & Hipler, U. C. (2008). Antifungal effect of high- and low-molecular-weight chitosan hydrochloride, carboxymethyl chitosan, chitosan oligosaccharide and N-acetyl-d-glucosamine against Candida albicans, Candida krusei and Candida glabrata. *Int. J. Pharm., 353*, 139–148.

Singh, U. V., & Udupa, N. (1998). Methotrexate loaded chitosan and chitin microspheres: In vitro characterization and pharmacokinetics in mice bearing ehrlich ascites carcinoma. *J. Microencapsul., 15*, 581–594.

Takeuchi, H., Thongborisute, J., Matsui, Y., Sugihara, H., Yamamoto, H., & Kawashima, Y. (2005). Novel mucoadhesion tests for polymers and polymer coated particles to design optimal mucoadhesive drug delivery systems. *Adv. Drug Deliv. Rev., 57*, 1583–1594.

Thongborisute, J., & Takeuchi, H. (2008). Evaluation of mucoadhesiveness of polymers by BIACORE method and mucin-particle method. *Int. J. Pharm., 354*, 204–209.

Tristan, P. L., Jane, L. B., Eddie, F., & Peter, C. S. (2008). Chitosan-based spray-dried respirable powders for sustained delivery of terbutaline sulfate. *Eur. J. Pharm. Biopharm., 68*, 224–234.

Ventura, C. A., Tommasini, S., Crupi, E., Giannone, I., Cardile, V., Musumeci, T., & Puglisi, G. (2008). Chitosan microspheres for intrapulmonary administration of moxifloxacin: Interaction with biomembrane models and in vitro permeation studies. *Eur. J. Pharm. Biopharm., 68*, 235–244.

Xu, Y. M., & Du, Y. M. (2003). Effect of molecular structure of chitosan on protein delivery properties of chitosan nanoparticles. *Int. J. Pharm., 250*, 215–226.

Zambito, Y., & Colo, G. D. (2003). Preparation and in vitro evaluation of chitosan matrices for colonic controlled drug delivery. *J. Pharm. Pharm. Sci., 6*, 274–281.

Zhang, Y. J., Ma, C. H., Lu, W. L., Zhang, X., Wang, X. L., Sun, J. N., & Zhang, Q. (2005). Permeation-enhancing effects of chitosan formulations on recombinant hirudin-2 by nasal delivery in vitro and in vivo. *Acta Pharmaco. Sin., 26*, 1402–1408.