Preparation of a Multistructural Film with CM-Chitosan and PVA, and *In Vitro* Ornidazole Release from the Carrier

Ling Chong Wang,[1] Xi Guang Chen,[1] Cheng Sheng Liu,[1] Li De Li,[1] Qiu Xia Ji,[2] Le Jun Yu[1]

[1]*College of Marine Life Science, Ocean University of China, Qingdao, People's Republic of China 266003*
[2]*The Affiliated Hospital of Medical College, Qingdao University, Stomatology, Qingdao People's Republic of China 266042*

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ABSTRACT: In this study, carboxymethyl-chitosan microspheres (CCMS) were prepared by a method of emulsification combined with two-step solidification and loaded ornidazole as model drug. The ornidazole loaded CCMS were incorporated into Poly(vinyl alcohol) (PVA) film to form a multistructure drug system for feasibly releasing drug in local site. The appearance, particle size, drug loading and encapsulation efficiency, and drug release profiles of CCMS could be tailored in the preparation. Appropriate enhancement of drug amount in the microsphere would bring optimum effect on drug loading percentage and release profile. The use of dimethylsulfoxide (DMSO) could produce spherical spheres with smooth surface and small size, but depress the drug loading and encapsulation efficiency and hasten the burst drug release. For the multistructure carrier materials, the outer PVA film rapidly breaks up to pieces after about 30 min of placement in water and then entirely dissolved. Drug release from the multistructure carriers was a little faster than that from the pure CCMS. Ornidazole release from the carriers performed a burst release in the initial 2 h then followed a gradual release. It could be achieved the controlled release pattern of ornidazole by dispersing the well prepared CCMS into PVA film. © 2008 Wiley Periodicals, Inc. J Appl Polym Sci 110: 1136–1144, 2008

Key words: CM-chitosan; PVA; ornidazole; multistructure films; local drug delivery system

INTRODUCTION

Controlled drug delivery technology using natural biodegradable polymers as carriers represents one of the most rapidly advancing areas of science. Chitin and its derivatives have been widely utilized as excipients in pharmaceutics to purposefully delivery drugs or nutriments.[1–3] The drug delivery system based on chitosan microsphere as potential carriers have been extensively investigated due to many advantages of this drug delivery system.[3,4] Comparing with commonly used chitosan, CM-chitosan shows several potentials in local drug delivery.[5] Examples of the advantages of site-specific employing CM-chitosan vehicles include pH-sensitivity, bioadhesive ability, solubility and absorbability, controllable biodegradability, nontoxicity of the degradation end products, sustained release potential and ease of administration.[6,7]

Local application of drug delivery system could be very advantageous, both in terms of raising drug concentration directly in the action site, and in preventing systemic side effects such as gastrointestinal complaints, depression, and tachycardia. For instance, periodontal diseases, which are initiated by the microorganisms that colonize on the tooth surface and infect the surroundings usually have been recommended to treat with the local delivery of antibiotics to maintain an effective therapeutic concentration in the gingival crevicular fluid of the periodontal pocket[8,9] using biodegradable carriers such as films, strips, gels, etc.[9–11] To be useful for periodontal therapy, it is desirable to have a bioerodible drug delivery system that can maintain an effective drug release rate in the periodontal pocket whereas simultaneously eroding throughout the duration of treatment over several days.[12,13]

Ornidazole is a metronidazole analogue, exhibiting great antiparasitic effect and prolonged half-lives and enhanced oral absorption capability; hence, are widely used in treatment of periodontitis and gingivitis.[14] However, high dosage systemic administration of the nitroimidazole to achieve a therapeutic level of periodontal pocket could result in unpleasant or toxic side effects.[15] Therefore, administration of this drug should be designed to the appropriate

Correspondence to: X. G. Chen (xgchen@ouc.edu.cn).
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formulations for convenient release in periodontal pocket.

Recently, we proposed a novel local drug delivery system designed to attain delayed and/or time-dependent periodontal pocket-specific release.[16] This system, loaded with ornidazole as model drugs, was prepared from film casting of the blend of PVA and carboxymethyl-chitosan (CM-chitosan). When in contact with periodontium of rat, the blend film system was of no any toxicity and kept a good retention at the application site. Following the erosion and degradation of polymers, ornidazole was released from the matrix at local site. However, owing to the strong hydrophilic properties of CM-chitosan,[17] the dispersed drugs released rapidly, and the blend film can not provided a long term pattern for drug delayed release.

In perspective of a forthcoming development of local drug delivery system used at oral cavity, the aim of the present work was designing a modified-release dosage form via still using CM-chitosan and PVA. For this purpose, CM-chitosan microspheres (CCMS) were prepared by water-in-oil emulsification and fixation methods, then the CCMS were dispersed into PVA films to incorporate a multistructural drug carrier. The outer PVA film was just a convenient resort for drugs administration in periodontal pocket, and it would be melted and eroded by body fluid after being put into the specific site; while CCMS, the drug vehicles loaded ornidazole, could warrant a sustained drug release and provide good retention effect at periodontal pocket. In this article, the performances of CCMS and the multistructural drug carrier were explored with the structural morphology and *in vitro* drug release at different pH conditions. We also checked the effect of DMSO addition on the performances of CCMS and the multistructural drug carrier, since it can enhance the solubility of ornidazole in water.

EXPERIMENTAL

Materials

CM-chitosan was prepared from reaction of chitosan (deacetylated degree is 89% and molecule-weight is 413 kDa) and chloroactic acid in our laboratory. The degree of carboxymethylation calculated from ^{1}H-NMR was found to be 93%. Its viscosity average molecular-weight, calculated based on Mark-Houwink equation[18] after measuring the intrinsic viscosities using an Ubbelohde viscosimeter in 25°C was 199.6 kDa. The following chemicals were used as received: PVA-124 (88% hydrolyzed, Wakopure Chemical Industry, Japan); ornidazole powder (Xi'an Bodyguard pharmaceutical, China); dimethylsulfoxide (DMSO, Boerhinger Ingelheim, Germany); Tween-80 and Span-80 (Sigma Chemical Co.); glutaraldehyde, toluene and sodium tripolyphosphate (TPP) (Aldrich Chemical). All other chemicals and solvents were of reagent grade.

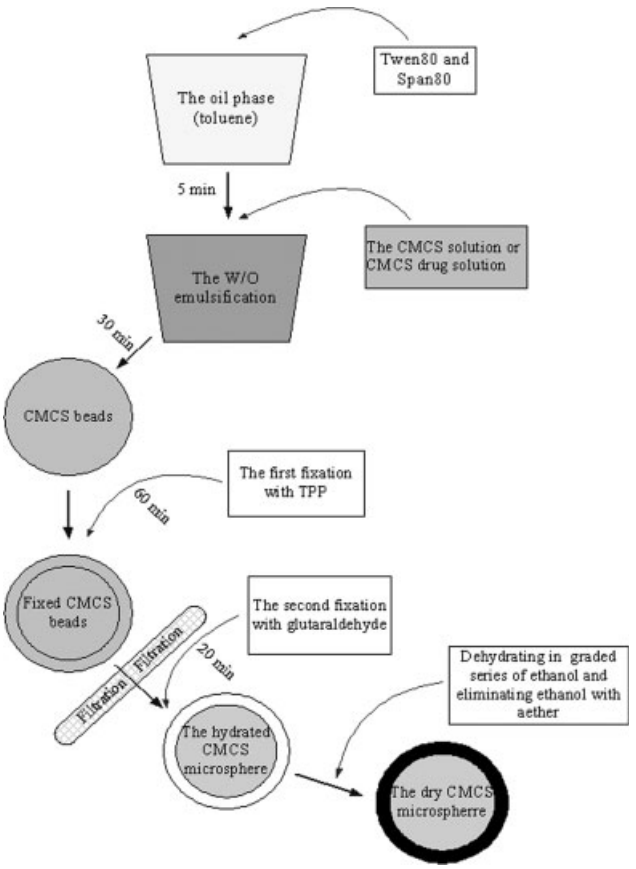

Figure 1 Schematic illustration of a process for preparation of CCMS by emulsification-fixation method. Ornidazole was loaded as model drug.

Preparation of CM-chitosan microspheres

A modified membrane emulsification technique combined with two-step solidification process was employed to prepare CCMS gel beads.[19] As shown in Figure 1, the W/O emulsion was performed with a CM-chitosan aqueous solution as the internal phase and toluene as the external oil phase, and the solidification of CM-chitosan gel beads were successively crosslinked with TPP and glutaraldehyde. Briefly, 40 mL of 4% (w/v) CM-chitosan solution was dispersed into 200 mL of toluene containing 0.5% (v/v) Span-80 and 0.5% (v/v) Tween-80 at a stirring speed of 1400 rpm at room temperature for 30 min. Next, 20 mL of 10% (w/v) TPP solution was added to the above emulsion and stirring continued for 60 min at a speed of 800 rpm. After the oil phase was filtered through a nylon sieve (200 mesh, Shanghai Jiede filtration materials, China), the CCMS gel beads was collected to disperse in 200 mL of

distilled waster containing 0.5 mL glutaraldehyde at a stirring speed of 500 rpm for 20 min. Then the hardened microspheres were washed several times with distilled water followed by 50% methanol aqueous solution, grade series of ethanol and finally, with ether.

Preparation of ornidazole-loaded spheres

For the preparation of drug-loaded CCMS, two methods were employed to manufacture the drug-contained CM-chitosan solution. One is directly dispersing certain amount of ornidazole powder in 40 mL of 4% (w/v) CM-chitosan solution; the other is dissolving ornidazole in 1 mL of DMSO solution (0.5% v/v) and then mixing it with the 40 mL of 4% (w/v) CM-chitosan solution. After the water phases containing drug were prepared, emulsification and crosslinking were carried out as previous. The drug-loaded microspheres were washed several times with 50% methanol aqueous solution, once with a 5% solution of TPP, thrice with distilled water and finally with grade series of ethanol and then dried with ether. According to how much ornidazole was initially added and whether DMSO was appended in the water phase, three types of drug-loaded microspheres, 10%-CCMS-O (10% of drug were initially loaded in CCMS without DMSO addition), 20%-CCMS-O (20% of drug were initially loaded in CCMS without DMSO addition) and 20%-CCMS-D (20% of drug were initially loaded in CCMS with DMSO addition), were prepared in this section.

Characterization of the CCMS

Morphological study and particle size distribution

The morphologies of the CM-chitosan beads and ornidazole-loaded beads, after the process of washing with ether, were observed under an optical microscope (LEITZ DM IL, Leica, Germany) equipped with a digital camera (Coolpix 995, Nikon, Japan). Particle size and size distribution of the blank CCMS and the three types of drug-loaded CCMS were determined by a particle size analyzer (Mastersizer S, Malvern, UK).

Determination of ornidazole loading and encapsulation efficiency

The ornidazole amount included in CM-chitosan microspheres was determined by crushing down 100 mg of ornidazole-loaded microspheres in an agate mortar. The crushed powder was placed in 57 mL phosphate buffer (0.1*M*, pH7.4) at 37°C and vigorous stirred for 72 h. At the end of extraction, the suspensions were centrifuged and the concentration of ornidazole in the supernatants was detected by UV–vis spectrophotometer (Hewlett–Packard 8453, Palo Alto, Canada) at 320 nm. The amount of ornidazole loaded in microspheres, given as a percentage, indicates the loading amount (mg) of ornidazole per 100 mg of microspheres. And the encapsulation efficiency of the process indicates the percentage of ornidazole encapsulated with respect to the total amount used in the preparation.

Tests of swelling behavior

Preweighed microspheres were placed in individual test tubes containing 5.0 mL of phosphate buffer (0.1*M*, pH ranged from 3.5 to 7.4). The tubes were kept in a thermostated shaking air bath (DNP-9052, Shanghai Jinghong Laboratory Equipment Company, China) that was maintained at 37°C and 120 rpm. After 6 h of incubation, the medium was removed from the vessel containing microspheres by centrifugation. Then, the centrifugated blocks were blotted with a piece of paper towel to absorb excess water on the surfaces. The swelling degree was calculated from mass ratio of the swelled CCMS and the pre-swelled CCMS.

Preparation of multistructural matrix

A 50 mL of 4% (w/v) PVA solution were prepared by dissolving poly(vinyl alcohol) (Mw: 4.96 kDa) in distilled water with continuous stirring at 90°C. When the solution was refrigerated to the room temperature, determined weights (0.4 or 1.0 g) of 20%-CCMS-O were added in it. To avoid the presence of air bubble during the stirring, the suspension was pumped to high vacuum. Then, the suspension was stirred 4 h at 500 rpm to uniformly disperse CCMS in the PVA solution. Finally, the suspension was cast onto a petri-dish (diameter = 12.5 cm) and the solvents were evaporated at 50°C for 12 h. By this process, a multistructure system was prepared by incorporating ornidazole-loaded CCMS in PVA film. Two types of multistructure carrier with different amounts of CCMS were prepared. One is that of CCMS : PVA = 1 : 5 and the other is that of CCMS : PVA = 1 : 2.

To observe its structure, the multistructure carrier was cut into about 10 mm diameter pieces. The film pieces were stained to facilitate visualization with toluidine blue and brilliant green by immersion in 0.5 mg/mL of dye solution for 3 min. The stained comatrix was observed under an optical microscope equipped with a digital camera.

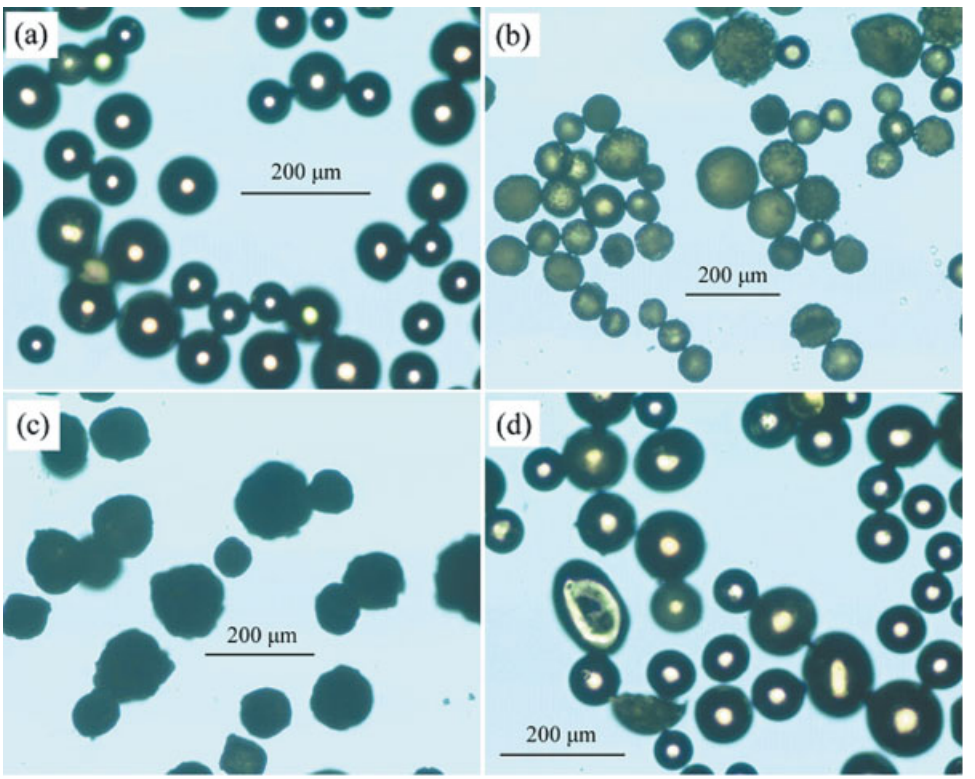

Figure 2 Optical images of CCMS beads produced by emulsification-fixation method. (a) The blank CCMS, (b) 10%-CCMS-O, (c) 20%-CCMS-O, and (d) 20%-CCMS-D. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

In vitro erosion of the multistructure matrix

Quadrate film disks of the multistructure comatrix were immersed into various 0.1*M* phosphate buffer solutions (pH ranged from 3.5 to 7.4 and ionic strengths ranged from 0 to 0.5*M* NaCl). Via keeping close watch on the behaviors of comatrix in the media, the two time points, at which the outer PVA films began to break up and was completely dissolved, were recorded as the index to evaluate the erosion of multistructure carrier.

In vitro ornidazole release tests

The *in vitro* ornidazole release profiles of the three types of drug-loaded CCMS and two types of multistructure comatrix were detected as follows. Determined amount of microspheres and comatrix pieces were incubated into a test tube containing 10.0 mL of phosphate buffer (0.1*M*, pH ranged from 3.5 to 7.4). These tubes were stored in the same shaking air bath and set at the same conditions as mentioned in swelling test. At appropriate intervals, 0.2 mL of release medium was collected centrifugation and 0.2 mL of fresh buffer was added back to the test tube. The amount of ornidazole released from systems was measured form absorbance at 320 nm using a UV–vis spectrophotometer (Hewlett–Packard 8453, Palo Alto, Canada) under the condition of calibration curves and regressive equations having been obtained.

RESULTS AND DISCUSSION

Characterization of the microspheres

The blank CCMS and three types of ornidazole-loaded CCMS (10%-CCMS-O, 20%-CCMS-O, and 20%-CCMS-D) were prepared by modified w/o emulsion combined with two-step solidification process. The micrographs of blank CCMS and drug-loaded CCMS are shown in Figure 2. The surface morphology of the blank CCMS prepared from pure carboxymethyl-chitosan was found spherical and smooth. After incorporation of ornidazole, the surface of CCMS became rough and the homogeneous spheres became chaotic. Also, the roughness and opacity of CCMS became worse off with the increase of amount of drug loaded. This may be due to the fact that ornidazole was not a strong solute in water[20] and easy to concentrate to crystals during the preparation of CCMS. And this may result in the shape of drug-loaded CCMS being irregular. To overcome such a demerit of CCMS and as more as possible enhance the drug loading percentage, we attempted to use DMSO to dissolve ornidazole

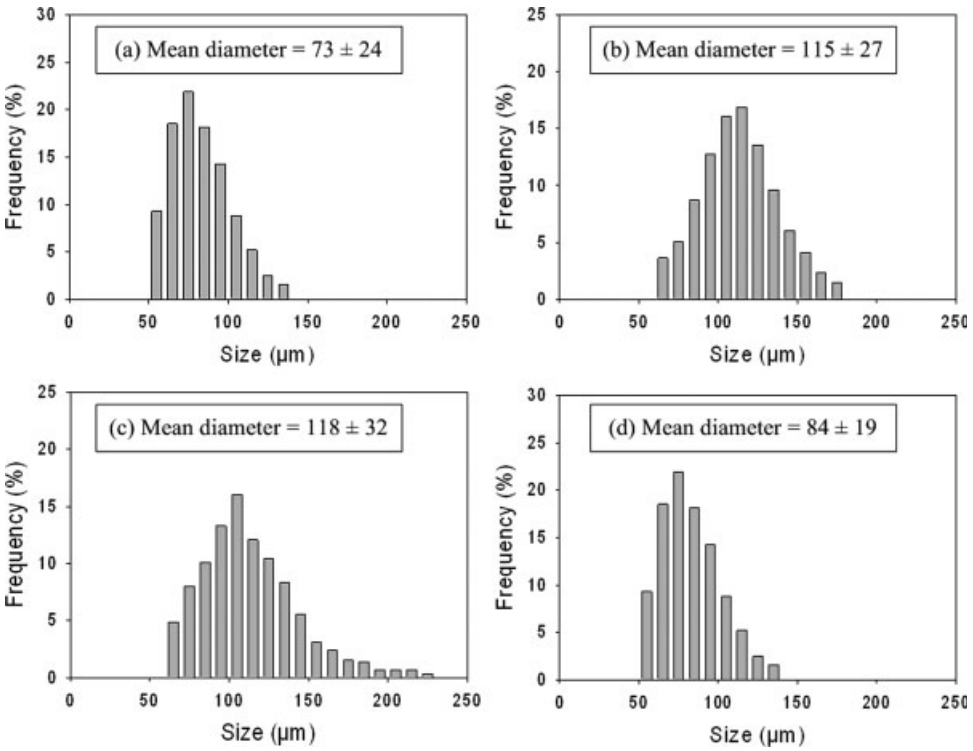

Figure 3 Size distribution and sphere mean diameter of the blank CCMS (a), 10%-CCMS-O (b), 20%-CCMS-O (c), and 20%-CCMS-D (d).

before adding the drug into the internal phase, because ornidazole has more solubility in DMSO than in water. In fact, dissolving ornidazole in DMSO would help the drug better dispersing in CM-chitosan matrix and make the 20%-CCMS-D with the same smooth spherical surface structure and homogeneous matrix as the blank spheres (Fig. 2d).

The size range, mean diameter and its standard deviation (SD) of these microspheres are exhibited in Figure 3. Unimodal distributions for all the microspheres were observed with peaks around the 100 μm as seen from the Figure 3. Microsphere diameters of both the blank and the three drug-loaded CCMS ranged from 50 to about 200 μm, which were small enough for the placement in the periodontal pocket. The smaller microspheres (<50 μm) were hardly found due to the filtration during the preparation. Drug-loaded microspheres without addition of DMSO showed higher mean diameters when compared with the blank CCMS. However, mean diameters of drug spheres decreased close to that of blank spheres after DMSO addition in the system.

The drug loading percentage and encapsulation efficiency of the three kinds of drug-loaded CCMS was determined by the above method and the results are illustrated in Table I. The microspheres had a relative high capacity in loading ornidazole. With the enhancement of drug initial loading ratio from 10 to 20%, the encapsulation efficiency little decreased from 79.5% to 70.9% while the drug loading percentage increased from 7.44% to 14.99%. However, DMSO addition halved the drug percentage and encapsulation efficiency. This phenomenon did not accord with our original idea, and might be attribute that more drugs were washed off by water during the CCMS preparation due to the enhanced solubility of ornidazole caused by the addition of DMSO.

The blank CCMS was allowed to swell in 5 mL of various pH (3.5–7.4) solution at 37°C (Fig. 4). The results indicated that the CCMS swelled acutely after 6 h incubation, due to the strong hydrophilicity of CM-chitosan. Although Chen et al.[21] reported that the matrix of CM-chitosan had the pH-sensitive swelling behaviors, our results exhibited that the CCMS have similar swelling degree (about 600%) at different pH conditions. This is because that the

TABLE I
Ornidazole Loading Percentage and Encapsulation Efficiency of CCMS

Type of CCMS	Loading percentage (%)	Encapsulation efficiency (%)
10%-CCMS-O	7.44 ± 0.93	79.5 ± 3.98
20%-CCMS-O	14.19 ± 0.44	70.9 ± 2.72
20%-CCMS-D	7.95 ± 0.38	37.2 ± 4.67

Results are mean ± SD ($n = 6$).

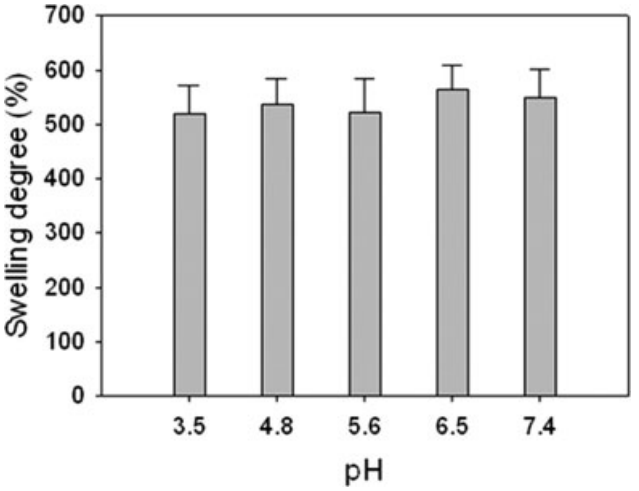

Figure 4 Swelling character of the carboxymethyl-chitosan microsphere in different pH media ($n = 3$).

network retardarce of polymeric crosslinking shielded the effect of electrostatic interaction among the ionic groups.

Preparation and characterization of multistructure carrier

Different approaches have been adopted to prepare PVA-based materials for biomedical application in the previous articles. In the present study, the system of CCMS included in PVA film were prepared using decentralization and casting method as previous depiction in earlier paragraphs. The drug microsphere chose to incorporate the PVA film was 20%-CCMS-O because of its high drug loading and encapsulation efficiency (the surface and particle size of 20%-CCMS-D are favorable, but the low ornidazole content can not satisfy the special using). To examine the dispersed (CCMS) and continuous phases (PVA matrix), CCMS domain of the multistructure matrix was dyed with brilliant green and toluidine blue. After staining, microscopic photographs of the multistructure system were visualized for us in Figure 5. It is found that the dispersed phase composed of caboxymethyl-chitosan is homogeneously formed in the continuous phases composed of PVA via the mechanical mix method. Hence, drug microspheres were successfully incorporated into PVA films. Films appear to be a suitable dosage form to deliver drugs into periodontal pocket, because the anatomic construction of the pocket allows for relatively easy insertion of such a delivery device.[22] Moreover, the use of bioerodable polymers can increase patient compliance, as the inserted film does not need to be removed. The composition of outer films of the system, PVA-124, is so hydrophilic (swells heavily in water) that can easy erode and dissolve in water. After the outer films breaking up into pieces in water, the drug-loaded CCMS was released to carry out the function of delivery drug at local site. Table II records the two time points, at which the outer PVA films began to break up and entirely dissolved in the media with various ionic strength and pH. As seen from Table II, the outer PVA film rapidly breaks up to pieces after about 30 min of placement in media and CCMS completely get rid of the bondage of PVA film need about 100 min. Furthermore, the increase of ionic strength or decrease of pH in the media would help to slightly accelerate the erosion of the outer PVA films.

Ornidazole release *in vitro*

The ornidazole release from CCMS and the multistructure carrier was investigated, and the effect of pH of the media on drug release was also studied.

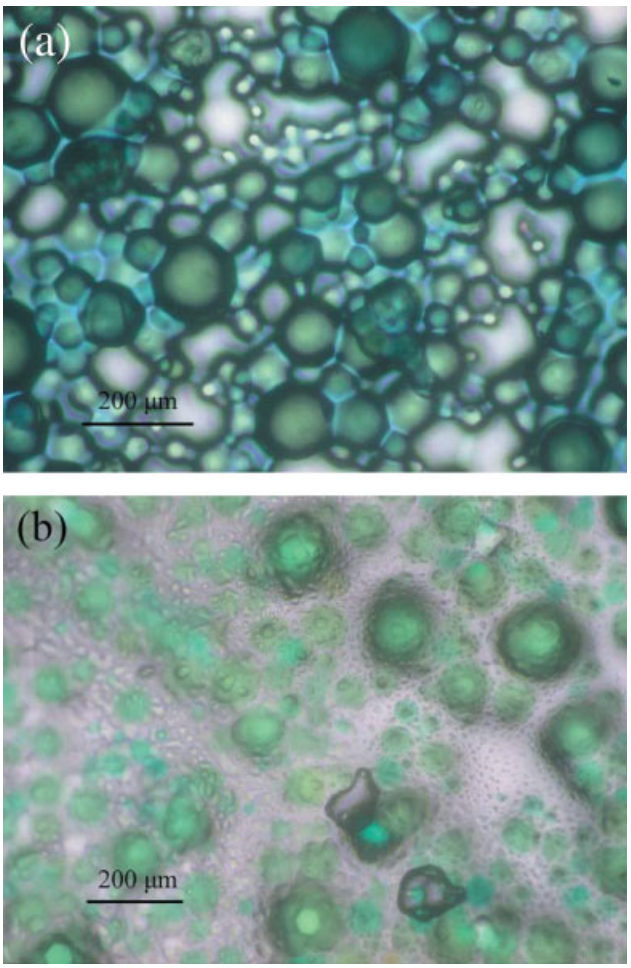

Figure 5 Optical images of the multistructure carrier after being stained with (a) toluidine blue and (b) brilliant green. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

TABLE II
Erosion of Outer PVA Films of the Multistructure Carrier in Different Media

Condition of media		Erosion time of the two multistructure carrier			
		CCMS : PVA = 1 : 5		CCMS : PVA = 1 : 2	
pH	Ionic strength	Erosion I*	Erosion B*	Erosion I*	Erosion B*
3.5	0	27	98	32	103
	0.1	25	90	35	112
	0.2	25	93	33	91
	0.5	23	92	34	107
7.4	0	29	99	40	112
	0.1	28	97	37	110
	0.2	30	109	39	101
	0.5	31	101	40	99

* Erosion I and Erosion B represent the time points at which the outer PVA films begin to crush up and entirely dissolved, respectively. Results are showed with the mean of thrice experiments.

The cumulative release percentage of ornidazole from all samples is depicted in Figures 6 and 7.

Figure 6 shows the percent release of ornidazole from all samples of CCMS (a) and the multistructure carrier (b) against incubation time at pH 7.4. The ornidazole release profiles of all samples consist of a burst release followed by a gradual release phase. 10%-CCMS-O microspheres at the initial phase is about 27%, which is higher than the 16% burst release of ornidazole from 20%-CCMS-O but lower than 36% burst release of ornidazole from 20%-CCMS-D. CCMS : PVA = 1 : 5 and CCMS : PVA = 1 : 2 multi structure carrier are 41 and 38% burst release in the first two hours, respectively, (Burst effect is defined here as the percentage released in the first 2 h as compared with the total ornidazole release amount because all samples of drug carrier shows obvious ornidazole release in the initial 2 h. Thus, the burst extent of all samples of drug carrier could be compared in the same condition.). As seen in Figure 6, the 20%-CCMS-O shows 25% ornidazole release within 12 h, whereas 10%-CCMS-O produce about 30% ornidazole release and 20%-CCMS-D produce almost 50% ornidazole release within 12 h. After 20%-CCMS-O being incorporated into PVA films, the drug released from the film form dosages with CCMS : PVA = 1 : 5 and CCMS : PVA = 1 : 2 would increase to 59 and 48% in 12 h, respectively. The release profiles of all samples are almost identical. This indicated that the complex formation may have similar influence on the ornidazole release. It was concluded that ornidazole burst release could be reduced and the sustained, gradual release profiles could be obtained by the CM-chitosan microsphere system.

It is thought that ornidazole release from the CCMS was controlled by the mechanisms of diffusion of drug molecules. The burst release of ornidazole was associated with those drug molecules dispersing close to the microspheres surfaces, which diffused out in the initial incubation time. The low ornidazole concentration around the CCMS surface

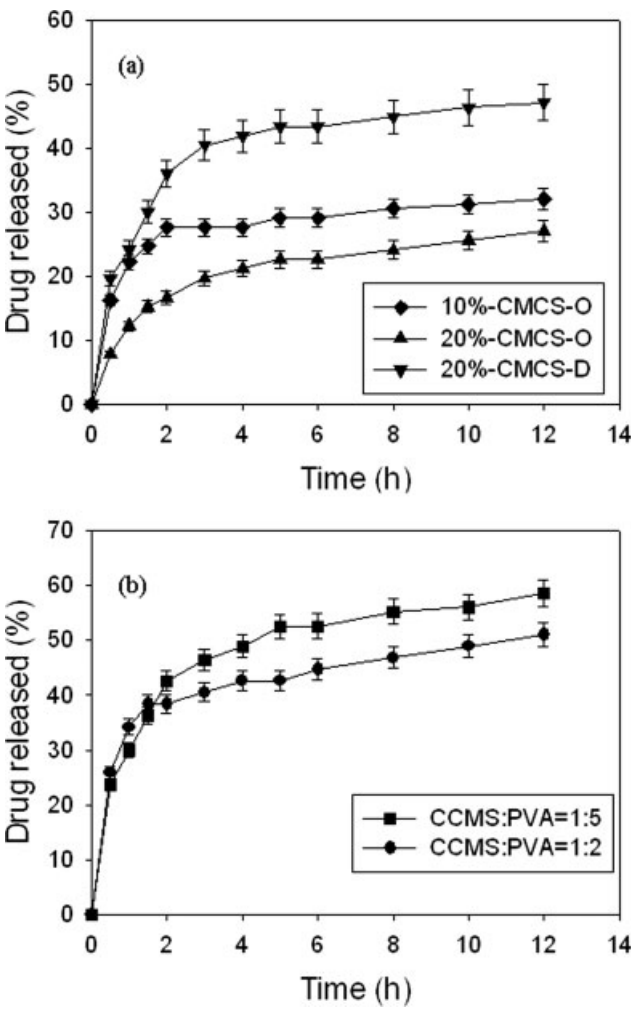

Figure 6 Ornidazole release from (a) drug-loaded CCMS and (b) the multistructure system at pH 7.4 condition *in vitro* ($n = 3$).

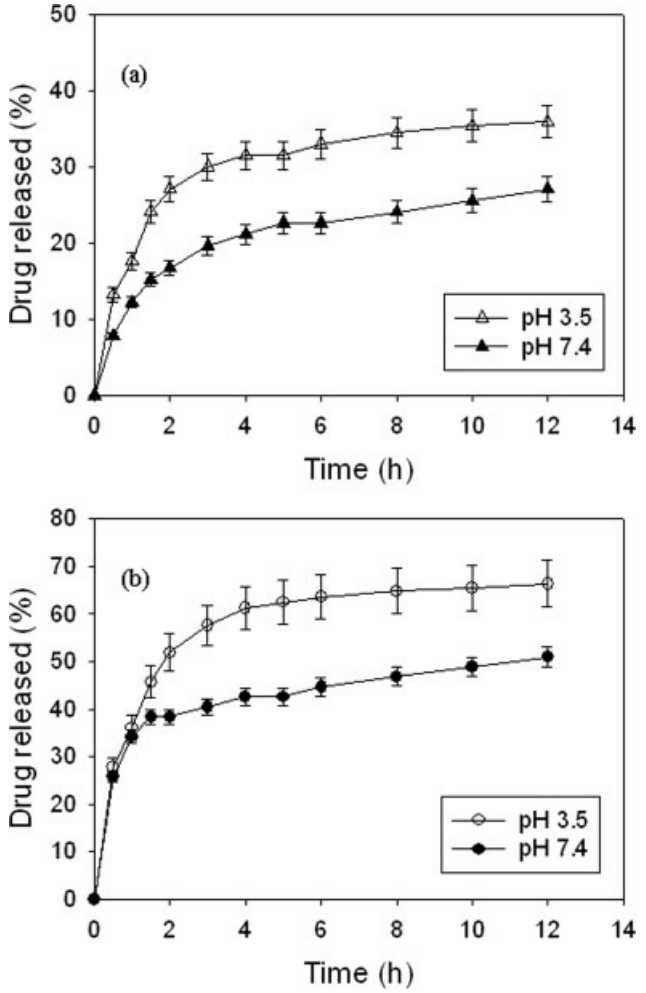

Figure 7 Ornidazole release from (a) 20%-CCMS-O and (b) the CCMS : PVA = 1 : 2 system in different pH media *in vitro* ($n = 3$).

would facilitate the drug diffusion from CCMS because of the poor solubility of ornidazole in water. On the contrary, the high concentration would inhibit the drug diffusion. Thus, the microspheres (as well as the multistructure carriers) with the lower drug loading amount exhibits the faster drug release velocity at initial incubation time. The use of DMSO would more enhance the drug diffusion and made the 20%-CCMS-D possessing the maximal drug release among all the carriers. Additionally, dispersing the 20%-CCMS-O in PVA solution for 4 h induced more ornidazole being extracted from the deep sections of microsphere, and this bring the multistructure carriers (CCMS : PVA = 1 : 5 and CCMS : PVA = 1 : 2) with higher drug release velocity than 20%-CCMS-O in initial time.

To better elucidate the diffusion mechanisms of drug release from these carriers, the drug release from the spheres and the multistructure carrier in both pH 3.5 and pH 7.4 of media was investigated. Figure 7 shows the ornidazole release profiles from the 20% CCMS (a) and the multistructure carrier with CCMS : PVA = 1 : 2 (b) at pH 3.5 and 7.4. As shown, with pH of the media being turned from 7.4 to 3.5, the amount of drug released from spheres and multistructure films increased from 25 to 37% and from 48 to 64%, respectively, within 12 h. It is well known that ornidazole is more soluble at acidic condition than at basic condition, and the H^+ ion can exchange with the ornidazole, which is associated with CM-chitosan by electrostatic affinity. Therefore, these ornidazole carriers show a rapid drug release in the acidic media.

CONCLUSIONS

In conclusion, a new drug dosage form based on drug microspheres incorporating in film was designed to treat periodontal diseases by using the CM-chitosan and PVA materials, and loading ornidazole as model drug. The film formulation may provide a convenient resort for drugs administration in local site and the core CCMS can stay in periodontal pocket to sustained release drug. The physical properties of CCMS and the multistructure carrier could be tailored by varying some factors, such as preparing conditions of CCMS, drug loading percentage, addition of DMSO, increase of CCMS content in the comatrix, and release media, etc. In our next studies on the multistructure drug carrier used in periodontal disease, the periodontitis animal model will be built to test the toxicity and the curative effect of the multistructure drug release system. It also needs us further compare this system with the commercially available dosage from.

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