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Release behavior of microspheres from cross-linked N-methylated chitosan encapsulated ofloxacin

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Abstract

A series of hollow microspheres encapsulated with ofloxacin have been successfully prepared, using cyclohexane droplets as a template and the N-methylated chitosan (NMC) cross-linked with glutaraldehyde as the shell. The structure and morphology were characterized by X-ray diffraction (XRD) and scanning electron microscopy (SEM). The swelling and releasing behaviors of the microspheres having different weight-average molecular weights (M_w) and the degree of quaternization of NMC at pH 1.2 and 7.4 media were investigated. The results revealed that microspheres exhibited a very smooth surface, and electrostatic interaction existed between cross-linked NMC and ofloxacin. Ofloxacin encapsulated in the microspheres was rapidly released into phosphate buffer solution (pH 7.4), whereas it was only slowly released in 0.1 M HCl (pH 1.2). The degree of swelling of microspheres at pH 7.4 was higher than that at pH 1.2. Moreover, ofloxacin was released more slowly from the microspheres having high M_w of NMC than that with low M_w. The release mechanism of the hollow microsphere was proposed to be a non-Fickan diffusion through the swollen microspheres, and to be controlled by the M_w and the cross-linking density of shell.

Keywords: N-Methylated chitosan; Microspheres; Drug delivery; Release behavior; Molecular weight

1. Introduction

Recently, microspheres or hollow microspheres have attracted great attention because of a variety of applications such as delivery vesicles for drugs, DNA, antigens, and protection proteins and enzymes, especially for controlled or sustained drug-delivering systems employing biopolymers as raw material (Chu, Yamaguchi, & Nakao, 2002; Clark & Wooley, 2001; Du et al., 2003; Foster & Hirst, 2005; Illum, Jabbal-Gill, Hinchcliffe, Fisher, & Davis, 2001; Jiang, Gupta, Deshpande, & Schwendeman, 2005; Peyratout & Dähne, 2004; Peter, Hutter, Stöllnberger, Kamer, & Hampel, 1997; Sanji, Nakatsuka, Ohnishi, & Sakurai, 2000; Sunder, Krämer, Hanselmann, & Mülhaupt, 1999; Tamber, Johansen, Merkle, & Gander, 2005; Zimmer & Kreuter, 1995). Usually, drugs are encapsulated to mask taste and odour, to stabilize the quality of the drug, to improve glutaraldehydestrointestinal (GI) tolerance and to provide sustained release after oral administration. Chitin, obtained from lobster, shrimp and crab shell waste, is the second most abundant polysaccharide found in nature. In the 21st century, chitin and its derivative (chitosan) face new opportunities to contribute functional materials and environmentally friendly materials to meet the diverse needs of today's society because of their nontoxic, biodegradable, biocompatible, antibacterial, etc. Therefore, chitosan has been extensively used in medical and pharmaceutical areas, as it would be advantageous to use them as drug formulations (Badawy et al., 2004; Peniche, Argüelles-Monal, Peniche, & Acosta, 2003; Ravi

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Kumar, Muzzarelli, Muzzarelli, Sashiwa, & Domb, 2004; Sinha et al., 2004).

It is well known that chitosan from the ocean resources has been found wide application in various areas as a result of its biocompatibility and nontoxicity, especially in the pharmaceutical and biomedical fields, such as drug delivery, wound dressing and antimicrobial agents (Knill et al., 2004; Kennedy, Methacanon, Lloyd, Paterson, & Knill, 1998; Liu, Du, Wang, Hu, & Kennedy, 2004). It is well known that microspheres and microcapsules prepared from chitosan matrices have found wide application in the controlled release delivery (Agnihotri, Mallikarjuna, & Aminabhavi, 2004; Du et al., 2004; Muzzarelli, Stanic, Gobbi, Tosi, & Muzzarelli, 2004), including hirudin (Chandy, Mooradian, & Rao, 1998), bovine serum albumin (Zhang, Guo, Peng, & Jin, 2004), and the nerve growth factor (Xu et al., 2003). However, the poor solubility of chitosan at neutral pH and higher limits its application as an adsorption enhancer in the basic environment of the large intestine, colon, and rectal mucosa. Moreover, some model drugs would be damaged by the acidic solution system of dissolved chitosan, the chitosan only can be dissolved in acidic conditions for the formation of the microspheres. To overcome these disadvantages of classical chitosan solutions, it is essential to prepare chitosan derivatives which are water-soluble around neutral pH. Some progresses have been reported in the preparation of water-soluble chitosan derivatives, such as: *N*-lauryl-*N*-methylene phosphonic chitosan (Ramos, Rodríguez, Rodríguez, Heras, & Agulló, 2003), α-D-mannoside branches at C-6 of chitin and chitosan (Kurita, Shimada, Nishiyama, Shimojoh, & Nishimura, 1998), tosyl- and iodo-chitin (Kurita, Inoue, & Nishimura, 1991), *N*-carboxymethylated chitosan (Zhang, Guo, Zhou, Guang, & Du, 2000), *N*-acetylchitosan (Sashiwa et al., 2002), and *N*-trimethyl chitosan chloride (Sieval et al., 1998).

N-Methylated chitosans (NMC), which are a kind of quaternized chitosan, are interesting for pharmaceutical applications because of their amphiphilic and water-soluble character at physiological pH caused by the hydrophilic groups $-N^{+}(CH_3)_3$ and hydrophobic groups $-N(CH_3)_2$ (Peng & Zhang, 2005). Moreover, quaternized chitosan has proved to be effective and safe for mucosal delivery of hydrophilic macromolecules (Van der Merwe, Verhoef, Verheijden, Kotzẽ, & Junginger, 2004; Van der Lubbe, Verhoef, Borchard, & Junginger, 2001) and safe for use as gene delivery vectors (Tahanou, Florea, Geldof, Junginger, & Borchard, 2002). In previous work (Peng & Zhang, 2005), we have reported the preparation and characterization of hollow spheres using cyclohexane droplets as template, and NMC cross-linked with glutaraldehyde as the shell. An oil-in-water emulsion system was chosen to prepare the hollow microspheres with smooth surfaces, because of the wide variability in both composition and physical parameters of water and oil phases and its potential for industrialization. The unique structure and function of microspheres was attractive for their potential for encapsulation of large quantities of guest molecules or large size guest molecules within the "empty" core domain. In the present work, attempts were made to prepare biocompatible hollow spheres from NMC with different weight-average molecular weights (M_w) and degrees of quaternization, and used an anti-infective drug ofloxacin as a model drug to test the release behavior of the microspheres. The release mechanism of ofloxacin from the microspheres was investigated to provide some meaningful information for applications in biomedical areas.

2. Materials and methods

2.1. Materials

All the general chemical reagents were obtained through normal commercial channels in China. Chitosan (case sensitive with chitosan) having M_w of 13.44×10^4 and 93% degree of deacetylation was purchased from Yuhuan Ocean Biochemistry Co Ltd., Zhejiang, China. An anti-infective drug ofloxacin was a gift from Kunshang Double-Crane Pharm Co., Jiangsu, China. Gultaraldehyde, 1-methyl-2-pyrrolidone, and methyl iodide were supplied by Shanghai Chemical Reagent Co., Shanghai, China. All chemicals were of analytical grade.

2.2. Preparation of microspheres

The CS samples having different M_w were prepared by hydrolysis with 30 mL of 3 M HCl (30 ml/1 g of CS) at 60 °C. By controlling the hydrolysis time to 0, 4, 8, and 12 h, four samples (coded as 0–3) were obtained. The hydrolysed samples were dialyzed against running water for 4 days, and then centrifuged 1447*g* for 20 min. The resultant precipitates were washed several times with distilled water and acetone, and then vacuum-dried at 40 °C.

N-Methylated chitosan (NMC) was prepared according to the procedure previously described (Peng & Zhang, 2005; Sieval et al., 1998). The NMC having different M_w was coded as NMC0–3. Their M_w values were determined by size-exclusion chromatography combined with multi-angle laser photometer (MALLS, DAWN-DSP, Wyatt Technology Co., Santa Barbara, CA, USA). A P100 pump (Thermo Separation Products, San Jose, USA) equipped with columns of G4000PWXL (MicroPak, TSK) and G6000PWXL (MicroPak, TSK) in CH_3COONa buffer solution at 25 °C was used as the SEC instrument. The degree of quaternization of the NMC samples was measured from their ^1H NMR spectra in a mixed solvent of CF_3COOD and D_2O on a Mercury 300 NMR spectrometer (Varian Inc., USA) according to Thanou's method (Thanou et al., 2000).

Microspheres were prepared from NMC0–3 by cross-linking with glutaraldehyde on the surface of the emulsion droplets according to the method described in previous work (Peng & Zhang, 2005). Portions of NMC powder

Table 1
The samples with different M_w and degree of quaternization of NMC

Sample	NMG	NMGF0	NMGF1		NMGF2	NMGF3
			NMGF1-I	NMGF1-II		
DD (%)	30.4	30.4	34.8	67.1	30.4	30.4
$M_w \times 10^{-4}$	8.345	8.345	5.233		4.953	3.897

(0.5 g) was dissolved in distilled water (18 mL), and then to the above emulsion system cyclohexane (6 ml) was added as the "oil phase" and 15% w/w ofloxacin acetic acid solution (1 mL) was added. The resultant mixture was stirred at 55 g and 40 °C for 1 h to form an oil-in-water (O/W) emulsion. After addition of 25% w/w aqueous glutaraldehyde aqueous (0.5 mL), the system was stirred at 10 g for 30 min to yield the emulsion droplets covered with cross-linked NMC and ofloxacin. The resulting products were precipitated by coagulating with acetone. The precipitated products prepared from NMC 0–3 were coded as NMGF 0–3, respectively. NMGF1 having different degrees of quaternization (there are two NMGF1 samples which have same M_w and different degree of quaternization) was coded as I and II. The product powder without ofloxacin was coded as NMG. The samples having different M_w values and degrees of quaternization of NMC are listed in Table 1.

2.3. Characterizations

To clarify the structure of the microspheres, WAXD measurements of ofloxacin, NMG, the mixture of ofloxacin, and NMG, NMGF0 were carried out on an X-ray diffractometer (D 8 ADVANCE BRUKER AXS GmbH, Karlsruhe, Germnany) with a Cu Kα radiation source at 40 kV and 50 mA. The diffraction angle ranged from 4° to 40°. The morphology of the microspheres was observed by a scanning electron microscope (SEM, S-570, Hitachi, Japan). The samples were coated with gold for SEM observation.

2.4. Swelling test

The dry microspheres encapsulated with ofloxacin (0.2 g) were immersed in either 0.1 M HCl (pH 1.2) or a phosphate buffer solution (which was composed of 500 mL of 0.1 M KH_2PO_4 and 391 mL of 0.1 M NaOH), and the mixture was diluted to 1000 mL (pH 7.4) for 48 h at room temperature until equilibrium of swelling had been reached. The swollen samples were collected by centrifugation, and then blotted with filter paper to remove the water on the surface, and immediately weighed. The degree of swelling (S_W) was calculated using the following equation:

$$S_W(\mathrm{wt}\%) = [(w - w_0)/w_0] \times 100\% \quad (1)$$

where w and w_0 are the weights of the microspheres at the equilibrium swelling state and at the dry state, respectively.

2.5. Release experiments

All release experiments were performed in a shaker model SHZ-82 incubator (Taichuang Factory, Jiangsu China) at 1 g. The desired amount of the microspheres (0.2 g) was suspended in release medium (20 mL) at 37 °C. The phosphate buffer solution (pH 7.4) and 0.1 M HCl solution (pH 1.2) were used as the releasing media. At the desired time interval, the microsphere dispersions were centrifuged for 10 min at 1005*g*, and the supernatants were assayed to detect any drug release by UV spectroscopy (UV-1600, Shimadzu, Japan) at 293 nm. Then fresh release medium was added to maintain a constant volume. The precipitates were re-dispersed in the same release medium as previously.

The release results were analyzed by using an empirical equation to estimate the value of n and k as follows (Ritger & Peppas, 1987):

$$M_t/M = kt^n \quad \text{or} \quad \log(M_t/M) = \log k + n \log t, \quad (2)$$

where M_t/M is the amount of the released ofloxacin (%) at time (h), n is a diffusion exponent which indicates the of the release mechanism, and k is a constant characteristic of the drug–polymer interaction (%/h). From the slope and intercept of the plot of log (M_t/M) versus log t, kinetic parameters n and k were calculated.

3. Results and discussion

3.1. Structure and morphology of microspheres

X-ray diffraction patterns of ofloxacin, NMG, the mixture of NMG, and ofloxacin, NMGF0 are shown in

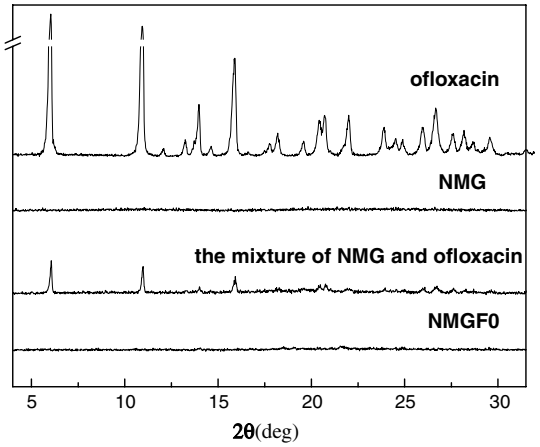

Fig. 1. WAXD patterns of ofloxacin, NMG, the mixture of NMG and ofloxacin, and NMGF0.

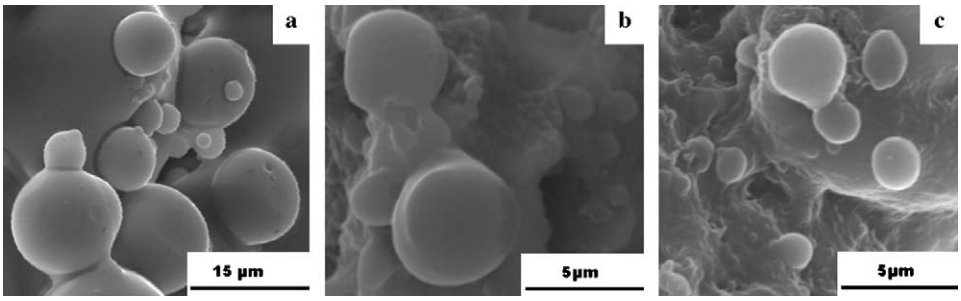

Fig. 2. SEM images of the NMGF0 microspheres, before releasing (a), after releasing by pH 1.2 medium (b) and by pH 7.4 medium.

Fig. 1. The diffraction peaks observed were at $2\theta = 6.0°$, 11.0°, 14.0°, 16.0°, 18.2°, 20.7°, 22.0°, 23.1°, 26.7°, 28.2°, and 29.6° for ofloxacin; this indicates that ofloxacin is crystalline. NMG does not exhibit any crystalline diffraction, indicating its amorphous nature. Compared with the ofloxacin, the mixture of NMG and ofloxacin exhibits some diffraction peaks corresponding to ofloxacin, located at $2\theta = 6.1°$, 11.0°, 15.1°, and 16.0°. These are only some of the ofloxacin peaks – because NMG is of an amorphous nature; this suggests that the mixture of NMG and ofloxacin is only simple and physical without any chemical reaction. In addition, no obvious characteristic crystalline peaks of ofloxacin in NMGF0 sample have been detected in the X-ray diffraction pattern. In view of the X-ray results mentioned above, it is believed that ofloxacin had been encapsulated inside the cross-linked NMC, and the electrostatic interaction existing between cross-linked NMC and ofloxacin prohibits the growth of ofloxacin crystal. The same result has been proved by FT-IR spectra as recorded in our previous works (Peng & Zhang, 2005).

As deduced from the SEM images of the microspheres of NMGF0 (Fig. 2) the NMGF0 microspheres, before releasing, exhibit smooth surfaces; this indicates that the cross-linked NMC has formed a tight exterior shell because the microspheres are formed by the surface fabrication, and ofloxacin has been encapsulated inside the cross-linked shell of the microspheres, as shown in Fig. 2a. The size of the microspheres lies in the range from 2 to 14 μm. After releasing, the microspheres remain spherical shapes, and have smooth surfaces. However, most of the microspheres become smaller, as shown in Figs. 2b and c. The size of the sample microspheres in pH 1.2 medium is larger than that of its counterpart in pH 7.4; this suggests the cross-linked shell is more stabilized pH 1.2 media than in pH 7.4 media. This could be because there exist stronger electrostatic interaction at acid media. The results indicate that ofloxacin that was in the microspheres has become released from the microspheres by diffusion and permeation, leading to the smaller size of microsphere. Therefore, the SEM results reveal that the ofloxacin release mechanism is one of diffusion through a swollen rubbery matrix by polymer chain relaxation.

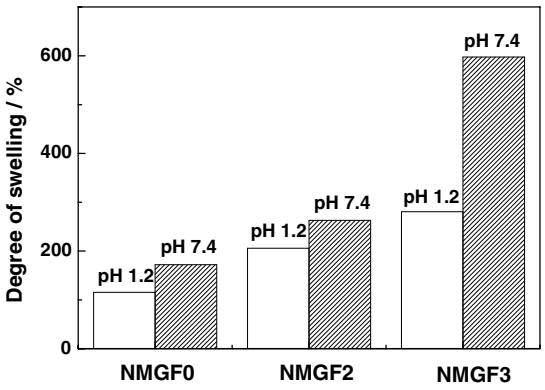

Fig. 3. Degree of swelling for microspheres with different M_w of NMC at pH 7.4 and 1.2 conditions.

3.2. Swelling ratio

Dependence of the degree of swelling on the M_w of NMC and pH of the medium for the microspheres is shown in Fig. 3. The microspheres in 0.1 M HCl (pH 1.2) have a lower percentage swelling degree than those in phosphate buffer pH 7.4. Therefore, the swelling ability of the microspheres is weakened in an acid environment. This can be explained in terms of the electrostatic interaction between the cross-linked NMC and the –COOH groups of ofloxacin, and the Schiff's base of the cross-linked network (as a result of the reaction between the –CHO groups of GA and the $–NHCH_3$ groups of NMC) is easily destroyed in pH 7.4 medium. With an increase of the M_w of NMC, the swelling degree of the microspheres decreases, because the microspheres with high M_w have a higher density of shell, leading to a decrease of solvent resistance.

3.3. Release behavior of drug

In vitro release behaviors of ofloxacin from the microspheres with different M_w at pH 7.4 and 1.2 media are shown in Figs. 4 and 5, respectively. The release curve suggests that the drug is included inside of the cross-linked shell because only 11–28 wt% ofloxacin is released within 30 min. If it was a case of release from the surface, most of the adsorbed drug is released within 10 min when the microspheres come in contact with the release medium,

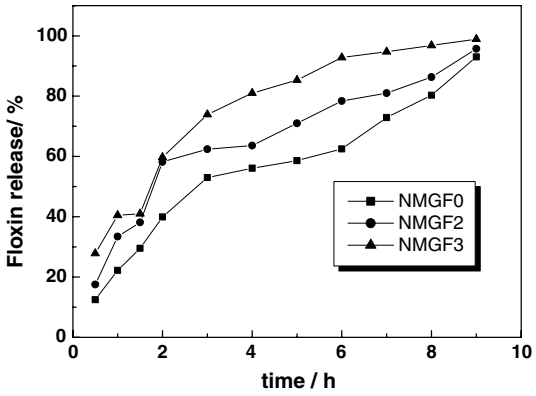

Fig. 4. Time dependence of ofloxacin release from the microspheres with different M_w of NMC at pH 7.4.

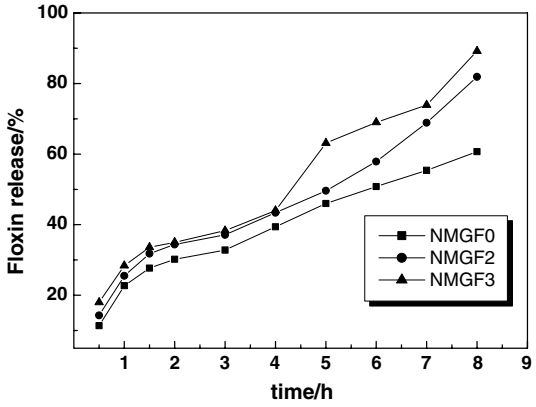

Fig. 5. Time dependence of ofloxacin release from the microspheres with different M_w of NMC at pH 1.2.

leading to a burst or disrupt in microsphere effect in the early stages of dissolution (He, Davis, & Illum, 1999). According to the release curve in pH 7.4 medium, ofloxacin releases more slowly from the microspheres having high M_w of NMC than from that of low M_w for the same release time period. This can be attributed to both lower solubility of high M_w and higher density of shell around the drug particles. These results support the above conclusions of the swelling degree; this suggests the increase of M_w of NMC leads to a decrease of the swelling degree, and further results in the decrease of release rate. Furthermore, the same results are shown in the release curve at pH 1.2 medium (Fig. 5).

Interestingly, ofloxacin releases more rapidly at pH 7.4 than at pH 1.2, the release half times t_{50} (time required for releasing 50 wt% of drug) for NMGF0, NMGF2, and NMGF3 are 2.8, 1.8, and 1.7 h at pH 7.4, and 6.0, 5.0, and 4.4 h at pH 1.2, respectively. More than 80 wt% ofloxacin is released from microspheres at pH 7.4 within 8 h, whereas less than 44 wt% of the drug is released at pH 1.2 within 4 h. This suggests that the drugs in the microspheres can be used to be suitable for the basic environment of the large intestine, colon, and rectal mucosa for which there are different emptying times. The results can be explained that cross-linked shell has different swelling ratio, i.e., the ratio of swelling at the two pH values, in both pH 7.4 and 1.2 medium, but the electrostatic interaction of microspheres is more easily broken at pH 7.4 than at pH 1.2, leading to ofloxacin being released more rapidly at pH 7.4 than 1.2.

The n value is an empirical parameter characterizing the release mechanism (Ritger et al., 1987). On the basis of the diffusion exponent, an n value of 0.5 indicates the drug release mechanism approaches to a Fickian diffusion controlled release, whereas n equal to 1.0 indicates the drug release mechanism approaches to zero-order release. The n value from 0.5 to 1 is a drug release mechanism for non-Fickian diffusion or chain relaxation control release. From the logarithmic plot of release data $\log(M_t/M)$ versus $\log t$, the diffusion exponent (n) and kinetic constant (k) have been calculated, as shown in Figs. 6 and 7, respectively. Table 2 summarizes the values for the microspheres in pH 1.2 and 7.4 medium. The n value is in the range from 0.46 to 0.64 at pH 7.4 medium, and increases with increasing M_w. It is believed that the ofloxacin release mechanism is non-Fickian diffusion, and is controlled by the cross-linking density of the shell, as proved by SEM images.

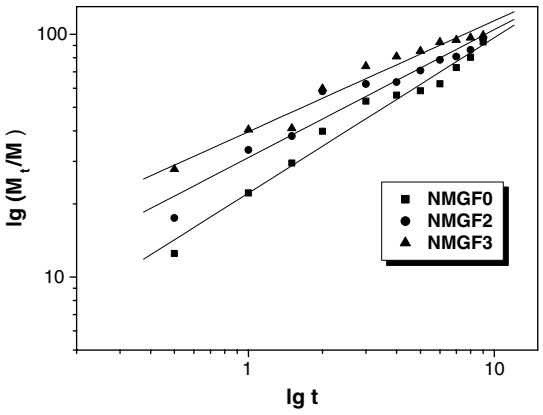

Fig. 6. Plots of release data $\log(M_t/M)$ versus $\log t$ at pH 7.4 for NMGF microspheres.

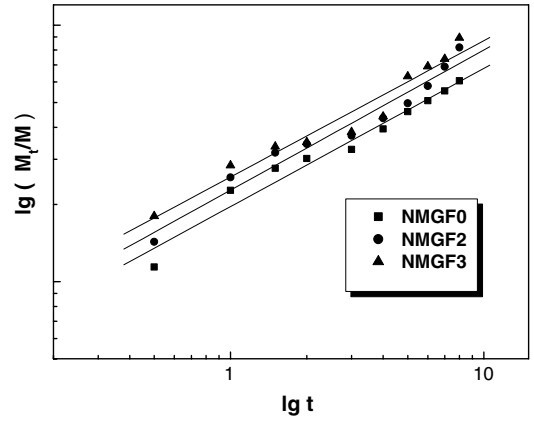

Fig. 7. Plots of release data $\lg(M_t/M)$ versus $\lg t$ at pH 1.2 for NMGF microspheres.

Table 2
The kinetic constants (k), diffusional exponents (n), and correlative coefficients (r^2) following linear regression of release data of microspheres at pH 1.2 and 7.4 medium

Sample	pH 1.2			pH 7.4		
	n	k	r^2	n	k	r^2
NMGF0	0.54	19.5	0.9653	0.64	22.4	0.9757
NMGF2	0.55	22.9	0.9598	0.53	30.9	0.9442
NMGF3	0.53	25.7	0.9495	0.46	39.8	0.9641

The n value is in the range from 0.53 to 0.56 at pH 1.2; this suggests that ofloxacin release is non-Fickian diffusion and mainly controlled by the swollen microspheres at pH 1.2 medium.

The results of the ofloxacin release amount from microspheres having different degrees of quaternization of NMC at pH 7.4 and 1.2 are shown in Figs. 8 and 9, respectively. It is obvious that the release speed of ofloxacin from the microspheres increases rapidly with an increase of the degree of quaternization of NMC as a result of the cross-linking density of microspheres shell formed by the reaction between glutaraldehyde and NMC (Mi, Kuan, Shyu, Lee, & Chang, 2000; Peng & Zhang, 2005). With an increase of the degree of quaternization of NMC, the cross-linking density decreases, leading to a loose structure shell. Moreover, ofloxacin in the high degree of quaternization of NMC (NMGF1-II) is released more speedily than that in the NMCF1-I with low degree of quaternization in both pH 1.2 and 7.4 media. This further confirms the existence of ofloxacin in the cross-linked shell and the sensitivity of the releasing amount to the quaternization degree. If the shell has not been cross-linked completely (the NMGF1-II sample with high degree of quaternization has little cross-linked network), ofloxacin easily diffuses from the microspheres on account of the loose structure.

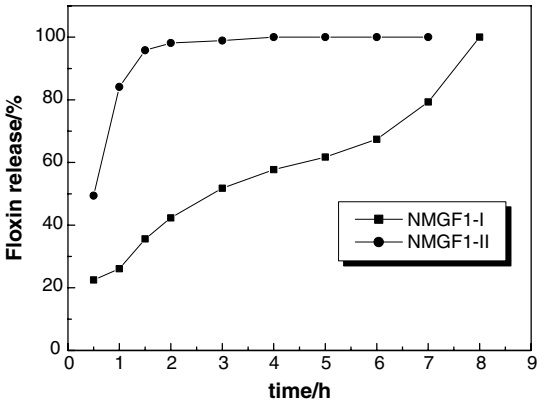

Fig. 8. Time dependence of ofloxacin from the microspheres with the different degree of quaternization of NMC at pH 7.4.

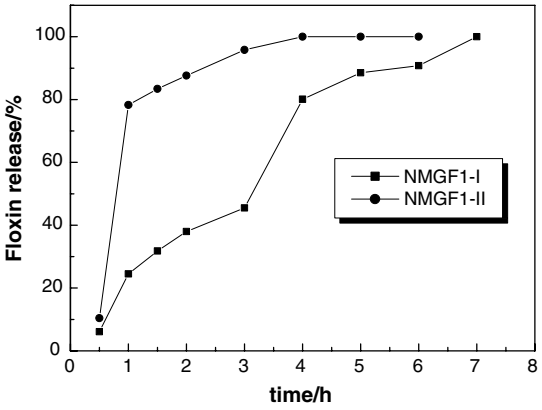

Fig. 9. Time dependence of ofloxacin release from the microspheres with different degree of quaternization of NMC at pH 1.2.

Because most of ofloxacin has been released within 1 h in both pH 1.2 and 7.4 media in the microspheres with high degree of quaternization of NMC, there is the burst release in the microspheres with high degree of quaternization of NMC. Seventy-eight to 84 wt% ofloxacin has been released within 1 h in both pH 1.2 and 7.4 media as a result of the low cross-linking density shell, in which all ofloxacin has been released within 4 h. This suggests that the microspheres with high degree of quaternization of NMC might be useful in stomach specific drug delivery. From the above results, ofloxacin is included inside the cross-linked shell of microspheres, and gradually releases from the microsphere with the permeation of solvent. The release mechanism is a diffusion through the swollen macrostructure of the microspheres.

4. Conclusions

A series of microspheres encapsulated with ofloxacin were prepared from NMC having different M_w and degree of quaternization. The degree of swelling of microspheres in phosphate buffer solution, pH 7.4, was much higher than that in 0.1 M HCl (pH 1.2), and decreased with an increase of M_w of the NMC ofloxacin in the microspheres was more speedily released at pH 7.4 than at pH 1.2. In addition, release speed of ofloxacin in the microspheres having high degree of quaternization of NMC was higher than that with low degree of quaternization. The results indicated that ofloxacin was included in inside the shell the cross-linked shell of microspheres, and gradually releases from the inside microsphere, accompanying the permeation of solvent. The ofloxacin release mechanism was a kind of non-Fickan diffusion through the swollen macrostructure of microspheres, and was controlled by the M_w, the cross-linking density of shell and the degree of quaternization of NMC. Such biocompatible and biodegradable hollow microspheres exhibited fascinating release behavior and pH sensibility, and the high degree of quaternization of the NMC of the microspheres might be useful in stomach specific drug delivery.

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