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Chitosan microcapsules loaded with either miconazole nitrate or clotrimazole, prepared via emulsion technique

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ABSTRACT

In this paper, a simple and versatile coacervation technique has been developed by using an ultrasound-assisted oil/water emulsion method for the preparation of antifungal agent-loaded microcapsules. Two types of chitosan microcapsules are successfully prepared. The mean particle size of the chitosan/miconazole nitrate microcapsules is 2.6 μm and that of the chitosan/clotrimazole microcapsules is 4.1 μm. The encapsulation efficiency of the chitosan/miconazole nitrate microcapsules (77.58–96.81%) is relatively higher than that of the chitosan/clotrimazole microcapsules (56.66–93.82%). The in vitro drug release performance of the microcapsules shows that the chitosan/miconazole nitrate microcapsules release about 49.5% of the drug while chitosan/clotrimazole microcapsules release more than 66.1% of the drug after 12 h under a pressure of 5 kg at pH 5.5, which is similar to the pH of human skin. The prepared drug-loaded microcapsules could be applied onto bandages or socks, and will continuously release antifungal drugs in a controlled manner under pressure.

1. Introduction

Fungi cause a wide spectrum of diseases. The most common examples are localized infections of the skin and mucous membranes, such as tinea pedis, vaginal yeast infections and infections of keratinized nails. Pharmacological treatments of these diseases involve the use of topical antifungal agents, such as terbinafine, clotrimazole, miconazole or ciclopirox creams (VandenBossche, Willemsens, Marichal, Cods, & Lauwers, 1983). The treatments are applied onto normal skin about 2 cm beyond the affected area. However, the data have shown that this kind of treatment fails to cure about one-third of the patients with tinea pedis (Bell-Syer et al., 2001) because it does not last long enough. In order to increase the success rate of curing patients with tinea pedis and reduce the chances of relapse, there is a strong need to design and develop a convenient pharmacological treatment and protective measure which will reduce suffering from the disease symptoms.

Microencapsulation provides an effective and long lasting method for the release of antifungal drugs, and has drawn a great amount of attention due to its potential applications in the fields of medicine (Chu, Park, Yamaguchi, & Nakao, 2002; Huang, Sui, Wang, & Jiao, 2010; Luca et al., 2003), biomedicine (Tan, Ren, & Yao, 2011; Wang, Hu, et al., 2011; Wang, Zhang, & Wang, 2011) and environmental engineering (Ngah & Fatinathan, 2008), and as a phase change material (Bayés-García et al., 2010; Wang, Hu, et al., 2011; Wang, Zhang, et al., 2011), coating (Cho, White, & Braun, 2009; Wang, Yu, & Chen, 2010), and catalysis (Parthasarathy & Martin, 1996), etc. The microcapsules are produced by depositing a thin polymer coating onto small solid particles or liquid droplets, or dispersions of solids in liquids. The core content may be released by friction, pressure, change of temperature, diffusion through the polymer wall, dissolution or biodegradation of the polymer wall coating after deliberately tailoring the capsule interior or wall properties (An, Möhwald, & Li, 2006; Cheng, Yuen, Kan, & Cheuk, 2008; Ibarz, Dähne, Donath, & Möhwald, 2001; Khopade & Caruso, 2002; Petrov, Gavryushkin, & Sukhorukov, 2003; Sukhorukov, Antipov, Voigt, Donath, & Möhwald, 2001; Zhu & McShane, 2005). Micro- or nano- particles with liquid cores and solid shells have been previously prepared and widely studied by an oil-in-water emulsion method due to its fast and simple preparation and great potential in applications (Loxley & Vincent, 1998; Park, Shin, & Lee, 2001; Suryanarayana, Chowdoji Raob, & Kumara, 2008). Chitosan is a good shell material for microcapsules due to its good biodegradability, high biocompatibility, effective antibacterial activity, low toxicity, and versatile chemical and physical properties (Hudson & Smith, 1998; Muzzarelli et al., 2012; Ravi Kumar, Muzzarelli, Muzzarelli, Sashiwa, & Domb, 2004). In recent years, drug-loaded chitosan microcapsules, which have great potential in the tableting process and for wound dressings, have been developed (Genta et al., 2003). As two kinds of widely used antifungal drugs, both

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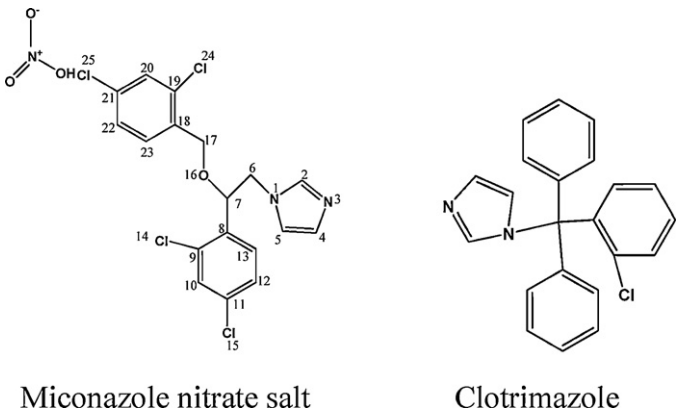

Fig. 1. Structures of miconazole nitrate salt and clotrimazole.

miconazole nitrate and clotrimazole can be used in the treatment of fungus infected diseases, such as oral thrush, ringworm, athlete's foot and so on. The combination of chitosan and antifungal drugs by an encapsulation technique is a good strategy to improve their effectiveness and expand the applications.

In this paper, we would like to develop a type of microcapsule which could be further applied onto bandages or socks and release antifungal drugs in a controlled manner under pressure in order to heal skin disease caused by fungi. Two types of microcapsules, including chitosan/miconazole nitrate and chitosan/clotrimazole microcapsules, are successfully prepared. The microcapsules prepared have a mean particle size of 2.6 μm for the chitosan/miconazole nitrate microcapsules and 4.1 μm for the chitosan/clotrimazole microcapsules. The drug loading and encapsulation efficiency is determined by high-performance liquid chromatography (HPLC). The prepared drug-loaded microcapsules could be applied onto bandages or socks, and will continuously release antifungal drugs in a controlled manner under pressure. Patients with tinea pedis or other infections by fungi can undergo medical treatment by the simple action of wrapping a bandage around the body area or putting on socks.

2. Experimental

2.1. Materials

Jojoba oil (100% pure natural and no additives) was purchased from Easy Creation Asia. The cloth fabric was purchased from Uniqlo, and washed (AATCC Test Method 61-2010). Chitosan from shrimp shells, practical grade with catalog number C3646, miconazole nitrate salt, clotrimazole, acetic acid and all other chemicals were purchased from Sigma–Aldrich Co. Ltd., Hong Kong, and used as received. The structures of miconazole nitrate and clotrimazole are shown in Fig. 1.

2.2. List of instruments

2.2.1. Scanning electron microscopy (SEM) study

The external morphology of the microcapsules was observed with a Nikon FX-35DX microscope and a JEOL JSM 6490 scanning electron microscope. The microcapsules were first diluted with deionized water at a ratio of 1:10 and then dropped onto a glass wafer followed by observation under the Nikon optical microscope.

For observation under the scanning electron microscope, the microcapsules were diluted with deionized water at a ratio of 1:10 and surfactant Tween 60 was added. The microcapsule emulsion was then dropped onto a silicon wafer followed by drying through freeze-drying before measurement.

The particle sizes were determined by optical photos of the wet microcapsules of M-25 and C-25. The size distribution was characterized by numbers in a different range of sizes.

2.2.2. Fourier transform infrared (FTIR) spectroscopy measurement

The infrared (IR) spectra were recorded by a Perkin Elmer Spectrum 100 spectrometer. The microcapsules were vacuum-dried at 50 °C for 24 h. A sample of about 10 mg was mixed with 120 mg of potassium bromide (KBr) and pressed into a pellet under 10 tons of pressure. Then, the IR test was conducted.

2.2.3. High performance liquid chromatography (HPLC) study

Wet microcapsules with an accurate weight were added into 50 mL of methanol (CH_3OH), first stirred for more than 48 h and then sonicated for 20 min in order to dissolve the drugs. The 5 mL samples extracted from 50 mL mixture were further sonicated and filtered before analysis. Analyses were carried out by a Waters Breeze 2 HPLC system equipped with a UV–vis detector Varian Varichrom UV2550 and 2 Agilent Bimodal Columns (6.2 mm × 250 mm × 5 μm) at certain wavelengths (230 nm for chitosan/miconazole nitrate microcapsules and 240 nm for chitosan/clotrimazole microcapsules). The mobile phase was a 75:25 (v/v) mixture of CH_3OH and 0.02 M of dipotassium phosphate (K_2HPO_4). The flow rate was 1.2 mL/min and the injection volume was 20 μL The concentration of drug was recorded by using a calibration curve (0.05–0.5 mg/mL) and the drug loading was calculated.

The drug loading of the wet microcapsules was calculated by using Eqs. (1) and (2).

$$\text{Drug loading of wet microcapsules (\%)} = \left(\frac{\text{weight of drug in wet microcapsules}}{\text{total weight of wet microcapsules}}\right) \times 100 \quad (1)$$

$$\text{Encapsulation efficiency of wet microcapsules (\%)} = \left(\frac{\text{actual loading}}{\text{original drug}}\right) \times 100 \quad (2)$$

As the microcapsules were wet, drug loading was greatly affected by the moisture content. It is difficult to compare drug loading between different batches that have different moisture contents. Hence, we introduced the moisture content to calculate the drug loading for pseudo dry microcapsules.

The prepared microcapsules (5 g) were weighed and entirely dried in an oven at 50 °C for 48 h. The moisture content rate was derived from Eq. (3) and the drug loading of the pseudo dry microcapsules (DLdry) was calculated by using Eq. (4):

$$\text{Moisture content rate (\%)} = \frac{W_w - W_d}{W_w} \times 100 \quad (3)$$

$$\text{DLdry} = \text{Drug loading of wet microcapsules} \times (1 - \text{Moisture content rate}) \quad (4)$$

where W_w is the weight before total dryness and W_d is the weight after total dryness.

3. Results and discussion

3.1. Preparation of microcapsules

Different amounts (25–100 mg) of the antifungal agent (miconazole nitrate salt or clotrimazole) were first dissolved in 5 g of a jojoba oil medium. The mixture of antifungal agent/oil was poured into 50 mL of chitosan solution (1% chitosan in 1% acetic acid

Table 1
Composite of antifungal agent and chitosan during preparation in each sample.

Microcapsule sample name	Chitosan/miconazole nitrate				Chitosan/clotrimazole			
	M-25	M-50	M-75	M-100	C-25	C-50	C-75	C-100
Drug (mg):chitosan (g)	25:0.5	50:0.5	75:0.5	100:0.5	25:0.5	50:0.5	75:0.5	100:0.5

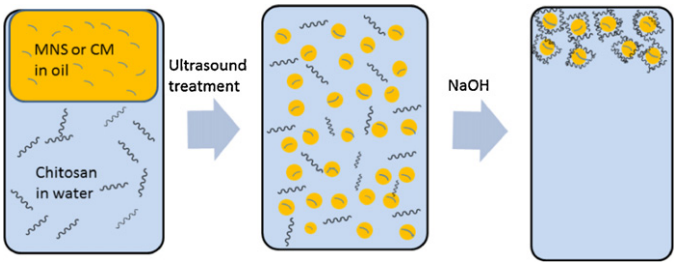

Fig. 2. Schematic diagram that shows the formation of anti-fungal drug loaded chitosan microcapsules (MNS: miconazole nitrate salt; CM: clotrimazole).

solution) followed by intermixing for 10 min. The emulsion was then further mixed by using an ultrasonic processor (Sonics Vibra-Cell™ Ultrasonic Processor VCX 750 with a CV33 probe at 95% amplitude) for 1 min to form an oil-in-water emulsion. A sodium hydroxide solution (1%) was slowly dripped into the fine emulsion for precipitation, and precipitates formed when the pH value reached 7.5. Afterwards, the pH value was further adjusted to pH 10.5 with the sodium hydroxide solution to ensure complete precipitation. The precipitates (i.e. chitosan/miconazole nitrate or chitosan/clotrimazole microcapsules) floated onto the surface of the water after centrifugation. Fig. 2 shows the procedure for the formation of the microcapsules. Then, the wet microcapsules were collected and rinsed with hexane followed by deionized water to remove unwanted materials, including uncapsulated oil and antifungal agents as well as excess chitosan which were deposited into the water medium. The ratio of antifungal agent to chitosan is listed in Table 1.

3.2. Morphology and size distribution of microcapsules

3.2.1. External morphology of microcapsules

Optical microscopy images of the wet microcapsules are shown in Fig. 3. It can be seen that the drug loading does not affect the external morphology of the microcapsules. All the chitosan/miconazole nitrate microcapsules with different drug loadings are similar and the chitosan/clotrimazole microcapsules with different drug loadings are also similar. The chitosan/miconazole nitrate microcapsules are generally smaller than the chitosan/clotrimazole microcapsules.

The SEM images of the wet microcapsules are shown in Fig. 4. Most of the microcapsules have a relatively smooth surface. Some of the capsules have collapsed during the drying process. The particle size of the dry capsules is much smaller than the wet capsules which was observed by optical microscopy. This is because some of the oil in the capsules leaked out of the chitosan shell which led to the shrinking and deformation of the capsules.

3.2.2. Size distribution of microcapsules

The size distribution of the wet microcapsules is shown in Fig. 5, which is a normal distribution. The mean particle size of the chitosan/miconazole nitrate microcapsules is 2.6 μm and that of the chitosan/clotrimazole microcapsules is 4.1 μm. The former is smaller than the latter. This may be attributed to the miconazole nitrate salt which has a negative charge and could work like a surfactant, causing the oil emulsion to be somewhat smaller and finer. Meanwhile, the reason that the chitosan/miconazole nitrate microcapsules are smaller can only be speculated while other factors, including the viscosity of the drug solution in oil and the preparation process, may also have an effect on the particle size of the microcapsules.

3.3. Fourier transform infrared spectroscopy

Fig. 6 shows the IR spectra of chitosan, miconazole nitrate, mixture of miconazole nitrate and chitosan, and chitosan/miconazole nitrate microcapsules (M-25).

For the FT-IR of chitosan, the N-H stretching and bending vibrations in polyamide usually appear at 3100–3500 cm^{-1} and 1550–1640 cm^{-1} respectively, depending on the type of amide (primary or secondary), chemical environment (solid or liquid) and hydrogen bond (intra- or inter-molecular). The C=O stretching

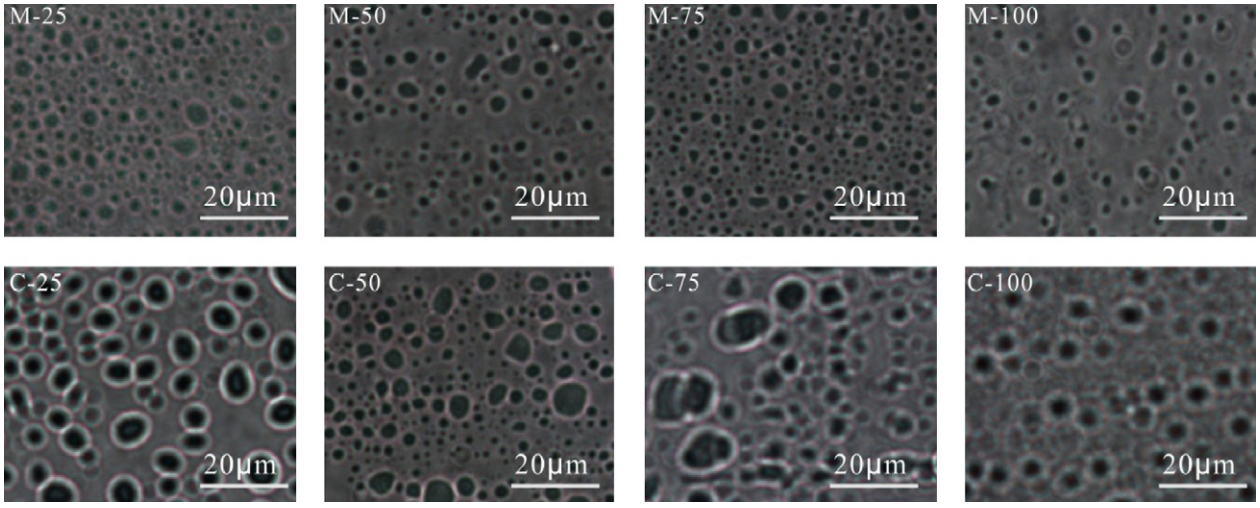

Fig. 3. Optical microscopy images of the chitosan/miconazole nitrate (M-25, M-50, M-75 and M-100) and chitosan/clotrimazole microcapsules (C-25, C-50, C-75 and C-100).

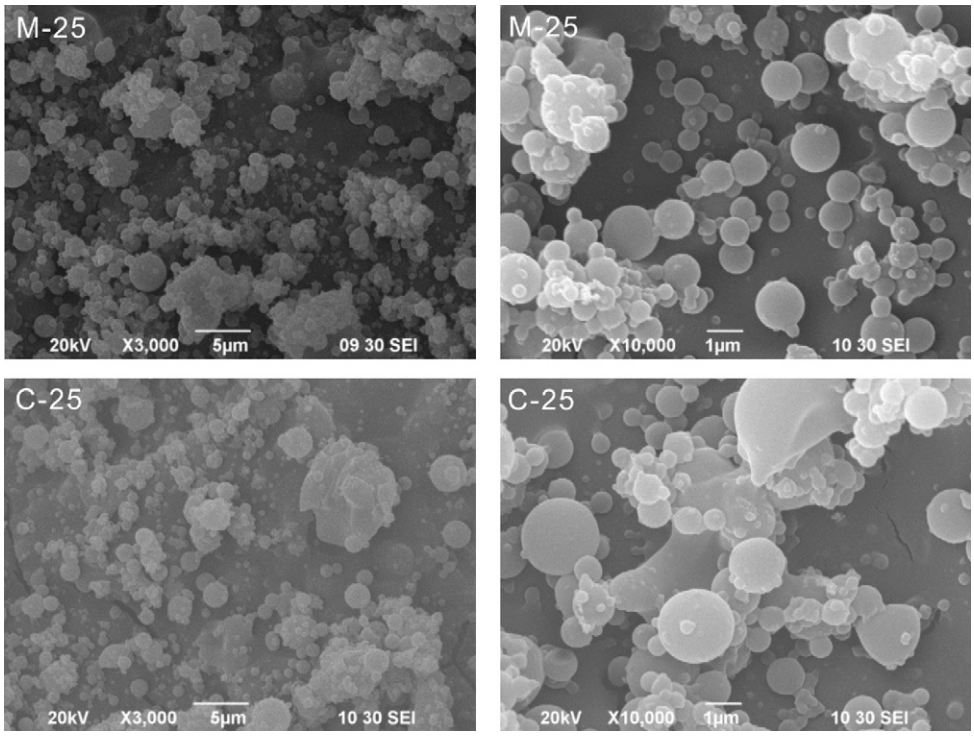

Fig. 4. SEM images of the chitosan/miconazole nitrate (M-25) and chitosan/clotrimazole microcapsules (C-25) at magnifications of 3000× and 10,000×.

vibration band usually appears in the normal region between 1640 and 1670 cm^{-1}.

For pure miconazole nitrate salt, four characteristic bands are observable. Bands 1 and 2 at 1585 cm^{-1} and 1473 cm^{-1} respectively in the spectrum are assigned to the C—C stretching vibration of the two dichlorobenzene groups. Band 3 which is observed at 1385 cm^{-1} corresponds to the C—C stretching vibration of the imidazole group as well as the C—H bending of the imidazole group, the C6 of the aliphatic part of miconazole (Fig. 1). Band 4 at 1327 cm^{-1} corresponds to the C—H bending of the two dichloronezene groups and C—H bending of C6 and C17 (Fig. 1) (Barillaro et al., 2008).

In the FT-IR of the chitosan/miconazole nitrate microcapsules, the peak at 3452 cm^{-1} is increased. The peak at 2344 cm^{-1} in the FT-IR of the chitosan/miconazole nitrate microcapsules responds to the peak at 2336 cm^{-1} in the FT-IR of the miconazole nitrate. We can conclude that the miconazole nitrate has been encapsulated in the microcapsules and there is no obvious covalent bond between the chitosan, oil and the drug.

Fig. 7 shows the IR spectra of chitosan, clotrimazole, mixture of clotrimazole and chitosan, and chitosan/clotrimazole microcapsules (C-25). For the pure clotrimazole, strong bands appear at 751 cm^{-1}, 994 cm^{-1}, 1671 cm^{-1} and 1645 cm^{-1}.

In the FT-IR of the chitosan/clotrimazole microcapsules, the increasing intensities of the bands at 1676 cm^{-1} and 1630 cm^{-1} as well as the bands at 979 cm^{-1} clearly indicate the existence of clotrimazole. We can conclude that clotrimazole has been encapsulated in the microcapsules.

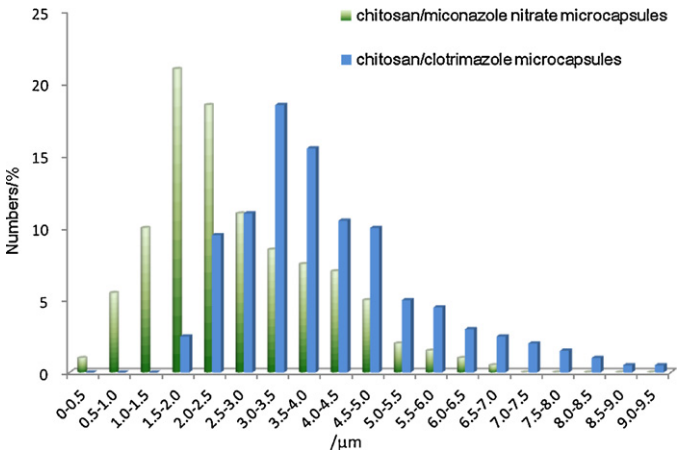

Fig. 5. Particle size distributions of chitosan/miconazole nitrate and chitosan/clotrimazole microcapsules.

3.4. Drug loading and in vitro drug release studies

3.4.1. Drug loading

The drug loading and encapsulation efficiency of the microcapsules is shown in Table 2. The drug could not be totally dried, because the prepared microcapsules would release part of the oil and drug along with the leaking of the oil during the drying process. Moreover, the moisture retention is greatly affected by the centrifuge, washing and filtration processes, so it is impossible to reproduce every time and can only be controlled within a certain range.

We used the drug loading of the pseudo dry microcapsules to compare the results. The drug loading increases and the encapsulation efficiency decreases as the antifungal agents are increased.

We noticed that the encapsulation efficiency of the chitosan/miconazole nitrate microcapsules (77.58–96.81%) is relatively higher than that of the chitosan/clotrimazole microcapsules (56.66–93.82%). This is probably due the structural differences between the miconazole nitrate salt and clotrimazole.

There are mainly two steps in the formation of microcapsules. The first is to emulsify a mixture of jojoba oil, oil soluble antifungal agents, and chitosan solution. The oil phase forms emulsion-droplets under ultrasound treatment. Hydrophobic chains of antifungal agents are inserted in the oil phase while hydrophilic

Table 2
Drug loading, encapsulation efficiency and moisture retention of the microcapsules.

Microcapsule sample name	Chitosan/miconazole nitrate				Chitosan/clotrimazole			
	M-25	M-50	M-75	M-100	C-25	C-50	C-75	C-100
Drug loading (wet microcapsules) (%)	0.2067	0.4012	0.5003	0.5342	0.2215	0.4054	0.4919	0.5517
Total wet microcapsule weight (g)	11.71	11.25	13.25	14.52	10.59	10.30	10.40	10.27
Actual loading (mg)	24.20	45.14	66.31	77.58	23.46	41.76	51.16	56.66
Encapsulation efficiency (%)	96.81	90.27	88.42	77.58	93.82	83.55	68.21	56.66
Moisture retention (%)	60.21	62.85	61.98	63.16	65.07	64.22	67.85	64.60
Total pseudo dry microcapsules weight (g)	4.66	4.18	5.04	5.35	3.70	3.69	3.34	3.64
Drug loading (pseudo dry microcapsules) (%)	0.5195	1.080	1.316	1.450	0.6341	1.133	1.530	1.558

groups of chitosan are immersed in the water phase. In the second step, by adding a sodium hydroxide solution into the chitosan solution, a chitosan layer will precipitate on the surface of the oil droplet and form the microcapsule. The encapsulation efficiency of both drugs is satisfied when the drug loading for pseudo dry microcapsules is above 1.2%.

A possible reason for the higher encapsulation efficiency of chitosan/miconazole nitrate microcapsules is that chitosan could be protonated in acidic conditions while miconazole nitrate salt demonstrates a more negative charge in the solution. The electrostatic attraction between the $-NH_3^+$ of chitosan and negative charge of miconazole nitrate could improve encapsulation efficiency.

3.4.2. In vitro drug release studies

A study of the in vitro drug release of the microcapsules was performed as follows. First, two squares of cloth sized 4 cm × 4 cm were prepared and soaked in a sodium phosphate buffer solution at pH 5.5, 7 or 8.5 (cloth pick up = 75%). Then, 0.6 g of the microcapsules (M-25 and C-25) was placed between the 2 cloth squares. Following that, the cloth-microcapsule-cloth "sandwich" was wrapped with cling wrap, subjected to a perspirometer on which three different pressures were applied (0.028, 1.5 and 5 kg). After 0.25, 0.5, 1, 2, 4, 6, 8, 10 and 12 h intervals, sampling was completed. The microcapsules were removed from the "sandwiches" and the cloth squares were thoroughly washed with CH_3OH. The drug content in the cloth squares was tested and drug release percentage was calculated. The experiment was repeated three times and the average of three values was recorded as the drug release percentage.

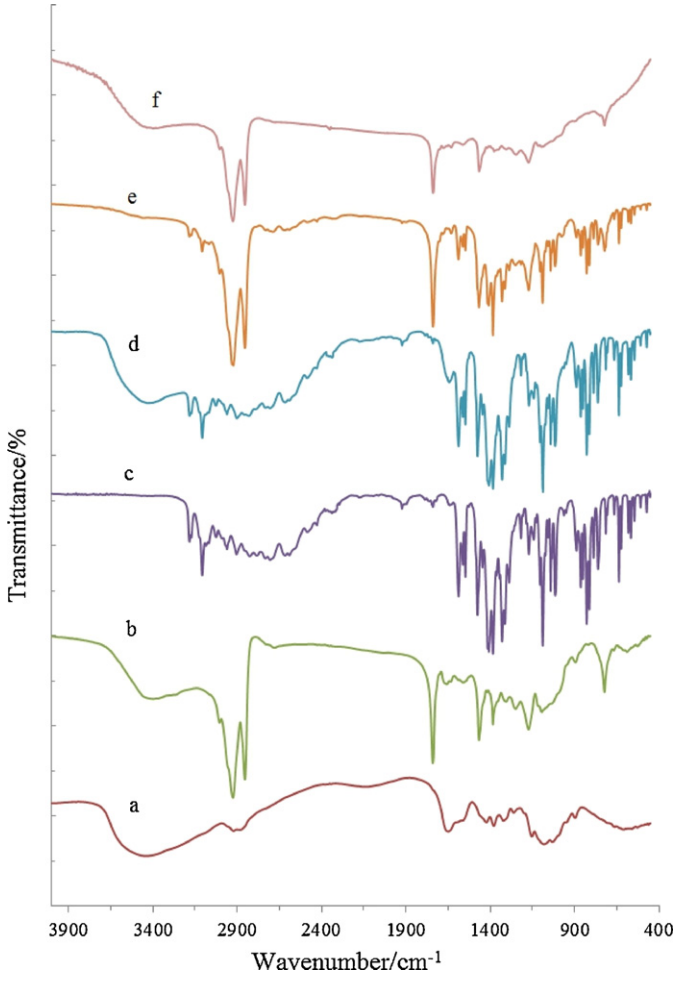

Fig. 6. FTIR spectra of (a) chitosan, (b) oil, (c) miconazole nitrate, (d) miconazole/chitosan mixture, (e) miconazole/oil mixture and (f) chitosan/miconazole nitrate microcapsules.

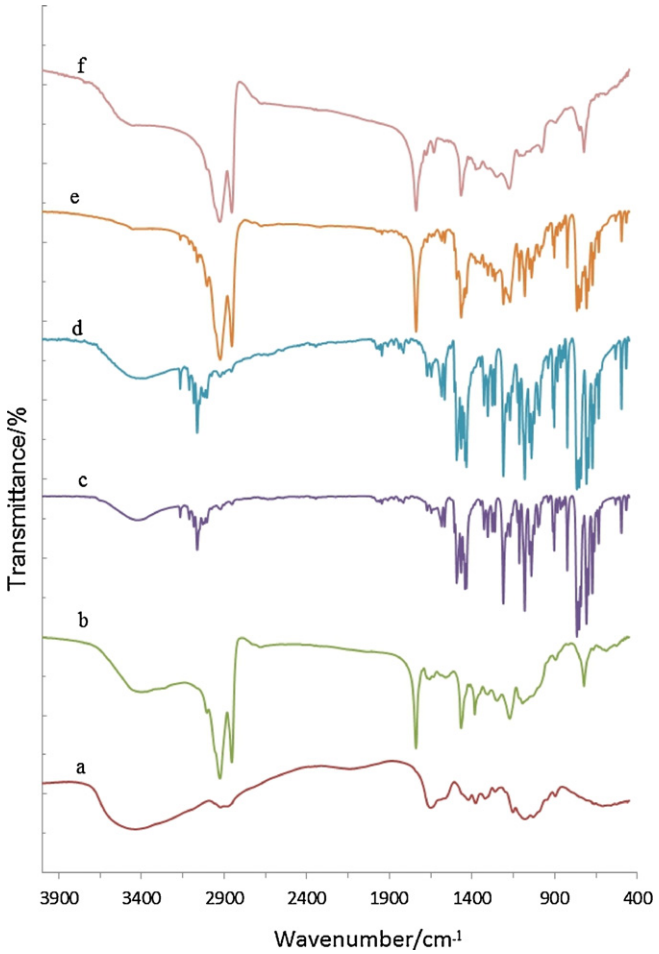

Fig. 7. FTIR spectra of (a) chitosan, (b) oil, (c) clotrimazole, (d) clotrimazole/chitosan mixture, (e) clotrimazole/oil mixture and (f) chitosan/clotrimazole microcapsules.

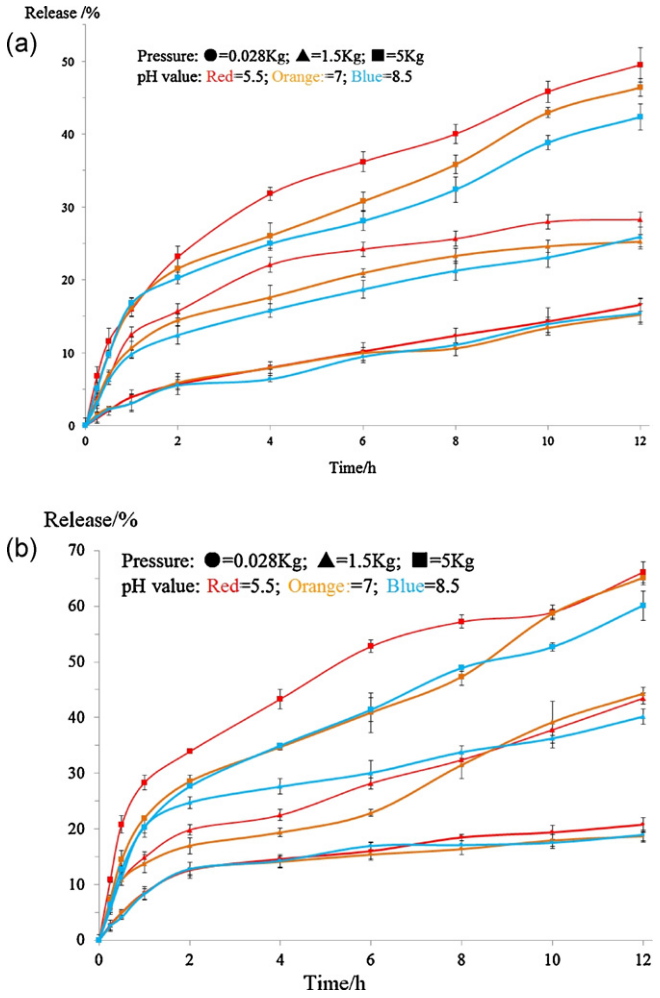

Fig. 8. In vitro drug release percentage of the (a) chitosan/miconazole nitrate (b) chitosan/clotrimazole microcapsules under different pressures at different pHs.

The in vitro drug release performance of the microcapsules is shown in Fig. 8a and b. The result shows that prepared microcapsules could provide a continuous release of drugs for more than 12 h under various pressures and pHs. The chitosan/miconazole nitrate microcapsules release about 49.5% of the drug while the chitosan/clotrimazole microcapsules release about 66.1% of the drug after 12 h under 5 kg of pressure at pH 5.5.

The drug release percentage of microcapsule is mainly controlled by pressure while the effect of pH has a less dominant effect. When the pressure is increased from 0.028 to 5 kg, the release percentage obviously increases, which implies that the microcapsules can be released during normal wear of socks. Moreover, the release rate is only minimally affected with different pH values, which means that the microcapsules prepared by us can be used in different pH value conditions. Therefore, even though the sweat of patients has different pH values, this will not affect the release percentage of the anti-fungal drugs from the microcapsules.

In the drug release process, the release rate is controlled by both the crushing of the microcapsules and the diffusion of the oil in the swelling chitosan. During the first half an hour of the drug release experiment, the microcapsules clasped very quickly and the release rate of the chitosan/clotrimazole microcapsules was 41.58%/h ($R^2 = 0.9994$) and the release rate of the chitosan/miconazole nitrate microcapsules was 23.14%/h ($R^2 = 0.9904$) under 5 kg of pressure at pH 5.5. After 1 h into the drug release experiment, the release rate of the microcapsules became much slower. The release rate of the chitosan/miconazole nitrate microcapsules was 3.31%/h ($R^2 = 0.9547$) and the release rate of the chitosan/miconazole nitrate microcapsules was 2.87%/h ($R^2 = 0.9603$) during the time period of 1–12 h in the release experiment.

The release rate of the chitosan/miconazole nitrate microcapsules is slower than that of the chitosan/clotrimazole microcapsules in the same conditions. This may also be attributed to the electrostatic attraction between the $-NH_3^+$ of chitosan and negative charge of miconazole nitrate.

4. Conclusion

In this paper, two types of microcapsules, including chitosan/miconazole nitrate and chitosan/clotrimazole microcapsules, with different drug loadings, have been successfully prepared by a novel ultrasound-assisted oil/water emulsion method. The most suitable conditions for the preparation of antifungal agent-loaded chitosan microcapsules are studied. It is found that miconazole nitrate-loaded microcapsules have better encapsulation efficiency than clotrimazole-loaded microcapsules in the same conditions. The in vitro drug release performance of the microcapsules shows that the chitosan/miconazole nitrate microcapsules release about 49.5% of the drug while chitosan/clotrimazole microcapsules release more than 66.1% of the drug after 12 h under a pressure of 5 kg at pH 5.5, which is similar to the pH of human skin. The prepared drug-loaded microcapsules could be applied onto bandages or socks, and will continuously release antifungal drugs in a controlled manner under pressure. Patients with tinea pedis or other infections by fungi can undergo medical treatment by the simple action of wrapping a bandage around the body area or putting on socks.

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References

An, Z. H., Möhwald, H., & Li, J. B. (2006). pH controlled permeability of lipid/protein biomimetic microcapsules. *Biomacromolecules*, *7*, 580–585.

Barillaro, V., Dive, G., Ziemons, E., Bertholet, P., Evrard, B., Delattre, L., et al. (2008). A Theoretical and experimental vibrational study of miconazole and its dimers with organic acids: Application to the IR characterization of its inclusion complexes with cyclodextrins. *International Journal of Pharmaceutics*, *350*(1–2), 155–165.

Bayés-García, L., Ventolà, L., Cordobilla, R., Benages, R., Calvet, T., & Cuevas-Diarte, M. A. (2010). Phase Change Materials (PCM) microcapsules with different shell compositions: Preparation, characterization and thermal stability. *Solar Energy Materials & Solar Cells*, *94*, 1235–1240.

Bell-Syer, S. E. M., Hart, R., Crawford, F., Torgerson, D. J., Young, P., Tyrrell, W., et al. (2001). A systematic review of oral treatments for fungal infections of the skin of the feet. *Journal of Dermatological Treatment*, *12*, 69–74.

Cheng, S. Y., Yuen, C. W. M., Kan, C. W., & Cheuk, K. K. L. (2008). Development of cosmetic textile using microencapsulation technology. *Research Journal of Textile and Apparel*, *12*(4), 41–51.

Cho, S. H., White, S. R., & Braun, P. V. (2009). Self-healing polymer coatings. *Advanced Materials*, *21*, 645–649.

Chu, L. H., Park, S. H., Yamaguchi, T., & Nakao, S. (2002). Preparation of micron-sized monodispersed thermoresponsive core–shell microcapsules. *Langmuir*, *18*, 1856–1864.

Genta, I., Perugini, P., Modena, T., Pavanetto, F., Castelli, F., Muzzarelli, R. A. A., et al. (2003). Miconazole-loaded 6-oxychitin–chitosan microcapsules. *Carbohydrate Polymers*, *52*(1), 11–18.

Huang, L., Sui, W., Wang, Y., & Jiao, Q. (2010). Preparation of chitosan/chondroitin sulfate complex microcapsules and application in controlled release of 5-fluorouracil. *Carbohydrate Polymers*, *80*, 168–173.

Hudson, S. M., & Smith, C. (1998). Polysaccharide: Chitin and chitosan: Chemistry and technology of their use as structural materials. In D. L. Kaplan (Ed.), *Biopolymers from renewable resources* (pp. 96–118). New York: Springer-Verlag.

Ibarz, G., Dähne, L., Donath, E., & Möhwald, H. (2001). Smart micro- and nanocontainers for storage, transport, and release. *Advanced Materials*, *13*, 1324–1327.

Khopade, A. J., & Caruso, F. (2002). Stepwise self-assembled poly(amidoamine) dendrimer and poly(styrene sulfonate) microcapsules as sustained delivery vehicles. *Biomacromolecules*, *3*, 1154–1162.

Loxley, A., & Vincent, B. (1998). Preparation of poly(methylmethacrylate) microcapsules with liquid cores. *Journal of Colloid and Interface Science*, *208*, 49–62.

Luca, G., Basta, G., Calafiore, R., Rossi, C., Giovagnoli, S., Esposito, E., et al. (2003). Multifunctional microcapsules for pancreatic islet cell entrapment: Design, preparation and in vitro characterization. *Biomaterials*, *24*, 3101–3114.

Muzzarelli, R. A. A., Boudrant, J., Meyer, D., Manno, N., DeMarchis, M., & Paoletti, M. G. (2012). Current views on fungal chitin/chitosan, human chitinases, food preservation, glucans, pectins and inulin: A tribute to Henri Braconnot, precursor of the carbohydrate polymers science, on the chitin bicentennial. *Carbohydrate Polymers*, *87*, 995–1012.

Ngah, W. S. W., & Fatinathan, S. (2008). Adsorption of Cu(II) ions in aqueous solution using chitosan beads, chitosan–GLA beads and chitosan–alginate beads. *Chemical Engineering Journal*, *143*(1–3), 62–72.

Park, S. J., Shin, Y. S., & Lee, J. R. (2001). Preparation and characterization of microcapsules containing lemon oil. *Journal of Colloid and Interface Science*, *241*, 502–508.

Parthasarathy, R. V., & Martin, C. R. (1996). Enzyme and chemical encapsulation in polymeric microcapsules. *Journal of Applied Polymer Science*, *62*(6), 875–886.

Petrov, A. I., Gavryushkin, A. V., & Sukhorukov, G. B. (2003). Effect of temperature, pH and shell thickness on the rate of Mg^{2+} and O_x^{2-} release from multilayered polyelectrolyte shells deposited onto microcrystals of magnesium oxalate. *Journal of Physical Chemistry B*, *107*, 868–875.

Ravi Kumar, M. N. V., Muzzarelli, R. A. A., Muzzarelli, C., Sashiwa, H., & Domb, A. J. (2004). Chitosan chemistry and pharmaceutical perspectives. *Chemical Reviews*, *104*, 6017–6084.

Sukhorukov, G. B., Antipov, A. A., Voigt, A., Donath, E., & Möhwald, H. (2001). pH-controlled macromolecule encapsulation in and release from polyelectrolyte multilayer capsules. *Macromolecular Rapid Communications*, *22*, 44–46.

Suryanarayana, C., Chowdoji Raob, K., & Kumara, D. (2008). Preparation and characterization of microcapsules containing linseed oil and its use in self-healing coatings. *Progress in Organic Coatings*, *63*, 72–78.

Tan, J. Y., Ren, Y. R., & Yao, S. J. (2011). Preparation of micro-scaled multilayer capsules of poly-dimethyl-diallyl-ammonium chloride and sodium cellulose sulfate by layer-by-layer self-assembly technique. *Carbohydrate Polymers*, *84*, 351–357.

VandenBossche, H., Willemsens, G., Marichal, P., Cods, W., & Lauwers, W. (1983). The molecular basis of the antifungal activities of N-substituted azole derivatives. *Symposia of the British Mycological Society*, *9*, 321–341.

Wang, R., Hu, H., He, X., Liu, W., Li, H., Guo, Q., et al. (2011). Synthesis and characterization of chitosan/urea-formaldehyde shell microcapsules containing dicyclopentadiene. *Journal of Applied Polymer Science*, *121*, 2202–2212.

Wang, H., Yu, L., & Chen, C. (2010). Antibacterial and moisture retention evaluation of cotton fabric treated with chitosan incorporated ceramide microcapsules. *Sen-I Gakkaishi*, *66*(8), 204–208.

Wang, J. P., Zhang, X. X., & Wang, X. C. (2011). Preparation, characterization and permeation kinetics description of calcium alginate macro-capsules containing shape-stabilize phase change materials. *Renewable Energy*, *36*, 2984–2991.

Zhu, H. G., & McShane, M. J. (2005). Loading of hydrophobic materials into polymer particles: Implications for fluorescent nanosensors and drug delivery. *Journal of American Chemistry Society*, *127*, 13448–13449.