Antibacterial and Moisture Retention Evaluation of Cotton Fabric Treated with Chitosan Incorporated Ceramide Microcapsules

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Abstract: This study used chitosan as the main capsule wall material, and sodium tripolyphosphate as a cross-linking reagent, to encapsulate rose hip oil and ceramide. The experiments investigated three formulations with their moisture retention and antibacterial tests (JIS L 1902's). The results showed that the fabric treated (after washing test) with microcapsules incorporated with rose hip oil and ceramide had a moisture retention value of 13.72 %, which is improved 25.92 % as compared to blank chitosan. The same sample also expressed an inhibition zone in the qualitative testing of *Staphylococcus aureus* and *Candida albicans*; the sterilizing value were 4.4 and 4.04, respectively (quantitative test). The experimental results proved that the antibacterial effect was improved, and moisture retention increased by 47 %.

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1. Introduction

Chitosan is a type of polysaccharide, polymerized by glucosamine, derived from deacetylated chitin, which forms amino polysaccharide with positive charges when dissolved in acid flux. It has features of high tension strength, non-toxicity, biodegradability, good biological compatibility, and activity, and does not produce suspended matters [1]. Some studies reported that high concentration chitosan is antibacterial to the stored causative fungi in strawberries [2,3], and in-vitro chitosan can inhibit the growth of fungi better than chitin [3,4]. In addition, when cultivating *mucor racemosus* with addition of chitosans of different concentrations, after 1~2 days, the bacteriostasis effect was related to concentration, pH, and temperature [5,6]. The chitosan has an effect on the cell wall structure of bacteria when it presents acidity [6,7]. The higher the deacetylation degree of chitosan, the more the -NH_3^+ are carried, and thus, the stronger the acting force on the electronegative materials on the surface of bacteria, thereby, the better the bacteriostasis effect. The results also showed that the concentration of microcapsule has notable effect on antibiosis, and the amount of membrane material has no obvious impact on antibacterial effect [8,9]. However, when the concentration of polymer encapsulating on the outer membrane is increased, the molecular weight is reduced,

thus, the ripening time is prolonged, and the compression resistance and stability of microcapsule would be improved positively. It is obvious that different encapsulating technologies have significant effects on the preparation of microcapsules [10]. Since chitosan is able to kill bacteria, socks made of this fiber can inhibit molds from athlete foot, and it is a suitable material for the production of sports wear [8].

The sodium tripolyphosphate is a type of white glossy microparticle powder, its molecular formula is $\text{Na}_5\text{P}_3\text{O}_{10}$, molecular weight is = 376.86, and 1 % tripolyphosphate aqueous solution pH value is = 9.7. It can react with chitosan through positive and negative charges [11]. The free amino group NH_3^+ of chitosan and electronegative tripolyphosphate can create a cross linking effect [12].

The ceramide is a liposome derived from the incorporation of ceramide alcohol, fat, and sugar. It is produced by epidermal cells and stored in stratum granulosum cells, and maintains a balance of water content in the cutin using a complete obstruction system of intercellular lipids. The ceramide synthesis decreases as age increases, and the keratinized cells become larger as a result of aging skin, thus, badly linked skin looks dry and coarse [13].

The rose hip oil contains γ -linoleic acid, unsaturated fatty acid, palmitinic acid, citric acid, and vitamins. The

rose hip oil strengthens tissues as a cosmetics, activates tissues, and it is likely to penetrate into the dermis layer, retain moisture, and make the skin look smooth and fair.

Poly (lactic-*co*-glycolic acid) or PLGA or is a copolymer which is popularly used as shell material owing to its biodegradability and biocompatibility. According to Wu's research [14], microcapsule shell made with PLGA and chitosan can improve the encapsulation ratio and decrease microcapsule sudden leakage problem.

The main purpose of this study is to examine the bacteria repellency, moisture-retention, and washability of cotton fabrics after soaking in water-soluble chitosan, and mixed with liquid encapsulates ceramide oil phase and cross-links tripolyphosphate.

2. Materials and methods

2.1 Materials

The experimental materials were chitosan (deacetylated 95 %, molecular weight 100~200 KDa, Lytone Enterprise, Inc) ; poly (lactide-*co*-glycolide acid) (PLGA) (Toong Yeuan Enterprise Co., Ltd. Taiwan) ; rose hip oil, ceramide (Chung Hsing Industrial Chemicals Company, Taiwan) ; sodium tripolyphosphate (ACROS, U.S.A) ; KBr (Panreac, E.S) ; filter paper (Advantec : 4A/110 mm) ; and water purifier (Synergy Co., SIMPAKOR1). The cultures used in this experiment were purchased from the Culture Collection and Research Center, Food Industry Research and Development Institute (FIRDI), Taiwan, including staphylococcus aureus (ATCC6538P), the culture medium was Tryptic Soy Agar (40 g/L), the culture temperature was 37°C, and Candida albicans (ATCC 10231). The culture medium was Yeast Malt Agar (41 g/L), and the culture temperature was 24°C.

2.2 Production method for microcapsules

In this experiment, sample 1 was water soluble chitosan microsphere, without encapsulating or emulsification, samples 2 and 3 were resulted from 150 mL chitosan mixed liquid (1 g chitosan + 99 mL pure water : 0.4 g PLGA + 99.6 mL pure water), and stirred at 10,000 rpm in an homogenizer (Ultra turrax company, T25) for 30 minutes. The steps are shown in Table 1.

2.3 Encapsulation rate test and SEM morphology analysis

2.3.1 Encapsulation rate test

In encapsulation rate tests, liquid nitrogen was used as the medium for hardening the capsules, then the microcapsule was shattered in a short period ; the

Table 1 Preparation steps of microcapsules.

Sample	Production steps
	Use a spray bottle to spray 5 % tripolyphosphate into
Sample 1	150 mL chitosan mixed liquid for cross-linking, allow to set for three hours, clean with pure water, and filter out microspheres.
	Instill 3 mL rose hip oil into 150 mL chitosan mixed liquid, stir for 60 minutes, place ice cubes outside the beaker in order to cool to 20 °C below, then stir at 6,000 rpm, spray
Sample 2	5 % tripolyphosphate in after emulsification for cross-linking, clean it with pure water three hours later and filter out microspheres.
	Instill oil phase (0.4 g ceramide mixed with 3 mL rose hip oil heated by magnet to 80°C and stirred for 20 minutes) into 150 mL chitosan mixed liquid, stir for 60 minutes, place ice cubes outside the beaker in order to cool to 20 °C below, stir at 6,000 rpm, spray 5 % tripolyphosphate in after emulsification for cross-linking, clean with pure water three hours later and filter out microspheres.
Sample 3	
Remarks column : the aforesaid filtered microspheres are dried by vacuum freeze dryer (temperature -57 °C, vacuum 5 mTorr, 3 days).	

crushed microcapsules were cleaned by ultrapure water and filtered. The formula is :

$$\text{Encapsulation rate \%} = [(D_i - D_a) / D_i] \times 100 \% \quad (1)$$

D_i is the sample weight (g) ; D_a is the weight of crushed capsule (g).

2.3.2 SEM morphology analysis

Three types of microcapsule were observed through a scanning electron microscope (Hitachi, JSM-6700F, Japan) in order to know the morphologies of the microcapsules in different cross linking conditions.

2.4 Cracking temperature and moisture retention

The test range of cracking temperature was 30°C~600°C, the temperature is raised by 20°C per minute, this test would measure the heat resistance of microcapsule using a Thermogravimetric Analyzer (TGA : PERKUN ELMER PYRIS 7). As for the moisture retention, the cross linked microcapsule 0.5 g was placed in an infrared moisture balance (OHAUS, MB45), set in order to reach 110°C within 10 minutes. To find out the degree of moisture retention, the detection and results of simulated moisture retention effects were compared. This test was divided into three groups, such as microcapsule, microcapsule material, and washed microcapsule material (AATCC 61 Colorfastness To Launder). The moisture retention rate was derived from the following equation :

(Wi is the weight (g) before measurement ; Wa is the weight (g) after measurement) :

$$\text{Moisture retention rate \%} = [(W_i - W_a) / W_i] \times 100 \% \quad (2)$$

2.5 Treatment of cotton fabric with microcapsules

The treatment of cotton fabric with microcapsules used Dmdheu resin (4,5-dihydroxy-1,3-bis (hydroxymethyl) imidazolidin-2-one) as binding material, the solid content was 40 %, using $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ (10 % of resin) as a catalyst (without humectants or softener). The mixed binding agent and microcapsule were uniformly mixed, then mixed with pure water into a 10 % solvent, and heated to 40°C ; cotton fabric was placed into the mixture and soaked for 3 minutes, then soaked in a constant temperature vibrating tank at 100 rpm ; finally, gently pressed for 3 minutes under a hot suction and pressure mode (iron temperature 120°C), in order to adhere and dry the microcapsule processing agent and material.

2.6 JIS-L1902-1998

This test used the fiber antibiosis and bacteriostasis test method of JIS L 1902 for qualitative and quantitative testing. When the bacterial growth activity value is > 1.5 , it means the experiment is effective. Three microcapsule samples were pressed into 1.5 cm dia and 0.8 g quantified pancake-shaped substrate by oil pressure for qualitative testing of the inhibition zone. The qualitative test procedure is as follows : place 1 colony (take No.1~4 generations only) in a centrifuge tube containing 1mL liquid culture medium, stir uniformly, and then use a glass rod to uniformly smear the bacteria solution (0.1 mL) on the culture medium, finally, place all test pieces in the center of the culture dish encapsulated with a uniform bacteria solution, and allow to set for 18 ~ 24 hours, and observe the formation of the inhibition zone.

2.6.1 Measurement of bacteriostasis activity value

The number of bacteria washed from the standard unprocessed cloth after 18 hours cultivation was divided by the number of bacteria washed out immediately from the processed cloth. After 18 hours cultivation, the log value was recorded. According to the experimental regulations, the bacteriostasis activity value of inoculation on the processed cloth “log (Mb / Mc)” must be greater than or equal to 2.2, in order for the processed cloth to have bacteriostasis effect.

2.6.2 Measurement of bactericidal activity values

The number of bacterial washed out immediately from the standard unprocessed cloth without cultivation

was divided by the number of bacterial washed out immediately from the processed cloth after 18 hours cultivation, then the log values were recorded. According to the experimental regulations, the bactericidal activity value of inoculation on the process cloth “log (Mb/Mc)” must be greater than or equal to 0. Only this processed cloth has bactericidal action. In this experiment, the bacterial growth activity value = $\log M - \log M_a$, bacteriostasis value = $\log M_b - \log M_c$, sterilizing value = $\log M_a - \log M_c$ [15], M_a = number of bacteria washed out immediately from unprocessed samples after 0 hours, M_b = number of bacteria on unprocessed sample after 18 ~ 24 hours cultivation, and M_c = number of bacteria on processed sample after 18 ~ 24 hours cultivation.

3. Results and discussion

3.1 Encapsulation rate test and SEM morphology analysis

Sample 3 has the best oil phase encapsulation rate from among the three microcapsule samples (Table 2), which is 45 %, and its ceramide encapsulation rate accounts for 5.17 %. Observing the microcapsule morphology by 500x magnification, it is found that the surface of sample 1 is coarse, and presents a porous state (Figure 1). Samples 2 and 3 also have coarse surfaces ; however, their shapes are presented as oval, compared with sample 1. According to the above results, after emulsification of the microcapsule, the cross-linking density becomes tighter with the structure, thus, there are obvious differences in the appearance of the microcapsules. This structure would influence subsequent moisture retention and antibacterial tests.

3.2 Cracking temperature and moisture retention analysis

Regarding water loss of microcapsules, as shown in Table 3, sample 1 has the maximum moisture retention rate. As compared with Figure 1, the microcapsule surface of sample 1 presents multiple pores. Since it has not experienced emulsification, the moisture retention

Table 2 Comparison of encapsulation ratio of rose hip oil and ceramide. (n = 3, Mean $\pm$ S. D.)

Samples	Sample 1	Sample 2	Sample 3
Sample weight g	1.00 $\pm$ 0.01	1.00 $\pm$ 0.01	1.00 $\pm$ 0.02
Weight after spalling g	0.80 $\pm$ 0.02	0.64 $\pm$ 0.04	0.55 $\pm$ 0.02
Oil phase encapsulation rate %		36	45
Ceramide encapsulation rate %			5.17 $\pm$ 0.01

Fig. 1 SEM morphology analysis samples 1, 2, and 3.

rate would be higher than samples 2 and 3 [16]. When sample 2 is added with rose hip oil and emulsified, it can improve the moisture retention effect of the microcapsule by cross-linking the tripolyphosphate, as shown in Table 3. After water wash tests, 22.22 % of sample 2 is lower than the 25.92 % of sample 1. In sample 3, when the microcapsule is encapsulated with rose hip oil, emulsified, and added with ceramide, the moisture retention of material after wash is minimum, which can be proved by the cracking temperature of 35.5°C. As shown in Table 3, the moisture retention effect of material can be improved if the microcapsule is processed by oil phase emulsification and added with ceramide.

3.3 Quantitative test of antibacterial activity of material

Samples 1, 2, and 3 have inhibition zones in identification of *Staphylococcus aureus* and *Candida albicans* [3, 17]. It is found that treated fabric has a sterilizing effect after 2 hours cultivation, and no bacteria exist after 18 hours cultivation. Also, the occurrence of

Table 3 Decomposition temperature and moisture retention analysis. (n = 3, Mean ± S. D.)

Item \ Sample	sample 1	sample 2	sample 3
Cracking temperature °C	266.41	339.7	355.05
Moisture Retention test of microcapsule (%)	11.54 ± 0.01	9.61 ± 0.01	6.00 ± 0.01
Moisture Retention test of material before wash (%)	17.54 ± 0.08	10.90 ± 0.02	5.88 ± 0.07
Moisture Retention test of material after wash (%)	25.92 ± 0.04	22.22 ± 0.01	13.72 ± 0.02

sterilization of *Staphylococcus aureus* is faster than that of *Candida albicans*, thus, treated fabric has better antibacterial activity to *Staphylococcus aureus* than *Candida albicans* (Table 4). In this study, the antibacterial is caused by the cell surface of the bacteria, which is usually negatively charged, and surface adsorption of bacterial cells is increased when the positive charge density of the treated fabric surface increases. When the negatively charged cell surface located at the cell surface of the non-adsorption end moves to the adsorption end, cytomorphosis would occur in the bacterial cell surface due to pressure and potential differences of the adsorption and non-adsorption ends, which slows down cellular metabolism and accelerates bacterial death [6].

4. Conclusions

This experiment used chitosan as a shell material and rose hip oil as an oil phase to encapsulate the ceramide, and cross-links tripolyphosphate for analysis of SEM surface structure types for moisture retention and antibacterial activity. Based on the experimental results, the following conclusions are made :

1. The microcapsule surface is observed by SEM, the result shows that the surface of sample 1 has only a membrane like appearance because it has no

Table 4 Results of antibacterial tests (n = 2, Mean ± S. D.)

Test items	Staphylococcus aureus				Candida albicans			
	Control	Sample 1	Sample 2	Sample 3	Control	Sample 1	Sample 2	Sample 3
Bacteriostasis value	--	4.76	6.06	6.06	--	4.94	5.94	5.94
Sterilizing value	--	3.1	4.4	4.4	--	3.04	4.04	4.04
Remarks:								
1. Test method: JIS L1902-1998 quantitative method.								
2. of staphylococcus aureus: $0.89 \pm 0.1 \times 10^5$; of candida albicans: $0.91 \pm 0.1 \times 10^5$; the concentration of $1.0 \pm 0.3 \times 10^5$ (bacteria number / mL) means the experiment is effective.								

encapsulating or emulsification, and thus, the microcapsule type is incomplete. Although samples 2 and 3 have coarse surfaces, the microcapsule type is complete, this has resulted from microcapsule encapsulating ceramide, rose hip oil, and cross-linking tripolyphosphate.

2. As for moisture retention, the cracking degrees of samples 1, 2, and 3 changing with encapsulating materials, the acceptable temperature is 266.41°C, 339.7°C, and 355.05°C, respectively. The moisture retention effect of the sample 3 microcapsule material is higher than that of sample 1, by 47 %, after fastness by washing test, as it is encapsulated with ceramide after the addition of rose hip oil and emulsifier.
3. It is found that treated fabric has a sterilizing effect after cultivation for 2 hours, and no bacteria exist on the treated fabric after cultivation for 18 hours. Also, the occurrence of sterilization of *Staphylococcus aureus* is faster than *Candida albicans*. Thus, treated fabric has better antibacterial activity for *Staphylococcus aureus* than *Candida albicans*.

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