Journal of Applied Polymer Science

Alginate Hydrogel Sphere Improves the Alkali and Heat Resistances of Isothiazolinones with Long-Term Antibacterial Activity

Dong Ma,[1*] Xiang Cai,[2*] Qianming Lin,[1] Jinglin Zhang,[2] Wenjie Mai,[3] Shaozao Tan,[2] Wei Xue,[1] Ting Wu[4]

[1]Key Laboratory of Biomaterials of Guangdong Higher Education Institutes, Department of Biomedical Engineering, Jinan University, Guangzhou 510632, People's Republic of China

[2]Department of Chemistry, Jinan University, Guangzhou 510632, People's Republic of China

[3]Department of Physics and Siyuan Laboratory, Jinan University, Guangzhou 510632, People's Republic of China

[4]R&D Center, Guangzhou Liby Enterprise Group Company, Limited, Guangzhou 510170, People's Republic of China

Correspondence to: D. Ma (E-mail: asd-0077@163.com) or T. Wu (E-mail: angelwu2006@163.com)

ABSTRACT: To improve the alkali and heat resistances of Cl+Me-isothiazolinone (MCI/MI), a calcium alginate (CA) hydrogel sphere was used as the carrier for MCI/MI, and an MCI/MI/CA hydrogel sphere was prepared. Then, the release properties, alkali-resistance and heat-resistance properties, long-term antibacterial performance, and cytotoxicity of this novel hydrogel sphere were investigated. The results show that the CA hydrogel sphere released MCI/MI in a sustained manner with long-term antibacterial activity. Moreover, the CA hydrogel sphere improved the alkali and heat resistances of the MCI/MI. More importantly, this hydrogel sphere showed a slight cytotoxicity, which suggested that the MCI/MI/CA hydrogel sphere would be an easily added antibacterial hydrogel sphere for a wide range of products. © 2013 Wiley Periodicals, Inc. J. Appl. Polym. Sci. 130: 1554–1561, 2013

KEYWORDS: applications; drug-delivery systems; gels; stimuli-sensitive polymers; thermal properties

Received 11 January 2013; accepted 18 March 2013; Published online 30 April 2013

DOI: 10.1002/app.39293

INTRODUCTION

Kathon CG or Cl+Me-isothiazolinone (MCI/MI) is a combination of 2-methyl-4-isothiazolin-3-one [MI; CAS: 2682-20-4, Figure 1(a)] and 5-chloro-2-methyl-4-isothiazolin-3-one [MCI; CAS: 26172-55-4, Figure 1(b)]. MCI/MI is frequently combined as a broad-spectrum preservative, and the microbiological performance of the MCI/MI preservative in most personal care products is excellent.[1,2] A Danish study (Nielsen) found that MCI/MI was registered in 523 products, and the most frequently registered products were paints, hair shampoos, skin care products, and cleaning agents.[3]

For each chemical, it is important to ensure the stability of the active ingredient. However, there are some circumstances in which MCI/MI does not preserve the stability.[4] For example, temperatures in excess of 50°C accelerate the rate of degradation of MCI/MI. Second, some agents, such as oxidizing agents, amines (secondary amines, in particular), reducing agents, and mercaptans, are detrimental to the MCI/MI stability. Also, sulfated and sulfonated surfactants often contain residual sulfite or bisulfite, which can react with MCI/MI.[5] These weaknesses limit the use of MCI/MI during manufacturing, storage, and application. An effective way to avoid side effects is to use a carrier. For instance, montmorillonite[6] and graphite[7] were used as carriers to improve the thermal stability of quaternary phosphonium salts. However, to the best of our knowledge, MCI/MI has never been loaded into a hydrogel carrier.

Alginate is a well-known natural polysaccharide with a negative charge; it is composed of 1,4-linked-β-D-mannuronate (MM blocks) and 1,4-linked α-L-guluronate (GG blocks) residues in variable proportions, depending on its algal or bacterial origin.[8] Sodium alginate is soluble in aqueous solutions and forms a stable gel at room temperature in the presence of certain divalent cations (i.e., Ba^{2+}, Ca^{2+}), which can be complexed by carboxylate groups of guluronate residues in a tetradentate structure and form the well-known egg-box model.[9,10] Calcium-ion-crosslinked sodium alginate possesses excellent properties and has been widely used as a carrier to deliver drugs and albumin,[11] such as riboflavin,[12] interleukin-2,[13] berberine hydrochloride,[14] and antigens.[15] What is more, the calcium alginate

*D. Ma and X. Cai contributed equally to this article.

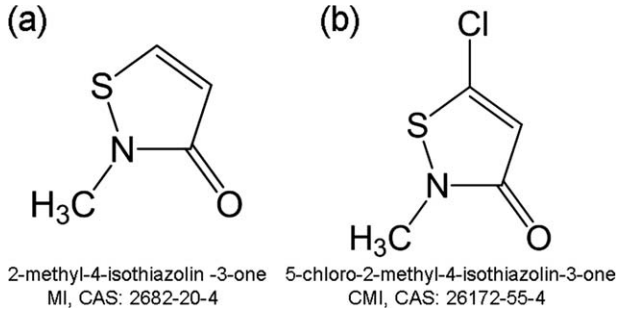

Figure 1. Chemical structures of MCI/MI.

(CA) hydrogel becomes more and more intelligent, with thermosensitivity[16] or pH responsivity.[17]

In this study, a CA hydrogel sphere was used as the carrier of MCI/MI. First, MCI/MI was mixed with sodium alginate. After mixture with $CaCl_2$, the MCI/MI-loaded CA (MCI/MI/CA) hydrogel sphere was prepared. The morphology, release properties, and alkali-resistance and heat-resistance properties of the MCI/MI/CA hydrogel sphere were studied. Then, the antibacterial performance of this novel hydrogel sphere was investigated for both Gram-positive and Gram-negative bacteria, and the sustained-release capability, long-term antibacterial effects, and lower cytotoxicity of this novel hybrid were also demonstrated.

The novelty of this study was that the use of the CA hydrogel sphere as an MCI/MI carrier should extend the application field of MCI/MI, and the specific benefits of this novel hybrid included (1) enhanced alkali resistance to achieve a more alkaline environment in manufacturing and use; (2) enhanced heat resistance to increase its usage temperature in manufacturing, storage, or application; (3) sustained-release capability to achieve long-term antibacterial effects; (4) easy addition in personal care products; and (5) application in a wider range of products.

EXPERIMENTAL

Materials

Sodium alginate was purchased from Sigma-Aldrich (Shanghai, China) and was used directly. $CaCl_2$ (analytical-reagent grade) was purchased by Guangzhou Chemical Industry Co., Ltd. (Guangzhou, China). A 1.2 wt % MCI/MI aqueous solution (containing inorganic magnesium salts, where the weight ratio of MCI/MI was 3:1) was supplied by Huankai Microorganism Co., Ltd. (Guangzhou, China). *Escherichia coli* ATCC 25922 and *Staphylococci aureus* ATCC 6538 were supplied by the Guangdong Institute of Microbiology (Guangzhou, China). Thiazolyl blue tetrazolium bromide (MTT) was purchased from Sigma-Aldrich (Shanghai, China). Human nasopharyngeal carcinoma CNE1 (CNE1) cells were supplied by the General Hospital of Guangzhou Military Command. Luria–Bertani broth and nutrient agar culture medium were supplied by Huankai Microorganism Co., Ltd. All other reagents and solvents were obtained from commercial suppliers. All of the aqueous solutions were prepared with ultrapure water (>18 MΩ) from a Milli-Q Plus system (Millipore).

Preparation and Characterization of the MCI/MI/CA Hydrogel Sphere

An amount of 1.00 g of sodium alginate was dissolved in 20 mL of MCI/MI aqueous solution to get a sodium alginate/MCI/MI solution, and then the solution was added dropwise to 20 mL of a 1 mol/L $CaCl_2$ aqueous solution through a 1-mL syringe. After the mixture was set aside for 12 h at room temperature, the MCI/MI/CA hydrogel sphere was obtained. The particle size was determined with a statistical method. In short, 200 individual MCI/MI/CA hydrogel spheres were picked out, and their sizes were measured by a microcalliper to obtain the normal distribution of the MCI/MI/CA hydrogel sphere. Two hundred individual MCI/MI/CA hydrogel spheres were picked out, and their diameters and size distribution were analyzed with image analyzer software (Image Plus 6).[18]

The load efficiency of MCI/MI on the MCI/MI/CA hydrogel sphere was studied by high-performance liquid chromatography (HPLC). In short, 100 mg of MCI/MI/CA spheres was immersed in 10 mL of phosphate buffer solution (PBS, pH = 7.0) at 37°C. Then, the mixture was stirred at 300 rpm for 48 h to obtain a clear solution; the spheres were dissolved, and the MCI/MI was completely released. Afterward, the mixtures were coupled with an analysis of the active ingredient by HPLC (Agilent 1260) with an external standard method. The chromatographic conditions of HPLC were as follow: the separation was performed on a AQ-C18 column (4.6 × 250 mm^2, Ultimate), the detection wavelength was set at 276 nm (the reference wavelength was set at 280 nm), the detection temperature was 25°C, the mobile phase was a 40% v/v methanol aqueous solution (the solvent was a 0.1% v/v acetic acid solution in ultrapure water) and was degassed with helium (purity 5.0), and the detection limit was 0.6 μg/mL. The retention times of MI and MCI were 3.46 and 6.63 min, respectively (Figure 2). The drug loading in the MCI/MI/CA hydrogel sphere was 12.8 mg/g.

In Vitro Drug Release

An amount of 2 g of MCI/MI/CA hydrogel sphere was immersed in 200 mL of ultrapure water at different temperatures, and the pH values of the release media were adjusted by 0.1 mol/L NaOH. At predetermined time points, 500 μL of the

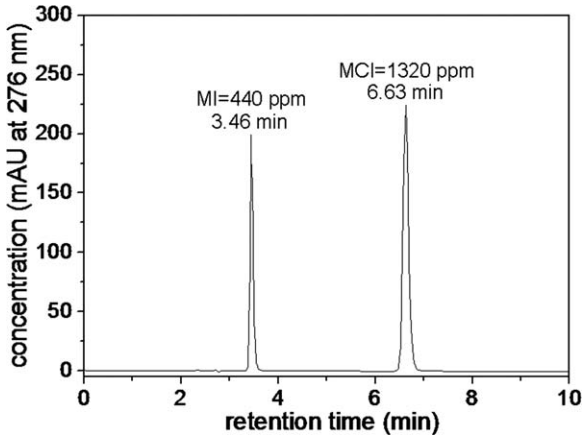

Figure 2. Retention times of 2-methyl-4-isothiazolin-3-one (MI) and 5-chloro-2-methyl-4-isothiazolin-3-one (MCI).

medium solution was taken out, and 500 μL of fresh ultrapure water was added back to maintain the same total solution volume. The amount of released MCI/MI was determined by HPLC. The cumulative percentage of released MCI/MI was calculated from a standard calibration curve. All of the release studies were carried out in triplicate.

Alkali-Resistance Analyses of the MCI/MI/CA Hydrogel Sphere

An amount of 5 g of the MCI/MI/CA hydrogel sphere (containing 64 mg of MCI/MI) or 64 mg of MCI/MI was immersed in 50 mL of sodium hydroxide solution at pH 7.0, 8.0, 9.0 or 10.0 at 25°C. At predetermined time points, the solution was neutralized to pH 7.0 with 0.1 mol/L HCl. Then the solution was immersed in 50 mL of PBS (pH 7.0) at 25°C and stirred at 300 rpm for 48 h to obtain a clear solution. The spheres were dissolved, and MCI/MI was completely released. After that, the mixtures were coupled with an analysis of the MCI/MI by HPLC.

Heat-Resistance Analyses of the MCI/MI/CA Hydrogel Sphere

An amount of 5 g of the MCI/MI/CA hydrogel sphere (containing 64 mg of MCI/MI) or 64 mg of MCI/MI was immersed in 50 mL of pH 7.0 ultrapure water at 25, 35, 45, or 60°C. At predetermined time points, the solution was cooled to 25°C, and then the solution was immersed in 50 mL of PBS (pH = 7.0) at 25°C and stirred at 300 rpm for 48 h to obtain a clear solution. The spheres were dissolved, and MCI/MI was completely released. After that, the mixtures were coupled with an analysis of MCI/MI by HPLC.

Antibacterial Activity Assay

The newly prepared samples (3.0 mg) were dispensed into 10 mL of sterile 0.8 wt % saline water containing about 10^6 cfu/mL *E. coli* or *S. aureus,* and then the mixture was shaken at 37°C. After 24 h of contact, 0.1 mL of the suspension was taken out of the test tube and diluted to a certain volume (to ensure the bacterial colonies could be counted easily and correctly) by 10-fold dilution. The diluted solution was plated on Luria-Bertani broth agar plates in triplicate and incubated at 37 ± 1°C for 24 h. The number of bacterial colonies on each plate was counted.[19] The killing rate (η) was related to the viable bacteria counts as follows: $\eta = (Y - X) \times 100\%/Y$, where Y is the number of microorganism colonies on the control tube (a sterile 0.8 wt % saline water without sample) and X is the number of microorganism colonies on the samples.[20]

Minimum Inhibitory Concentration (MIC)

The MIC of the newly prepared samples against *E. coli* or *S. aureus* was measured by a two-fold diluting method. Briefly, the newly prepared samples were suspended into a Mueller–Hinton broth medium to form homogeneous suspensions and then diluted two-fold into different concentrations. Each 1 mL of culture medium containing various concentrations of test sample was inoculated with 0.1 mL of 10^6 cfu/mL bacterial suspensions and cultured for 24 h at 37°C with shaking. Then the growth of the bacteria was observed. When no growth of bacteria was observed in the lowest concentration of the test sample, the MIC of the sample was defined as this value of dilution. The test for every MIC of the newly prepared samples was repeated three times.

Cytotoxicity Assay

The cytotoxicity of the MCI/MI/CA hydrogel sphere was tested with the MTT assay on the basis of the cellular uptake of MTT and its subsequent reduction in the mitochondria of living cells to dark blue MTT formazan crystals. CNE1 cells were seeded on 96-well plates (1.5–2 × 10^4 cells/well) in corresponding medium. Then, the cells were treated with the CA hydrogel sphere, MCI/MI, or MCI/MI/CA hydrogel sphere for 4 h. After that, MTT (5 mg/mL in PBS) was added to each well and incubated for an additional 4 h (37°C, 5% CO_2). The cells were then lyzed in dimethyl sulfoxide (150 μL/well), and the plates were allowed to stay in the incubator (37°C, 5% CO_2) to dissolve the purple formazan crystals. The color intensity reflecting cell viability was read at 490 nm with a model 550 enzyme-linked immunosorbent microplate (Bio-Rad), and the morphological changes in the CNE1 cells were photographed by a IX-70 inverted phase-contrast microscope (Olympus, Japan). All of the experiments were repeated four times, and Statistical Product and Service Solutions software (SPSS 11.0, United States) was used to assess the statistical significance of the differences among the treatment groups.

Statistical Analysis

Statistical analysis was performed with Statistical Product and Service Solutions software statistical software. The differences between the groups were assessed with the analysis of variance test. The results were considered statistically significant when the p value was less than 0.05 or less than 0.01.

RESULTS AND DISCUSSION

Synthesis of the MCI/MI/CA Hydrogel Sphere

The MCI/MI/CA hydrogel sphere was prepared by a two-step approach, as illustrated in Figure 3. After the sodium alginate/MCI/MI solution was dropped into the $CaCl_2$ aqueous solution, the drop turned cloudy and then formed a stiff white hydrogel sphere. This phenomenon was attributed to the inclusion complex formation between alginate and Ca^{2+}, which resulted in a white MCI/MI/CA hydrogel sphere.

Figure 4 shows the particle size distribution and morphology of the MCI/MI/CA hydrogel sphere. As shown, all of the particles were observed to have spherical morphologies. The resulting MCI/MI/CA hydrogel sphere had an average size of 1.86 ± 0.24 mm.

In Vitro Drug Release

Figure 5 shows the *in vitro* release profiles for MCI/MI, MCI, and MI released from the MCI/MI/CA hydrogel sphere. To represent the controlled and sustained drug release for the hydrogel sphere, the release profiles of MCI and MI from the hydrogel sphere are also indicated. It could be seen that the release rates of MCI/MI, MCI, and MI from the hydrogel sphere were almost the same in each case. The same release rates of MCI and MI indicated that the hydrogel sphere could simultaneously release MCI and MI, and their release was constant and steady.

We observed that with increasing pH value, all of the release rates of MCI/MI, MCI, and MI from the hydrogel sphere decreased. With increasing temperatures, all of the release rates of MCI/MI, MCI, and MI from the hydrogel sphere increased.

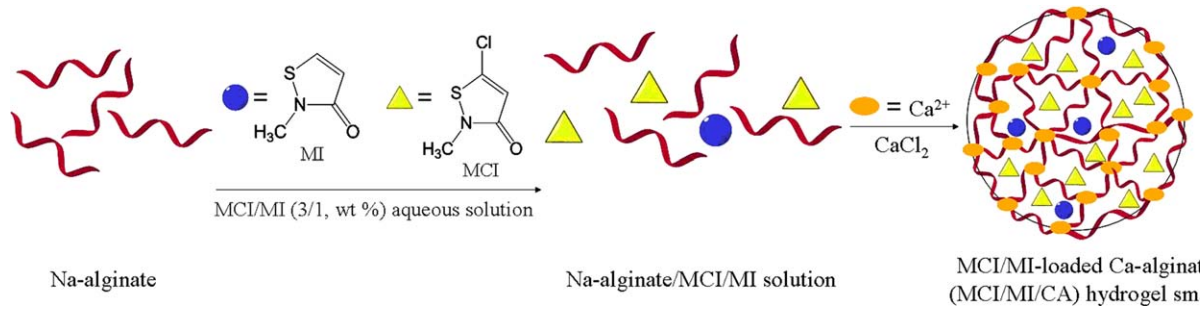

Figure 3. Preparation of the MCI/MI/CA hydrogel sphere. Step 1: the sodium alginate aqueous solution was mixed with MCI/MI. Step 2: the sodium alginate/MCI/MI solution was added drop by drop into the $CaCl_2$ aqueous solution in a mild condition to produce an MCI/MI/CA hydrogel sphere. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

In addition, no initial burst release was observed, and this indicated that the release of MIC and MI could be controlled and sustained from the hydrogel sphere.

To understand the MCI/MI, MCI, and MI release mechanisms of the MCI/MI/CA hydrogel sphere, we fitted the accumulative release data with the semi-empirical eq. (1):[21]

$$\frac{M_t}{M_\infty} = Kt^n \left(for \frac{M_t}{M_\infty} \leq 0.6 \right) \quad (1)$$

where M_t and M_∞ are the cumulative amounts of the drug released at time t and equilibrium, respectively; K is the rate constant relating to the properties of the hydrogel matrix and the drug; and n is the release exponent characterizing the transport mechanism.

By plotting $\log(M_t/M_\infty)$ versus $\log t$, the n values and the corresponding determination coefficients (R^2's) were obtained. The n values were between 0.53 and 0.72, and the R^2 values were all greater than 0.98. According to semi-empirical eq. (1), there are four distinguishable modes of diffusion:

1. A value of $n = 0.5$ suggests Fickian or case I transport behavior, in which the relaxation coefficient is negligible during transient sorption.
2. A value of $n = 1$ refers to a non-Fickian or case II mode of transport where the morphological changes are abrupt.
3. When $0.5 < n < 1$, the transport process is anomalous, corresponding to case III, and the structural relaxation is comparable to diffusion.
4. A value of $n < 0.5$ indicates a pseudo-Fickian behavior of diffusion where the sorption curves resemble Fickian curves, but the speed of approach to the final equilibrium is very slow.

In our case, the n values were found to be in the range from 0.5 to 1, showing a case III mechanism; this meant the structural relaxation was comparable to diffusion.

Alkali- and Heat-Resistance Analyses

Figure 6 shows the stability of the MCI/MI and MCI/MI/CA hydrogel sphere preservative in aqueous solution at 25°C and various pH values. As demonstrated in Figure 6, when the MCI/MI or MCI/MI/CA hydrogel sphere preservative was in pH 7.0 aqueous solution, the rate of degradation of MCI/MI was very slow. With increasing pH, the rate of degradation of MCI/MI was accelerated, and this meant that MCI/MI was stable up to a pH of 8.0 over the lifetime. What was more, the MCI/MI in the CA hydrogel sphere was more stable under basic conditions than the MCI/MI alone.

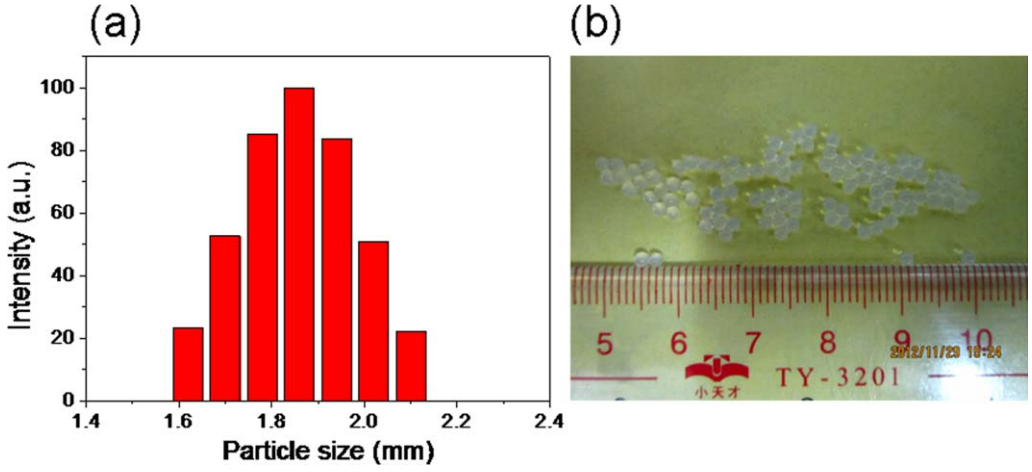

Figure 4. (a) Particle size distribution and (b) picture of the MCI/MI/CA hydrogel sphere. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Applied Polymer SCIENCE

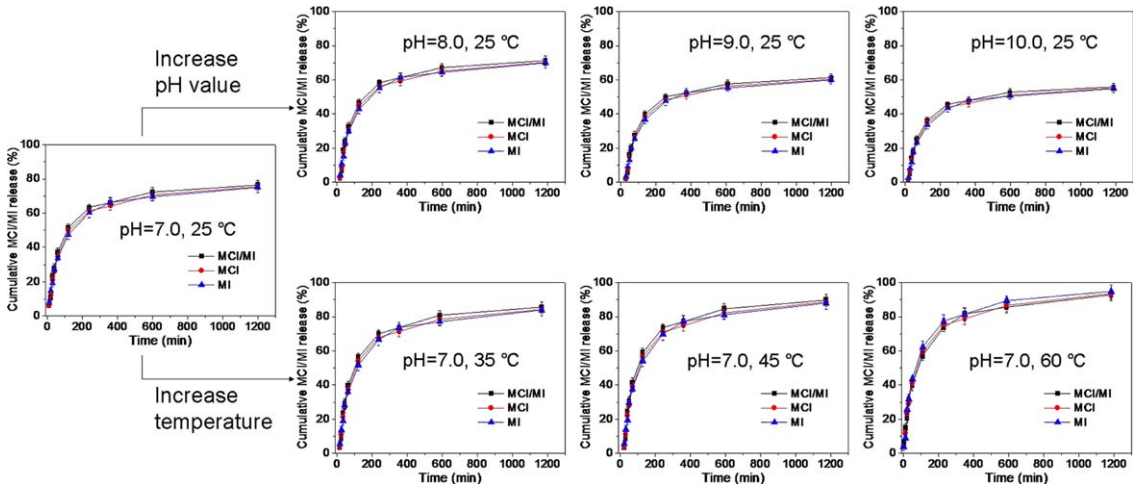

Figure 5. *In vitro* MCI/MI, MCI, and MI release profiles from the MCI/MI/CA hydrogel sphere at different pH values and temperatures. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

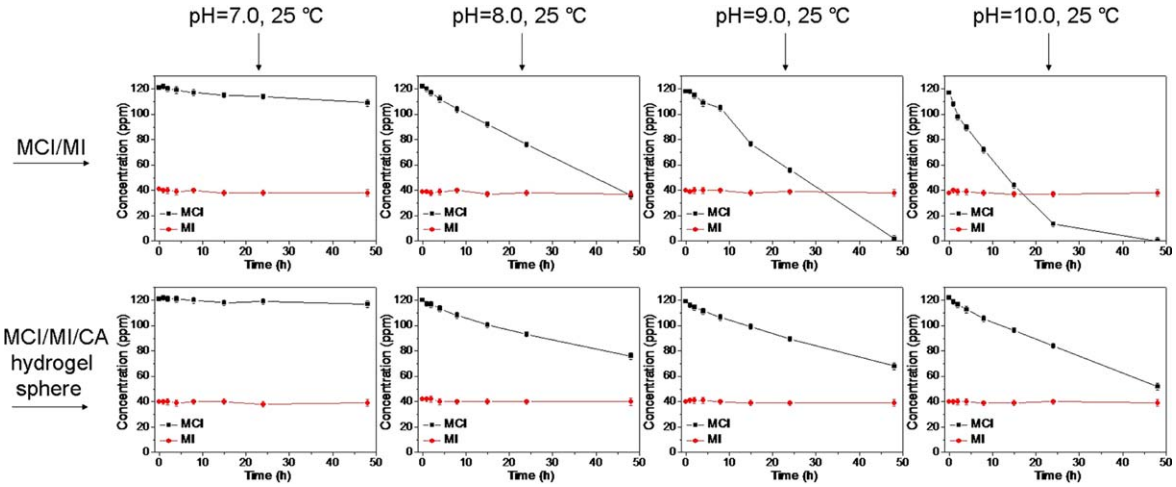

Figure 6. Stability of the MCI/MI and MCI/MI/CA hydrogel sphere preservative in an aqueous solution at 25°C and various pH values. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

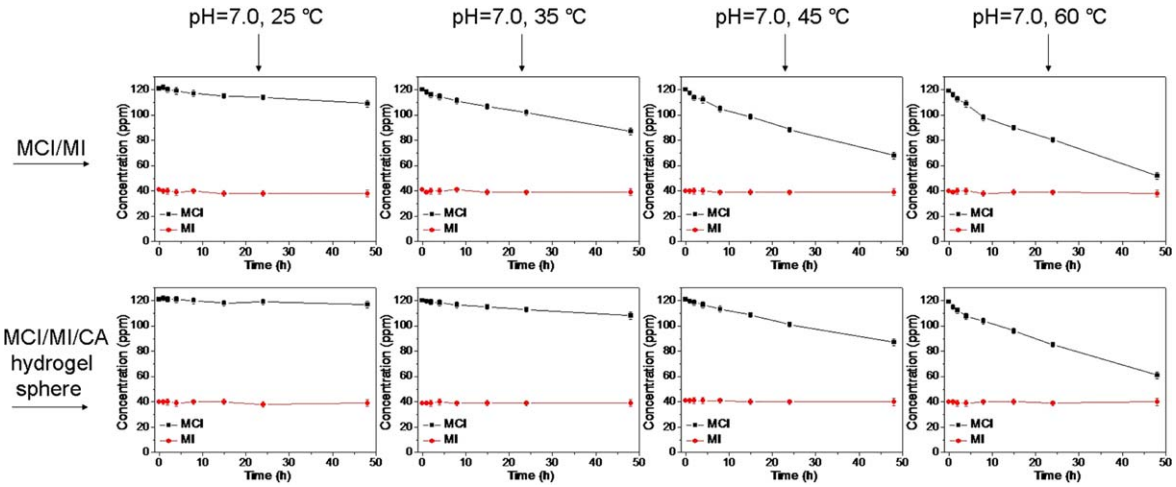

Figure 7. Stability of the MCI/MI and MCI/MI/CA hydrogel sphere preservative in a pH 7.0 aqueous solution at various temperatures. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

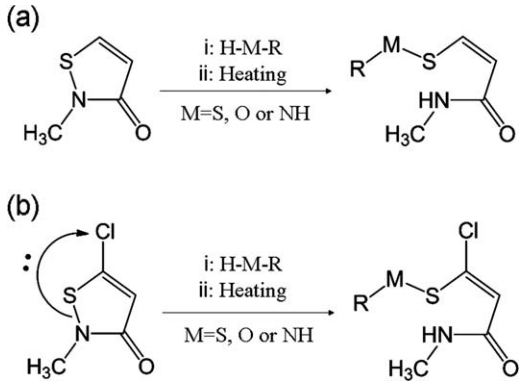

Figure 8. Chemical reactions of (a) MI and (b) MCI under basic conditions with H-M-R (reducing agents, M═S,O or NH) or under heating conditions.

As a general rule, a rise in temperature will accelerate the rate of degradation of chemicals. As demonstrated in Figure 7, MCI/MI was no exception. With increasing temperature, the rate of degradation of MCI/MI was accelerated. What was more, the MCI/MI in the CA hydrogel sphere was more stable under heating conditions than the MCI/MI alone.

As we know, under basic conditions, the MI could react with reducing agents, and heating conditions also led to the reaction between MI and water [Figure 8(a)]. For MCI, there was a 5-chloro; this made the N—S more easily react with reducing agents under basic conditions or with water under heating conditions [Figure 8(b)]. However, a simple reduction in pH to below 7, which converted the reducing agents to their corresponding acid salt, could normally resolve the problem. In addition, a lower temperature could also slow the rates of the chemical reactions of MI and MCI, which would have prolonged the stability of MI and MCI.

Because the CA hydrogel sphere was bundled the MCI/MI in the hydrogel sphere, the MCI/MI in the CA hydrogel sphere was isolated from the reducing agents; the basic conditions or heating conditions, which made the MCI/MI more stable than the MCI/MI alone, should extend the application field of MCI/MI.

Antibacterial Activity Analysis

Table I shows the antibacterial activity of the MI, MCI, MI/MCI, and MCI/MI/CA hydrogel sphere after 24 h of contact, and the MICs of the MI, MCI, MI/MCI, and MCI/MI/CA hydrogel spheres are also shown in Table I. MI showed poor antibacterial activity against *E. coli* and *S. aureus* because the viable colonies of *E. coli* or *S. aureus* remained essentially unchanged. In contrast, MCI showed excellent antibacterial activity against *E. coli* and *S. aureus*. There was a general agreement that the biological action of MCI was higher than that of MI because MCI could react with —SH of bacteria more easily than MI; as a result, η of MCI was higher than that of MI. For MI/MCI, the antibacterial activity was slightly lower than that of MCI, and after treatment under basic conditions (pH 10.0, 25°C, 48 h), the antibacterial activity was dramatically decreased (Table I).

In contrast, the MI/MCI in CA hydrogel sphere was still shown to have a relatively high antibacterial activity after treatment

Table I. Antibacterial Activity of the Samples

Strain	Sample	MCI concentration (ppm)	MI concentration (ppm)	Viable colonies of bacteria after contact for 24.0 h (cfu/mL)	Killing rate (%)[a]	MIC (ppm)
E. coli	Blank sample	0	0	1.12×10^8	No effect	—
	MI	0	4	$(1.05 \pm 0.01) \times 10^8$	6.53 ± 1.22	30
	MCI	12	0	$(7.62 \pm 1.80) \times 10^5$	99.32 ± 0.16	1.5
	MCI/MI	12	4	$(4.48 \pm 1.12) \times 10^4$	99.96 ± 0.01	2
	MCI/MI/CA hydrogel sphere	12	4	$(3.06 \pm 0.57) \times 10^6$	97.27 ± 0.51	160
	MCI/MI at pH 10.0 and 25°C for 48 h	0	4	$(1.03 \pm 0.01) \times 10^8$	8.29 ± 0.52	35
	MCI/MI/CA hydrogel sphere at pH 10.0 and 25°C for 48 h	6	4	$(8.80 \pm 2.09) \times 10^6$	92.14 ± 1.87	320
S. aureus	Blank sample	0	0	7.83×10^7	No effect	—
	MI	0	4	$(6.88 \pm 0.09) \times 10^7$	12.19 ± 1.14	20
	MCI	12	0	$(2.27 \pm 0.16) \times 10^5$	99.71 ± 0.02	1
	MCI/MI	12	4	$(7.83 \pm 0.00) \times 10^3$	99.99 ± 0.00	1.5
	MCI/MI/CA hydrogel sphere	12	4	$(6.11 \pm 0.08) \times 10^5$	99.22 ± 0.01	120
	MCI/MI at pH 10.0 and 25°C for 48 h	0	4	$(6.42 \pm 0.29) \times 10^7$	17.98 ± 3.76	25
	MCI/MI/CA hydrogel sphere at pH 10.0 and 25°C for 48 h	6	4	$(7.67 \pm 0.62) \times 10^5$	99.02 ± 0.08	240

[a] Plus or minus the standard deviation, $n = 3$.

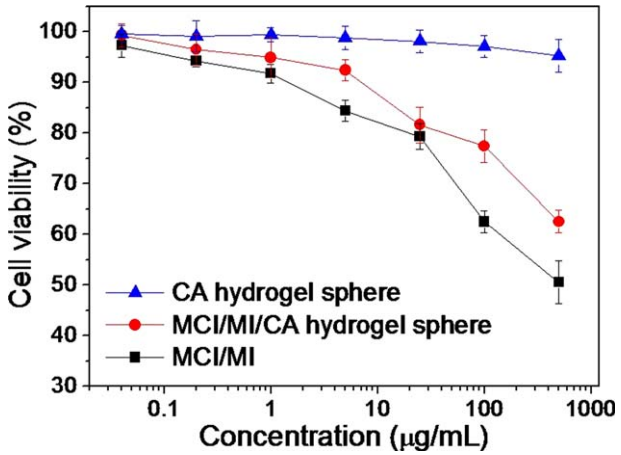

Figure 9. Cytotoxicity of the CA hydrogel sphere, MCI/MI/CA hydrogel sphere, and MCI/MI on the L929 cells. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

under basic conditions (pH 10.0, 25°C, 48 h). Because the CA hydrogel sphere had the ability to stabilize the MI/MCI, that is, end the antibacterial activity before dissolution and diffusion of hydrogel sphere, the MCI/MI/CA hydrogel sphere more easily achieved the long-term antibacterial activity than MI/MCI. These results were consistent with the MIC test. The MIC value of the MCI/MI/CA hydrogel sphere was higher than that of MI/MCI because the MI/MCI loading in the MCI/MI/CA hydrogel sphere was 12.8 mg/g.

In addition, all of the samples exhibited lower activity against *E. coli* than against *S. aureus*. The structure of the cytoderm of *E. coli* is more complicated than that of *S. aureus* because of the presence of another layer outside of the peptidoglycan layer called the *outer membrane*, which is composed mainly of lipopolysaccharides and phospholipids. The outer membrane takes a significant role in protecting bacterial cells against foreign compounds, such as MI or MCI. Thus, the lower sensitivity of these test samples toward *E. coli* was mainly due to the presence of the outer membrane.[22,23]

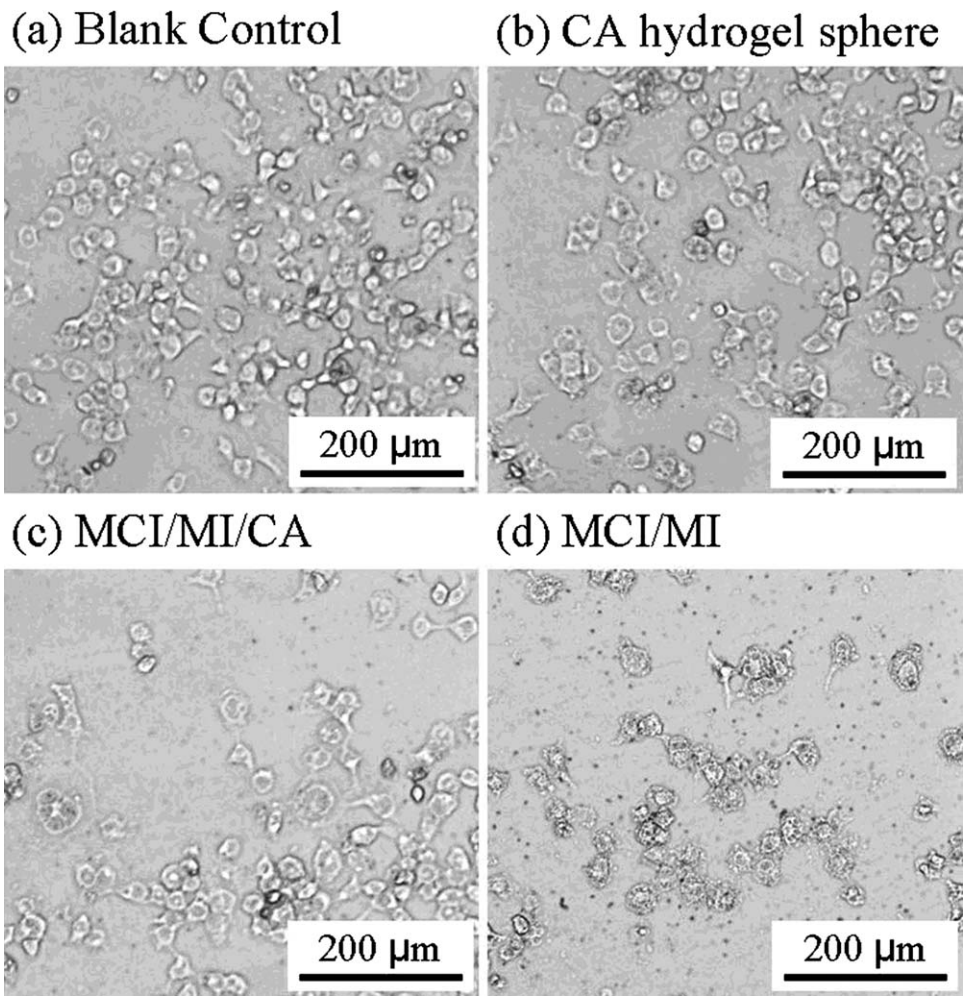

Figure 10. Morphologic changes of the L929 cells when the concentrations of the CA hydrogel sphere, MCI/MI/CA hydrogel sphere, and MCI/MI were set to 500 mg/L and after interaction for 24 h. In the blank control group (a), the L929 cells had good shapes, which presented as polygons. The presences of round dividing cells showed their vigorous growth. In the CA hydrogel sphere group (b), the L929 cells were basically the same as those in the blank control group. In the MCI/MI/CA hydrogel sphere group (c), the number of L929 cells decreased significantly. In the MCI/MI group (d), the number of L929 cells decreased significantly; the shapes of L929 cells became irregular, and the shapes of majority of the cells were seriously injured.

Given the enhanced alkali resistance and heat resistance, and the long-term antibacterial effect, we concluded that the MCI/MI/CA hydrogel sphere would have practical application in a wider range of products.

Cytotoxicity Test

We also carried out a cytotoxicity test on the CA hydrogel sphere, MCI/MI/CA hydrogel sphere, and MCI/MI. The MTT assays (Figure 9) showed that the CA hydrogel sphere exhibited almost no cytotoxicity to L929 within 24 h of incubation (the cell viability of L929 was reduced to 98.8 and 95.2% with 5 and 500 μg/mL of CA, respectively). MCI/MI exhibited cytotoxicity to L929 within 24 h of incubation (the cell viability of L929 was reduced to 84.4 and 50.5% with 5 and 500 μg/mL of MCI/MI, respectively). However, the cell viability of L929 was reduced to 92.4 and 62.5% with 5 and 500 μg/mL of the MCI/MI/CA hydrogel sphere, respectively.

Therefore, the cytotoxicity of the MCI/MI/CA hydrogel sphere was significantly lower than that of MCI/MI, and the use of an MCI/MI/CA hydrogel sphere would be safer than the direct use of MCI/MI; this was in accordance with the results of our inverted phase-contrast microscope measurements (Figure 10). In comparison with our previous report,[24–26] we concluded that the MCI/MI/CA hydrogel sphere was a relatively biocompatible hydrogel with a slight cytotoxicity.

CONCLUSIONS

In summary, CA hydrogel sphere was used as the carrier of MCI/MI, and a MCI/MI/CA hydrogel sphere was prepared. The results showed that the MCI/MI/CA hydrogel sphere had an average size of 1.86 ± 0.24 mm. With increasing pH value, the release rate of MCI/MI from the hydrogel sphere decreased. With increasing temperature, the release rate of MCI/MI from the hydrogel sphere increased. The release rates of MCI and MI from the hydrogel sphere were almost the same; they showed a case III mechanism, which meant that the structural relaxation was comparable to diffusion. The CA hydrogel sphere bundled the MCI/MI in the hydrogel sphere, and the MCI/MI in CA hydrogel sphere was isolated from reducing agents under basic conditions or heating conditions. This made the MCI/MI more stable than the MCI/MI alone and should extend the application field of MCI/MI. The MCI/MI/CA hydrogel sphere more easily achieved long-term antibacterial activity than MI/MCI. The MCI/MI/CA hydrogel sphere was composed of relatively biocompatible nanomaterials with a slight cytotoxicity; thus, the use of MCI/MI would be safer and more efficient. Given these advantages, we expect that the MCI/MI/CA hydrogel sphere would be an easily added antibacterial hydrogel sphere in a wide range of products.

ACKNOWLEDGMENTS

The authors thank the National Natural Science Foundation of China (contract grant numbers 21104099, 21006038, 21271087, 51172099, 20971028, and 21176100), the Natural Science Key Foundation of Guangdong Province of China (contract grant number 10251007002000000), Foundation of Science and Technology Projects of Guangdong Province (contract grant number 2011B010700080), and the Fundamental Research Funds for the Central Universities (contract grant number 21612109).

REFERENCES

1. Rafoth, A.; Gabriel, S.; Sacher, F.; Brauch, H.-J. *J. Chromatogr. A* **2007**, *1164*, 74.
2. Critchley, M.; Bentham, R. *J. Appl. Microbiol.* **2009**, *106*, 784.
3. Speksnijder, P.; van Ravestijn, J.; de Voogt, P. *J. Chromatogr. A* **2010**, *1217*, 5184.
4. Smaoui, S.; Hlima, H. B. *Biocontrol Sci.* **2012**, *17*, 135.
5. Collier, P. J.; Ramsey, A.; Waigh, R. D.; Douglas, K. T.; Austin, P.; Gilbert, P. *J. Appl. Microbiol.* **1990**, *69*, 578.
6. Cai, X.; Tan, S. Z.; Liao, M. H.; Wu, T.; Liu, R. F.; Yu, B. *J. Cent. South Univ. T.* **2010**, *17*, 485.
7. Xie, A. G.; Cai, X.; Lin, M. S.; Wu, T.; Zhang, X. J.; Lin, Z. D.; Tan, S. Z. *Mater. Sci. Eng. B* **2011**, *176*, 1222.
8. Abd El-Ghaffar, M. A.; Hashem, M. S.; El-Awady, M. K.; Rabie, A. M. *Carbohydr. Polym.* **2012**, *89*, 667.
9. Wang, L.; Shelton, R. M.; Cooper, P. R.; Lawson, M.; Triffitt, J. T.; Barralet, J. E. *Biomaterials* **2003**, *24*, 3475.
10. Xie, L.; Jiang, M.; Dong, X. G.; Bai, X.; Tong, J.; Zhou, J. *J. Appl. Polym. Sci.* **2012**, *124*, 823.
11. Wang, K.; Xu, X.; Wang, Y. J.; Guo, G.; Huang, M. J.; Luo, F.; Zhao, X.; Wei, Y. Q.; Qian, Z. Y. *Soft Mater.* **2010**, *8*, 307.
12. Chen, L. Y.; Subirade, M. *Biomaterials* **2006**, *27*, 4646.
13. Liu, L. S.; Liu, S. Q.; Ng, S. Y.; Froix, M.; Ohno, T.; Heller, J. *J. Controlled Release* **1997**, *43*, 65.
14. Zhu, A. M.; Chen, J. H.; Liu, Q. L.; Jiang, Y. L. *J. Appl. Polym. Sci.* **2011**, *120*, 2374.
15. Kim, B.; Bowersock, T.; Griebel, P.; Kidane, A.; Babiuk, L. A.; Sanchez, M.; Attah-Poku, S.; Kaushik, R. S.; Mutwiri, G. K. *J. Controlled Release* **2002**, *85*, 191.
16. Tan, R. W.; She, Z. D.; Wang, M. B.; Fang, Z.; Liu, Y. S.; Feng, Q. L. *Carbohydr. Polym.* **2012**, *87*, 1515.
17. El-Sherbiny, I. M.; Abdel-Mogib, M.; Dawidar, A.-A. M.; Elsayed, A.; Smyth, H. D. C. *Carbohydr. Polym.*, **2011**, *83*, 1345.
18. Lin, J. T.; Li, C. H.; Zhao, Y.; Hu, J. C.; Zhang, L. M. *Am. Chem. Soc. Appl. Mater. Interfaces* **2012**, *4*, 1050.
19. Ouyang, Y. S.; Xie, Y. S.; Tan, S. Z.; Shi, Q. S.; Chen, Y. B. *J. Rare Earth* **2009**, *27*, 858.
20. Lin, Y. G.; Yang, Z. R.; Cheng, J. *J. Rare Earth* **2007**, *25*, 452.
21. Franson, N. M.; Peppas, N. A. *J. Appl. Polym. Sci.* **1983**, *28*, 1299.
22. Cai, X.; Dai, G. J.; Tan, S. Z.; Ouyang, Y.; Ouyang, Y. S.; Shi, Q. S. *Mater. Lett.* **2012**, *67*, 199.
23. Wu, T.; Xie, A. G.; Tan, S. Z.; Cai, X. *Colloid Surf. B* **2011**, *86*, 232.
24. Cai, X.; Lin, M. S.; Tan, S. Z.; Mai, W. J.; Zhang, Y. M.; Liang, Z. W.; Lin, Z. D.; Zhang, X. *J. Carbon* **2012**, *50*, 3407.
25. Cai, X.; Tan, S. Z.; Yu, A. L.; Zhang, J. L.; Liu, J. H.; Mai, W. J.; Jiang, Z. Y. *Chem.-Asian J.* **2012**, *7*, 1664.
26. Cai, X.; Tan, S. Z.; Lin, M. S.; Xie, A. G.; Mai, W. J.; Zhang, X. J.; Lin, Z. D.; Wu, T.; Liu, Y. L. *Langmuir* **2011**, *27*, 7828.