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Preparation and release kinetics of carboxymethyl chitosan/cellulose acetate microspheres as drug delivery system

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ABSTRACT: Carboxymethyl chitosan, a water soluble chitosan derivative, was prepared from chitosan using monochloroacetic acid. Carboxymethyl chitosan/cellulose acetate microspheres (CCM) were prepared using the method of W/O/W and emulsification solvent evaporation as drug delivery system. The CCMs prepared were spherical, free-flowing, and nonaggregated with the smooth appearance and many small pores on the surface. All CCMs prepared had sustained release efficiency for acetaminophen and the optimal formulation was that carboxymethyl chitosan of 2.0% and 1360 KD. In addition, the release rate of drug from CCMs in dilute hydrochloric acid was much slower than that in phosphate buffer saline (pH 6.8) during 24 h. It is illustrated that the drug loaded in CCMs released slower in simulated gastric fluid than that in simulated intestinal fluid. Furthermore, the drug release data showed better fitness with the first order model which indicated that the drug release from CCMs was depended on the drug concentration in the polymeric networks. And the release of drug from CCMs indicated diffusion-controlled drug release based on Fickian diffusion and accompanied with anomalous transport (i.e., non-Fickian diffusion) according to the values obtained from Higuchi model and Peppas models. So it was shown that the CCMs might be an ideal sustained release system for acid-labile drugs both for the solubility of carboxymethyl chitosan and the release media. © 2015 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2015**, *132*, 42152.

KEYWORDS: biomaterials; composites; drug delivery systems; functionalization of polymers

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INTRODUCTION

Chitosan is a linear polysaccharide composed of randomly distributed β-(1–4)-linked D-glucosamine (deacetylated unit) and *N*-acetyl-D-glucosamine (acetylated unit). Chitosan has been extensively studied in the pharmaceutical industry for its potential in the development of controlled drug release delivery, its excellent bioactivity, biocompatibility, biodegradability and non-toxicity. And chitosan has been used to prepare various sustained release drug carriers such as microparticles,[1,2] microspheres,[3–5] films,[6,7] hydrogels,[8,9] and beads.[10,11] Chitosan microspheres have been investigated widely as controlled release delivery systems for antigens,[12] vaccines,[13] phytoalexin,[14] proteins,[15] cells,[16] vitamins,[17] and drugs.[18] Furthermore various chitosan composite microspheres have been prepared with different materials such as poly (vinyl alcohol),[19,20] tripolyphosphate,[21] alginate,[22,23] poly(lactic-*co*-glycolic acid),[24] polyvinylpyrrolidone,[25] ethyl cellulose,[26] and cellulose acetate.[27] In addition, different administration routes of microspheres are studied to enhance the bioavailability of drugs including via intranasal,[13,28] ocular,[22] inhalable,[29,30] subcutaneous,[13] colonic,[18,31] intra-articular,[32] and oral delivery.[33]

However, chitosan is insoluble in water and only soluble in acidic solution which limits its application in pharmaceutical industry, especially for the drugs unstable in acidic conditions. So carboxymethyl chitosan, a water soluble chitosan derivative, is prepared and chosen as a substitute to prepare drug carriers. The new composite microspheres (CCM) have been prepared with hydrophilic core and hydrophobic coating as drug delivery system. Cellulose acetate is selected as hydrophobic coating to entrap the hydrophilic carboxymethyl chitosan microcores. Cellulose acetate is one of the most important cellulose derivatives because of its wide range of industrial applications including thickeners in cosmetics and food products, membranes for drug release, adhesives and biomolecule immobilization. Cellulose acetate has been used to prepare carriers such as films,[34] microparticles,[35] nanofibers,[36] and microspheres.[37]

In this article, carboxymethyl chitosan was prepared with monochloroacetic acid and the CCM with cellulose acetate coating and carboxymethyl chitosan core were prepared by the methods of water in oil in water (W/O/W) and emulsification solvent evaporation as drug delivery system. The effect of

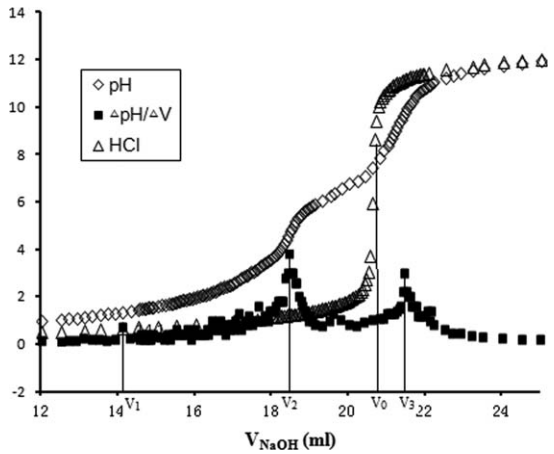

Figure 1. The integral and differential titration curves of HCl and CM_1.

carboxymethyl chitosan on sustained release was studied while the preparation parameters of coating were fixed. Acetaminophen is a popular analgesic and antipyretic drug which is used for the relief of fever, headaches, and other minor aches and pains. Although the side effects of acetaminophen are mild to nonexistent in recommended doses and for a limited course of treatment, acute overdoses of acetaminophen can cause potentially fatal liver damage according to the US Food and Drug Administration.[38] So acetaminophen is selected as model drug to evaluate the potential of the loaded microspheres as oral delivery system.

EXPERIMENTAL

Materials

Chitosan, derived from crab shell, molecular weight 1360 KD and deacetylation degree 75.1%, was obtained from Biochemical Medicine Plant of Qingdao (Qingdao, China). Cellulose acetate (viscosity 300−500 Pas, acetyl content 55%), methylene chloride, ethanol, and acetic acid glacial were all chemical reagents (analytical pure) provided by Shanghai Chemical Reagent Company (Sigma Co. ST. Louis, USA). Acetaminophen was kindly donated by the Xinhua Pharmaceutical Factory (Shijiazhuang, China).

Preparation and Characteristics of Carboxymethyl Chitosan

Carboxymethyl chitosan was prepared using the method of Chen *et al.*[39] In brief, chitosan (10 g), sodium hydroxide (12 g), and solvents (40 mL distilled water and 60 mL isopropanol) were added into a three-mouth flask (500 mL). The temperature was controlled by a water bath (Thermo-controller, Comabiotech. Co., Korea) to swell and alkalize at 50°C for 1 h. Monochloroacetic acid (12 g) was dissolved in isopropanol (20 mL), and added into the three-mouth flask by dropping equably and reacted for 7 h. Then the reaction was stopped by adding 70% v/v aqueous ethanol (200 mL). The solid was filtered and rinsed in 70−90% ethyl alcohol to desalt and dewater, and vacuum dried at room temperature.

Carboxymethyl chitosan of different molecular weights were prepared from chitosan of different molecular weights. Different molecular weights chitosan with the same deacetylation degree were prepared by the method of acetic acid hydrolysis of chitosan.[40] The molecular weights of chitosan samples prepared was 1360 kD, 1110 kD, 540 kD, 200 kD, and 38 kD, respectively, with the mean deacetylation degree of 75.1%.

Deacetylation degree and degree of substitution of carboxymethyl chitosan were analyzed by the method of potentiometric titration. Each dried carboxymethyl chitosan (0.1 g) dissolved in HCl (0.1 mol/L, 20 mL) and was titrated with the standard solution of NaOH (0.1 mol/L) using a pH meter (DELTA-320-S pH meter). V_1, V_2, and V_3 were the inflection points (seen in Figure 1). The differential volume ($\triangle V$) of NaOH between V_2 and V_1 corresponded to the alkali consumed by carboxymethyl groups and that between V_3 and V_2 corresponded to the alkali consumed by amino groups presented in the carboxymethyl chitosan, respectively.

Deacetylation degree and degree of substitution of carboxymethyl chitosan were calculated using following eqs. (1) and (2), respectively.

$$DS = \frac{0.203 \times (V_2 - V_1) \times C_{(NaOH)}}{m - m_1 + m_2} \quad (1)$$

$$DD(\%) = \frac{(V_3 - V_2) \times 0.1 \times 240.3 \times 100}{m \times 1000} \quad (2)$$

Where, DD was the deacetylation degree; DS was degree of substitution; $m_1 = (V_2\text{-}V_1) \times C_{(NaOH)} \times 0.080\text{-}(V_3\text{-}V_0) \times C_{(NaOH)} \times 0.022$; $m_2 = (V_3\text{-}V_2) \times C_{(NaOH)} \times 0.042$; V_0 was the volume of NaOH consumed by standard HCl solution. m_1 and m_2 were the weight of carboxymethyl groups and acetyl groups deviated from $-NH_2$ of carboxymethyl chitosan in the total sample while m was the initial weight of dried carboxymethyl chitosan sample.

The FTIR spectra of carboxymethyl chitosan were recorded on an FT/IR-430 Fourier Transform Infrared Spectrometer (Jasco, Tokyo, Japan). Pellets were formed from 2 mg of each sample and 100 mg of KBr.

Preparation of Microspheres

Carboxymethyl chitosan/cellulose acetate microspheres (CCM) were prepared using the method of W/O/W emulsification and solvent evaporation.[27] Briefly, carboxymethyl chitosan (0.2 g) was dissolved in 20 mL distilled water and 0.2 mL tween-80 was added into the solution. Then model drug, acetaminophen, was added into carboxymethyl chitosan solution under agitation to dissolve completely. Cellulose acetate (2.0%, w/v) were dissolved in 60 mL mixture of methylene chloride and ethanol ($v/v = 3/1$). The carboxymethyl chitosan solution was poured dropwise into the cellulose acetate solution with continuous stirring at 2000 rpm for 20 min to form the primary W/O emulsified solution. Then the emulsified solution was added into 240 mL of sodium polyphosphate solution (3%, w/v) under moderate stirring for 30 min to allow the evaporation of the solvent. Finally, the microspheres were filtered and dried at room temperature.

CCM with different concentrations and molecular weights of carboxymethyl chitosan were prepared and the characteristics were studied to evaluate the effects of carboxymethyl chitosan. The parameters were shown in Table I.

Table I. The Different Parameters of Carboxymethyl Chitosan and CCMs

	Carboxymethyl chitosan							
CCM Sample	Sample	DD/%	DS	C /%	MW/KD	Acetaminophen/g	LE/%	EE/%
CCM_0	CM_1	72.1	1.12	2.0	1360	/	/	/
CCM_1	CM_1	72.1	1.12	1.0	1360	0.32	9.8	4.93
CCM_2	CM_1	72.1	1.12	1.5	1360	0.36	13.5	6.96
CCM_3	CM_1	72.1	1.12	2.0	1360	0.40	17.1	8.64
CCM_4	CM_1	72.1	1.12	2.5	1360	0.44	11.1	4.06
CCM_5	CM_1	72.1	1.12	3.0	1360	0.48	12.7	5.14
CCM_6	CM_2	72.9	1.18	2.0	1110	0.40	13.4	6.84
CCM_7	CM_3	71.5	1.13	2.0	540	0.40	9.1	3.84
CCM_8	CM_4	72.7	1.16	2.0	200	0.40	8.2	3.76
CCM_9	CM_5	71.9	1.14	2.0	38	0.40	7.8	3.21

DD, deacetylation degree; DS, degree of substitution; C, concentration; MW, molecular weight; LE, loading efficiency; EE, entrapment efficiency.

Morphological Characterization of Microspheres

The size and the surface properties of the CCM were investigated with a scanning electron microscopy (JSM-840 scanning microscope, JEOL). Prior to observation, samples were coated with gold under vacuum.

Determination of Drug Loading Efficiency

The CCM (50mg) loaded acetaminophen were triturated and dispersed into 100 mL of distilled water. The suspension of CCM were stirred continuously at 160 rpm in a vibrating incubator at 37 ± 0.5°C (HZQ-F160 All-temperature Vibrating Incubator, Harbin Donglian Electronic & Technology Development, China) over night. The suspension was filtered and the filtrate was collected to measure the absorbance at 257 nm by ultraviolet spectroscopy (Tu-1800 uv-vis spectrophotometer, Beijing Purkinje General Instrument). Then the contents of acetaminophen were calculated using the standard curve method. The standard curve of acetaminophen achieved in distilled water was seen in Table II.

The drug loading efficiency was calculated from the eq. (3):

$$\mathrm{LE} = A/B \times 100\% \quad (3)$$

where LE was loading efficiency; A was the amount of drug loaded in the microspheres and B was the total amount of drug.

Then the entrapment efficiency was calculated from the eq. (4):

$$\mathrm{EE} = A/C \times 100\% \quad (4)$$

where EE was entrapment efficiency; A was the amount of drug loaded in the microspheres and C was the total amount of microspheres.

Table II. Standard Curves of Acetaminophen in Different Medium

Medium	Standard curve	R^2
Distilled water	$A = 0.072C - 0.009$	0.9998
dHCl	$A = 0.0430C + 0.0213$	0.9993
PBS (pH6.8)	$A = 0.0435C + 0.0123$	0.9992

Evaluation of Drug Release *In Vitro*

The microspheres, 100 mg, were placed into dialysis membrane with a molecular weight cut-off of 8000 ~ 15,000. Then the dialysis membrane was placed into 100ml release media in Erlenmeyer flasks (250 mL). Release media was phosphate buffer saline (PBS, pH 6.8) or dilute hydrochloric acid (dHCl, pH 1.0). The Erlenmeyer flasks were closed with plastic membrane and stirred continuously at 160 rpm in a vibrating incubator at 37 ± 0.5°C. Triplicate samples were run.

At predetermined time intervals, samples of 5 mL were taken out of the solution and replaced by equal volume of the same medium to maintain a constant volume. The samples were assayed by spectrophotometry at 257 nm. The model drug concentrations were determined by UV absorption at each collecting time point according to standard curve method. The standard curves of acetaminophen achieved in PBS (pH 6.8) and dHCl (pH 1.0) were seen in Table II. The accumulated release rates were calculated by using the eq. (5):

$$Q\% = (C_n \cdot V + V_i \cdot \sum_{i=0}^{n-i} C_i)/m \times 100\% \quad (5)$$

where Q was cumulative release rate, %; C_n was the model drug concentration collected at n time point, mg/mL; V was the volume of release medium, mL; V_i was the sample volume per time point ($V_0 = 0$), mL; C_i was the concentration of collected sample per time point ($C_0 = 0$), mg/mL; m was the mass of model drug in microspheres, mg; n was sampling frequency of release medium.

The assays were performed in at least triplicate on separate occasions. The data collected in this study were expressed as the mean value ± standard deviation (SD).

Release Kinetics

Several mathematical models of drug release, such as zero order kinetic equation, first order kinetic equation, Higuchi equation and Peppas kinetics equation are used to fit the *in vitro* release of acetaminophen in PBS and dHCl from CCMs.

The zero order kinetic equation describes the systems, where the drug release did not depend on the concentration.[41,42] Zero order model is described as eq. (6):

$$M_t = M_0 + k_0 t \quad (6)$$

where M_t is the percentage of drug released at time t and k_0 is the release rate constant;

The first order kinetic equation describes the dependency on the drug concentration in the polymeric networks.[41,42] First order model is described as eq. (7):

$$Ln(100 - M_t) = Ln100 - k_1 t \quad (7)$$

where k_1 is the release rate constant for the first order kinetics;

Higuchi model equation proposes a direct relation of the drug release from the matrix to the square root of time and is based on the Fickian diffusion.[43,44] Higuchi model is described as eq. (8):

$$\frac{M_t}{M_\infty} = k_H t^{1/2} \quad (8)$$

where M_t is the amount of drug release at time t; M_∞ is the amount of drug release after infinite time; K_H is the rate constant which represents the internal structure and the shape of the matrix as well as the drug solubility and concentration. When a plot of cumulative drug release to $t^{1/2}$ yields a straight line, the particular system is considered to follow Higuchi kinetics of drug release.[44,45]

When the drug release is controlled both by diffusion and dissolution, a simple, semi-empirical model is developed by Ritger and Peppas[44] which described as Peppas model seen eq. (9).

$$\frac{M_t}{M_\infty} = Kt^n \quad (9)$$

where n is a parameter that depends on the release mechanism and is used to characterize the mechanism. If the n value is 0.5, the release mechanism follows Fickian diffusion according to Higuchi model drug release. If the n value is 1, the release is independent of time which corresponded to zero-order release kinetics. When the n values are between 0.5 and 1.0, the diffusion is termed as Non-Fickian release. The n value could be obtained from the slope of the plot of lg M_t/M_∞ vs. lg time.

RESULTS AND DISCUSSION

Characteristics of Carboxymethyl Chitosan Samples

The parameters of carboxymethyl chitosan prepared were shown in Table I. The series carboxymethyl chitosan were successfully synthesized from different molecular weights of chitosan and all samples had water solubility. The deacetylation degree and degree of substitution of carboxymethyl chitosan were estimated using potentiometric titration with eqs. (1) and (2). It could be seen in Table I that the carboxymethyl chitosan samples prepared had the almost same deacetylation degree from 71.5% to 72.9% and degree of substitution from 1.12 to 1.18. The integral titration and differential curves of CM_1 were shown in Figure 1. Molecular weight of carboxymethyl chitosan was depended on its degree of substitution and the molecular weights of corresponding chitosan. For the degree of substitution of carboxymethyl chitosan prepared were almost the same and the deacetylation degree was almost changeless with chitosan, so the molecular weights of chitosan was used to represent the different molecular weights of carboxymethyl chitosan.

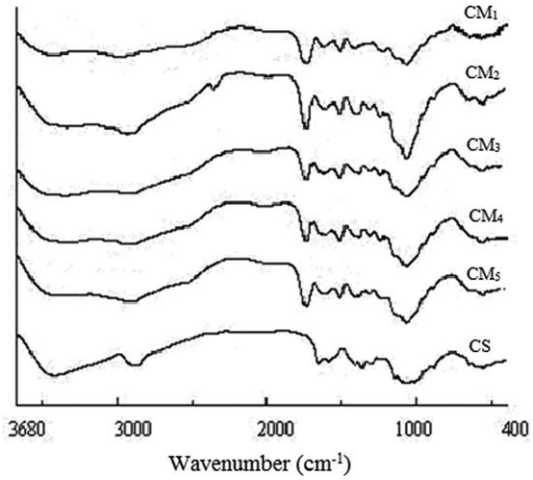

Figure 2. The FTIR spectra of carboxymethyl chitosan and chitosan.

The FTIR spectra of carboxymethyl chitosan and chitosan were shown in Figure 2. It could be seen that all synthesized carboxymethyl chitosan had similar structure. All carboxymethyl chitosan had large -COOH group peak at 1741 cm^{-1} and $-NH_3$ group peak at 1506 cm^{-1}. The C—O stretching band at 1030 cm^{-1} corresponding to the primary hydroxyl group of chitosan disappeared, which verified a high carboxymethylation of 6-OH. The characteristic peak of second hydroxyl group at 1080 cm^{-1} was not changed. So carboxymethyl chitosan were successfully prepared and the FTIR spectra were in agreement with the former report.[46,47]

Characteristics of CCMs

The scanning electron micrographs of CCM were shown in Figure 3. It could be seen that the CCM prepared by the W/O/W method were spherical, free-flowing, and nonaggregated with the smooth appearance and many small pores on the surface. Figure 3(A,B) were the micrographs of CCM without model drugs (CCM_0), Figure 3(C−H) were that of CCM-loaded acetaminophen as CCM_3, CCM_1, and CCM_8, respectively.

The adding of model drug had no effects on the size of CCM seen in Figure 3(A,C). However, the appearance of CCM_3 became more compact and the pores became less compared with CCM_0 seen in Figure 3(B,D). Furthermore, the crystal could be seen in the surface of CCM_3 which were the model drug exuded from the core material during the preparation of microspheres [Figure 3(D)].

The size and appearance of microspheres were affected by the concentration and molecular weight of carboxymethyl chitosan which could be seen in Figure 3. The microspheres made from carboxymethyl chitosan of concentration (1.0%) had a smaller size than that made from higher concentration (2.0%) seen in Figure 3(C,E). The microsphere size became bigger with the carboxymethyl chitosan concentration increased from 1.0% to 3.0%. These results could be explained by the increase of

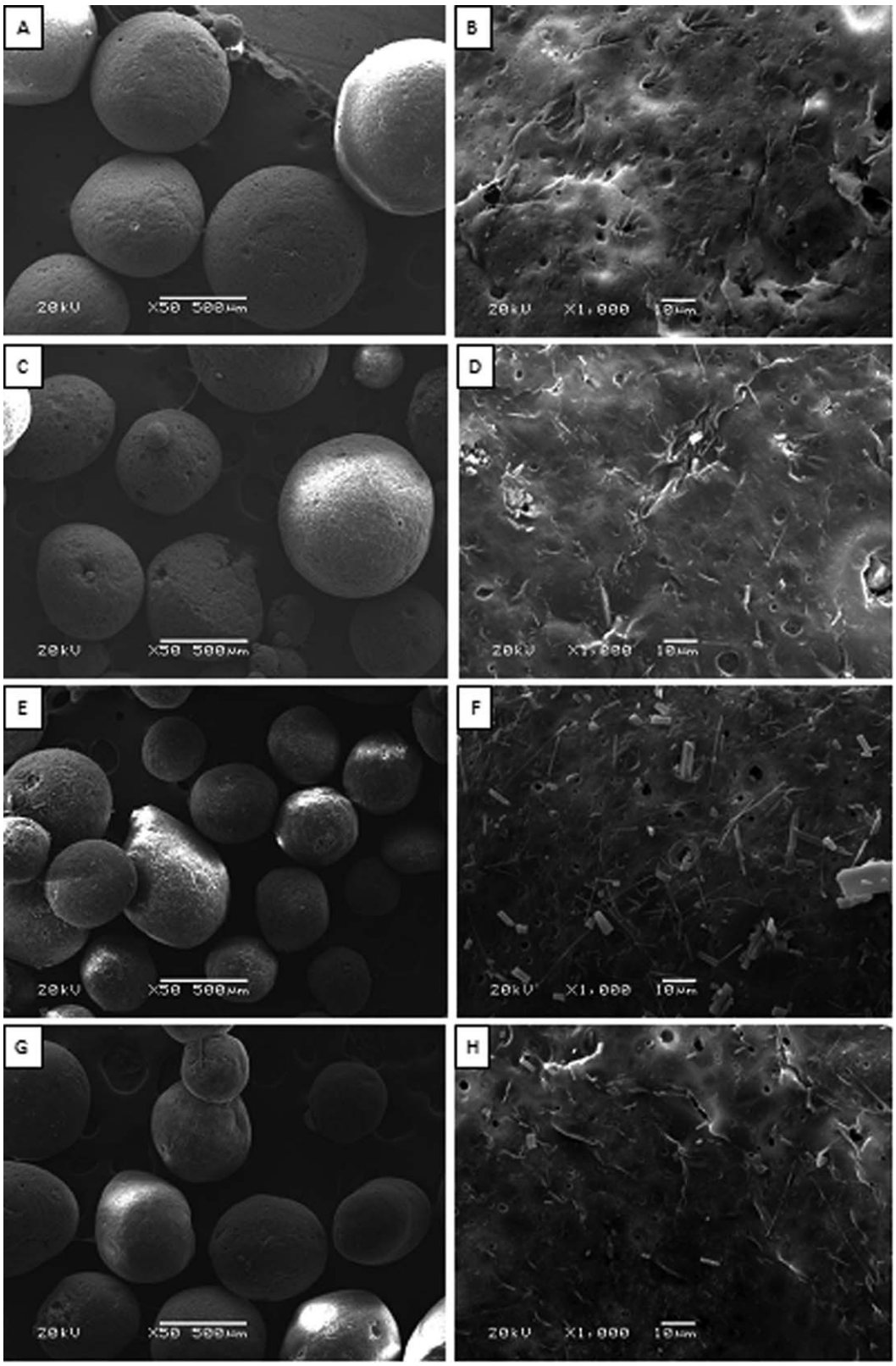

Figure 3. Scanning Electron Micrograph of CCMs: A:CCM_0 (×50); B: the appearance of CCM_0 (×1000); C: CCM_3 (×50); D: the appearance of CCM_3 (×1000); E: CCM_1 (×50); F: the appearance of CCM_1 (×1000); G: CCM_8 (×50); H: the appearance of CCM_8 (×1000).

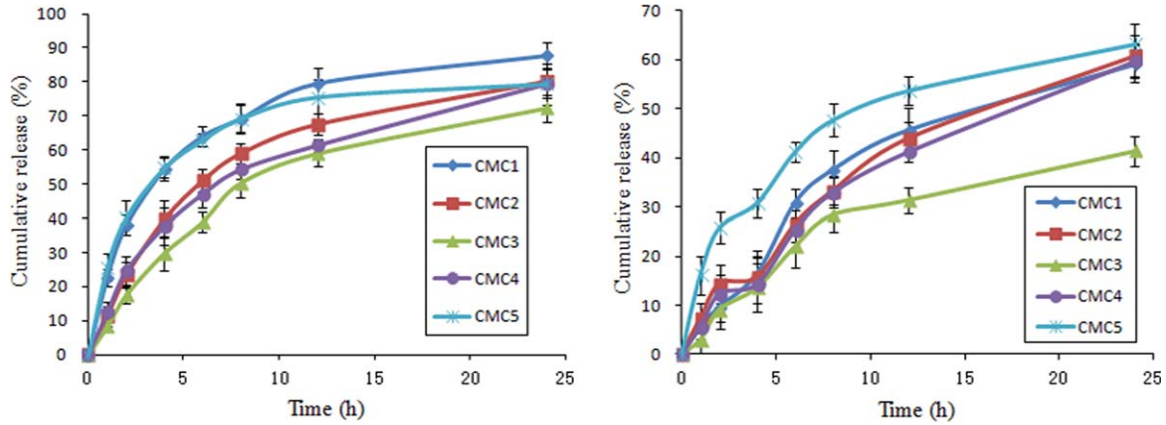

Figure 4. Release profile of the model drug from CCMs prepared with different concentration of carboxymethyl chitosan. A: Released in PBS (pH 6.8); B: released in dHCl (pH 1.0) (date shown were the mean ± SD, n= 3 for each sample). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

viscosity which increased the mean droplet size. It could be seen in Figure 3(D,F) that the appearance of microspheres was almost the same except that the crystal in the surface of CCM_1 was obviously more than that of CCM_3. The results might be because that the parameters (concentration and volume) of coat material (cellulose acetate) were constant for all CCM preparation. So the model drug diffused quickly as the concentration of carboxymethyl chitosan was lower for its lower viscosity which resulted in more crystal of drug in the CCM surface.

Different molecular weights carboxymethyl chitosan (38 KD, 200 KD, 540 KD, 1110 KD, and 1360 KD) at 2.0% concentration were used to make CCMs. Figure 3(C) showed the micrographs of the microspheres of carboxymethyl chitosan (1360 KD) and Figure 3(G) showed that of carboxymethyl chitosan (200 KD). It could be seen that the CCMs made from lower molecular weight carboxymethyl chitosan had the smaller size and more loose appearance and the microspheres made from the lowest molecular weight chitosan (38 KD) had the smallest size. The microspheres structure became more compact with the increasing molecular weights of carboxymethyl chitosan [seen in Figure 3(D,H)]. Similarly, the lower molecular weights of carboxymethyl chitosan had lower viscosity which resulted in the quickly diffuse of model drug and more crystal of drug in the CCM surface.

Loading Efficiency of CCMs

Acetaminophen was selected as a amphoteric drug to investigate the loading efficiency and entrapment efficiency which were shown in Table I. It could be seen that the loading efficiency and entrapment efficiency were increased with the increase of molecular weights of carboxymethyl chitosan and the CCM prepared with largest molecular weights of carboxymethyl chitosan had the highest loading efficiency and entrapment efficiency. Meanwhile, the loading efficiency and entrapment efficiency were increased with the increase of concentration of carboxymethyl chitosan from 1.0% to 2.0%. The results could be explained by the diffusion of drug that the lower molecular weights and concentration of carboxymethyl chitosan had lower viscosity and quicker diffusion which were in accordance with the results of scanning electron micrographs. However, the loading efficiency and entrapment efficiency were decreased with the

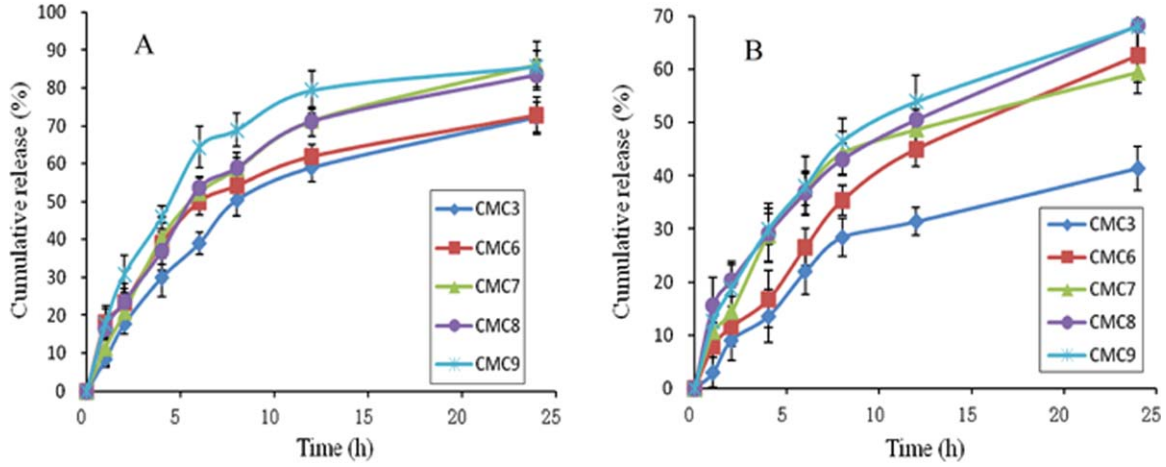

Figure 5. Release profile of the model drug from CCMs prepared with different molecular weights of carboxymethyl chitosan. A: Released in PBS (pH 6.8); B: released in dHCl (pH 1.0) (date shown were the mean ± SD, n = 3 for each sample). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

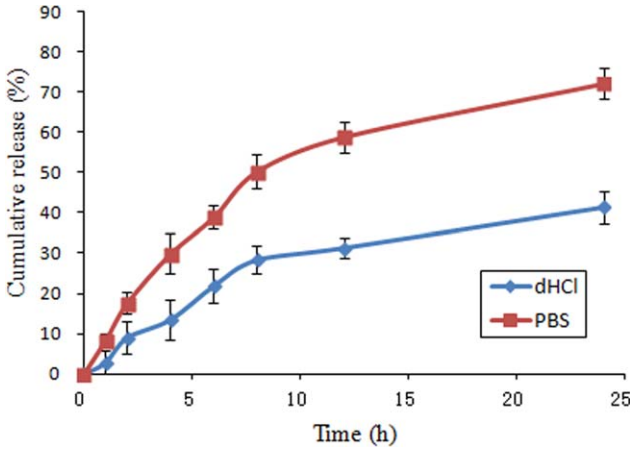

Figure 6. Release profile of the model drug from CCM_3 in different release media. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

increase of concentration of carboxymethyl chitosan from 2.0% to 3.0%. The results might be attributable to that the parameters of coat material (cellulose acetate) were constant for all CCM preparation. The coating layer might become thin and loose with the concentration of carboxymethyl chitosan became large enough which resulted in the more diffusion and lose of drug. So the 2.0% and 1360 KD of carboxymethyl chitosan were the optimal parameters to prepare CCM microspheres.

It was shown in Table I that the highest loading efficiency was not more than 20%. The results might be related to that water was exuded from the microspheres along with the dissolved drug during the dispersion and hardening process of the primary W/O emulsion in the outer aqueous phase.

In Vitro Release

The release profile of the CCMs was evaluated in different media *in vitro*. Phosphate buffer saline (PBS, pH 6.8) and dilute hydrochloric acid (dHCl, pH 1.0) were selected as release media. PBS (pH 6.8) was used as simulated intestinal fluid and dHCl (pH 1.0) as simulated gastric fluid to evaluate the effect of gastrointestinal tract on the release of oral drug.

The release profiles of CCM prepared with different concentrations of carboxymethyl chitosan were shown in Figure 4. It could be seen that the release of acetaminophen from CCM characterized by a slow release phase which indicated the sustained release efficiency of the microspheres prepared. The release rate of acetaminophen had the same variation tendency with the change of carboxymethyl chitosan concentration both in PBS and dHCl. Firstly, the release rate decreased with the increase of carboxymethyl chitosan concentration from 1.0% to 2.0%. Then it increased with the increase of carboxymethyl chitosan concentration from 2.0% to 3.0%. CCM prepared with 2.0% carboxymethyl chitosan had the slowest release rate of acetaminophen, and the CCM prepared with higher or lower concentration of carboxymethyl chitosan had relatively higher release rate. So the CCM prepared with different carboxymethyl chitosan concentrations had sustained release efficiency for acetaminophen and the optimal condition was 2.0%.

Figure 5 showed the release profile of acetaminophen from the CCMs prepared with different molecular weights of carboxymethyl chitosan *in vitro*. Figure 5(A) was the released profile of acetaminophen from microspheres in PBS (pH 6.8) and Figure 5(B) was that in dHCl (pH 1.0). The results showed that all CCM samples had sustained release efficiency on acetaminophen. It could be seen that the release rate of acetaminophen was decreased with the increase of molecular weights of carboxymethyl chitosan no matter in PBS or dHCl and CCM_3 prepared with carboxymethyl chitosan of 1360KD had the slowest release rate. The release rate of acetaminophen from CCM_3 was not more than 75% in PBS and 42% in dHCl during 24 h, respectively. So for all samples used in this paper the optimal formulation to prepare CCMs was that carboxymethyl chitosan concentration and molecular weights was 2.0% and 1360 KD, respectively.

It could be seen in Figures 4 and 5 that the release rate was different in PBS from that in dHCl, although the variation tendency was the similar. So the release profiles of the CCM_3 were selected to evaluate the effect of different release media which was shown in Figure 6.

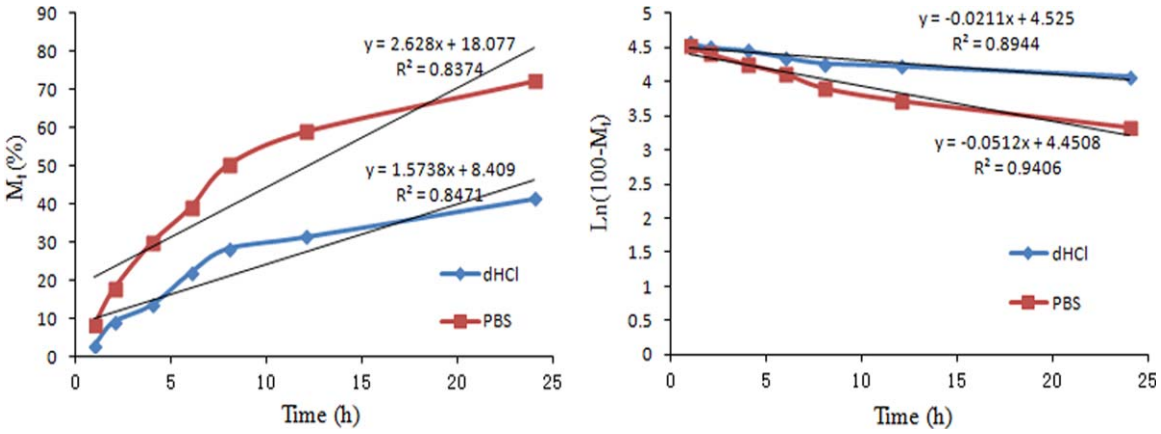

Figure 7. Release kinetics of CCMs in PBS (pH 6.8) and dHCl (pH 1.0): A: zero order release kinetics; B: first order release kinetics. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

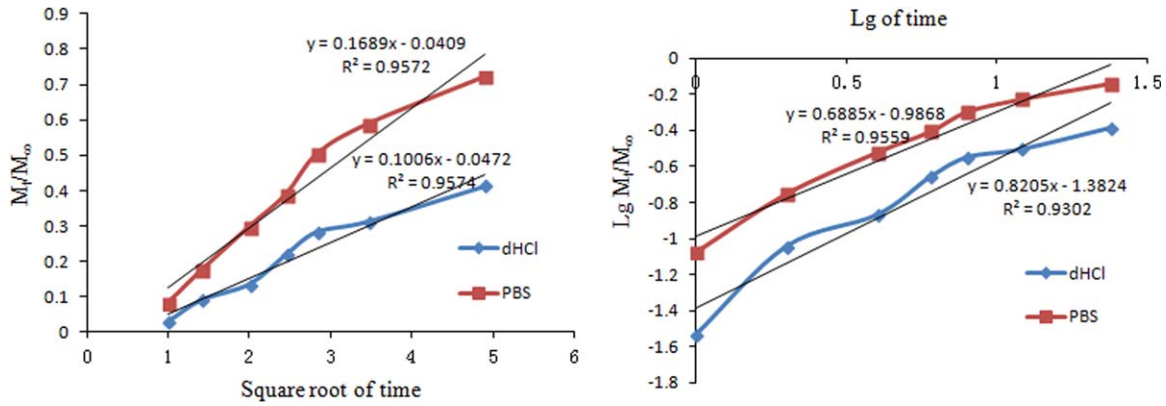

Figure 8. Release kinetics of CCM s in PBS (pH 6.8) and dHCl (pH 1.0): A: Higuchi kinetics; B: Peppas kinetics. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

It could be seen that all release rates in dHCl (pH 1.0) were much slower than that in PBS (pH 6.8). The release rate of acetaminophen was only a little more than 10% in dHCl and that was almost 30% in PBS in 4h. In addition, the release rate of acetaminophen was a little more than 40% in dHCl during 24 h and that was more than 70% in PBS during the same time. This result might be for the difference of solubility or swelling of carboxymethyl chitosan and the different effects of cellulose acetate under different pH values. It is illustrated that the drug loaded in CCMs released slower in simulated gastric fluid than that in simulated intestinal fluid. So CCMs might be the ideal carrier for acid-labile drugs by using carboxymethyl chitosan, water soluble chitosan derivative, both for the solubility of carboxymethyl chitosan and the release media.

Release Kinetics

Different kinetic models were selected to evaluate the release kinetics and the mechanism from the CCMs. The drug release data of the optimized formulation CCM_3 were fitted to zero order and first model kinetic models (seen in Figure 7). To evaluate the release mechanism, the release data were fitted to the Higuchi model and Peppas model equation (seen in Figure 8). The kinetic rate constants k, release exponent n and $t_{1/2}$ of each model were calculated by linear regression analysis, respectively. Coefficients of correlation (R^2) were used to evaluate the accuracy of the fitness. Based on the best correlation coefficient values, the most appropriate model was selected to explain the release behavior of the drug. The fitting equation, values of the release exponent (n), kinetic rate constant (k), the correlation coefficient (R^2) and $t_{1/2}$ were tabulated in Table III. It could be seen that the $t_{1/2}$ predicted from different models were 9.9−12.1 h for the release in PBS and 20.8−29.6 h for that in dHCl, respectively.

Generally speaking, the formulations did not seem to obey a zero order kinetics based on the low R^2 values obtained compared to those of the first order profiles of the drug release. It was shown from Figure 7 and Table III that CCMs showed better fitness with the first order model in different media, especially the release in PBS where the R^2 reached 0.9406. So the drug release from CCMs was depended on the drug concentration in the polymeric networks.

It could be seen in Figure 8 and Table III that the values obtained from Higuchi model and Peppas models were found to be very close to each other both in PBS and dHCl. The value of "n" determined for microspheres studied was 0.8205 in dHCl and 0.6855 in PBS which were between 0.5 and 1.0 as tabulated in Table III. So the release of drug from CCMs indicated diffusion-controlled drug release based on Fickian diffusion and accompanied with anomalous transport (i.e., non-Fickian diffusion).

Table III. Fitting Results and Relative Parameters of *In Vitro* Release of Acetaminophen from CCMS in Different Release Media

			Relative parameters			
Fit method		Equation	n	k	R^2	$T_{1/2}$
zero-order	pH1.0	M_t=1.5738t+8.409	/	1.5738	0.8471	26.4
	pH6.8	M_t=2.628t+18.077	/	2.628	0.8374	12.1
first-order	pH1.0	$Ln(100-M_t)$=−0.0211t+4.525	/	−0.0211	0.8944	29.1
	pH6.8	$Ln(100-M_t)$=−0.0512t+4.4508	/	−0.0512	0.9406	10.5
Higuchi	pH1.0	M_t=0.1006$t^{1/2}$−0.0472	/	0.1006	0.9574	29.6
	pH6.8	M_t=0.1689$t^{1/2}$−0.0409	/	0.1689	0.9572	11.9
Peppas	pH1.0	LgM_t/M_∞=0.8205Lgt−1.3824	0.8205	0.0415	0.9302	20.8
	pH6.8	LgM_t/M_∞=0.6885Lgt−0.9868	0.6885	0.1031	0.9559	9.9

CONCLUSIONS

In this article, the CCMs were prepared by the methods of W/O/W and emulsification solvent evaporation as drug delivery system. The series carboxymethyl chitosan with almost the same deacetylation degree and degree of substitution were successfully synthesized from different molecular weights of chitosan and all samples had water solubility. It could be seen in the FTIR spectra that all synthesized carboxymethyl chitosan had similar structure. The prepared CCMs were spherical, free-flowing and nonaggregated with the smooth appearance and many small pores on the surface. Although the adding of model drug had no effects on the size of CCMs, the appearance of CCMs-loaded acetaminophen became more compact, the pores became less and the crystal could be seen in the surface. The loading efficiency and entrapment efficiency were increased with the increase of molecular weights of carboxymethyl chitosan, with the increase of concentration of carboxymethyl chitosan from 1.0% to 2.0% and then decreased with the increase of concentration of carboxymethyl chitosan from 2.0% to 3.0%. The release of acetaminophen from CCMs characterized by a slow release phase which indicated the sustained release efficiency of the microspheres prepared. The release rate decreased with the increase of carboxymethyl chitosan concentration from 1.0% to 2.0%, and then increased with the increase of carboxymethyl chitosan concentration from 2.0% to 3.0%. Furthermore, the release rate of acetaminophen was decreased with the increase of molecular weights of carboxymethyl chitosan no matter in PBS or dHCl. All release rates in dHCl (pH 1.0) were much slower than that in PBS (pH 6.8). The release rate was not more than 75% in PBS and 42% in dHCl during 24 h, respectively. It is illustrated that the drug loaded in CCMs released slower in simulated gastric fluid than that in simulated intestinal fluid. The drug release data were fitted to different kinetic models to analyze the release kinetics and the mechanism from the microspheres. CCMs showed better fitness with the first order model in different media which indicated that the drug release from CCMs was depended on the drug concentration in the polymeric networks. On the other hand, the values obtained from Higuchi model and Peppas models were found to be very close to each other both in PBS and dHCl. So the release of drug from CCM indicated diffusion-controlled drug release based on Fickian diffusion and accompanied with anomalous transport (i.e., non-Fickian diffusion). So CCMs might be the ideal carrier for acid-labile drugs by using carboxymethyl chitosan, a water soluble chitosan derivative, both for the solubility of carboxymethyl chitosan and the release media.

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