Study on preparation of chitosan microcapsule

Jae-Don. Cha, Cheon-Il. Lee, Geun-Soo. Lee, Tae-Hun. Kim

HANBUL COSMETICS Co. Ltd., R & D Center, Chung-buk, Korea

Keywords: Chitosan microcapsule, retinol, zeta-potential, stabilization

SUMMARY

Unstable cosmetic active ingredients could be degraded rapidly by chemical and photochemical process. Particularly, some of active ingredients like retinol are known to cause skin irritation when applied on the skin excessively. Therefore, it has become a very important issue to encapsulate cosmetic actives for the stabilization and skin protection.

This study was performed in order to prepare a chitosan microcapsule containing liposoluble cosmetic actives and to investigate the stabilization effect of actives when chitosan microcapsule was applied in cosmetic formulation. Chitosan, deacetylated form of chitin, has been of interest in the industrial applications due to its biocompatibility, biodegradability, non-toxicity, antimicrobial activity and also used as a wall material of capsule. Retinol was used as a core material and was stabilized by a wall of chitosan and antioxidants. The chitosan microcapsule containing retinol(CMR) was prepared by using coacervation method and $W_1/O/W_2$ emulsification techniques. The CMR has 0.5~10.0 ㎛ size distribution and a long-term stability of more than an year inside the cosmetic formulation(O/W). Remaining retinol percentages at 45℃ after 8 weeks in the CMR dispersion were 15.6%(pH 4.0), 59.8%(pH 6.0) and 65.0%(pH 6.0 with antioxidant) respectively. Retinol stability when added CMR inside a O/W emulsion was better than that of O/W emulsion added non-capsulated retinol. As a result, remaining retinol at 45℃ after 8 weeks in O/W emulsion added non-capsulated retinol and O/W emulsion containing CMR was 12.7%, 70.5% respectively.

It appeared that chitosan treated microcapsule may be used for a potential encapsulation method of unstable active ingredients.

INTRODUCTION

Microcapsules are widely used in medical, agricultural and cosmetic field.[1,2] Encapsulation of actives usually targets stabilization of unstable ingredients, control of internal actives release and dispersion of insoluble materials. The currently used microcapsules in cosmetic fields are prepared by a complex coacervation using gelatin/acacia gum/sodium alginate/carboxy methyl cellulose and o/w/o emulsification method using hardly gelling agent like agar.[3,4,5] Gelatin is normally the cationic polymer used. A variety of natural and synthetic anionic water-soluble polymers interact with gelatin to form complex coacervates suitable for encapsulation.

Chitin and chitosan are well-known natural biopolymers that occur in many organisms, such as crustaceans, insects, spiders, fungi and algae. Chitin is one of the most common natural polysaccharides, second abundant natural biopolymer after cellulose. Chitosan is , the deacetylated form of chitin, a biodegradable, biocompatible, non toxic polymer which is composed of acetyl-d-glucosamine and is soluble in various organic acid solutions, but becomes insoluble at pH values >6.5.[6,7,8] At low pH, the chitosan is present as a cationic polymer, with very high charge density, and therefore may function as a good flocculant for negatively charged particles.

In the past, chitosan was used for microencapsulation by spray drying method and complexation the positively charged chitosan with negatively charged polymer, such as carboxy methyl cellulose and sodium alginate.[9,10] In this study, the chitosan microcapsule containing liposoluble actives was prepared by interacting the chitosan with a negatively charged surfactant(SLS, Sodium laurylsulfate) and $W_1/O/W_2$ emulsification method. For cosmetic application of microcapsules, the retinol used as a core ingredient. The kinetics of the degradation of free retinol in chitosan microcapsule dispersion were measured as a function of pH, temperature.

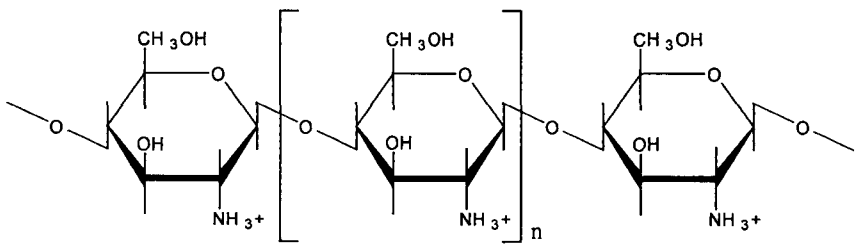

Figure 1. Chemical structures of chitosan in acid solution

Materials and methods

Materials

Retinol 10S(BASF, Germany), Sodium lauryl sulfate(Aldrich, USA), Dimethylmethoxy chromanol (Lipotec, France), Dimechicone Copolyol(N-emulsion, Japan), Squalane, Tocopherol(Kanto chemical, Japan), Chitosan(80.0% deacetylation, AMW 300~400 Kda, Jakwang , Korea).

Measurement of Zeta-Potential

Zeta potential was measured by the Zeta-Sizer 3000HS(Melvern, U.K), after diluting the sample in a buffer solution at pH 4.85

HPLC analysis

An HPLC system equipped with a photodiode-array detector(Waters 996), Waters 2695, was used to analyze vitamin A(Retinol). Chromatographic conditions for retinol were as follows: a reversed-phase HPLC column, Waters symmetry C18, 4.6×250mm; methanol(HPLC grade) as a mobile phase; flow rate, 0.8ml/min; detection at 325nm; column oven temperature at 20℃.

Size and size distribution

Size and its distribution measurements were performed by the Mastersizer 2000 (Melvern, U.K), after diluting in D.W.

Preparation of chitosan capsules containing actives

Chitosan microcapsules containing actives were prepared by a 2-step emulsification method. In the first step, we prepared W/O emulsions; a small quantity of water, in which SLS was dissolved, was dispersed in oil phase that was composed of squalane, retinol and antioxidants. In the second step, the W/O emulsion was emulsified in chitosan solution that was added dimethicon copolyol as a dispersant. Finally, $W_1/O/W_2$ emulsion prepared, and water insoluble membrane was formed by interacting the chitosan with a negatively charged surfactant(SLS) at outer O/W interface. The formulas and preparation method were showed in Figure 2 and 3.

	Components	Contents(w/w%)
Inner water phase (W_1)	Water	0.5 ~ 2.0
	Sodium lauryl sulfate	0.0 ~ 0.12
Oil phase	Squalane	15
	Retinol 10S(BASF)	10
	Ethyl hexyl methoxy Cinnamate	0.5
	Antioxdants	-
Outer water phase (W_2)	Chitosan 1.0% solution(pH 4.0, 6.0)	30~50
	Dimechicone copolyol(KF 6011)	1
	Water	to 100

Figure 2. Formulas for the chitosan microcapsule preparation

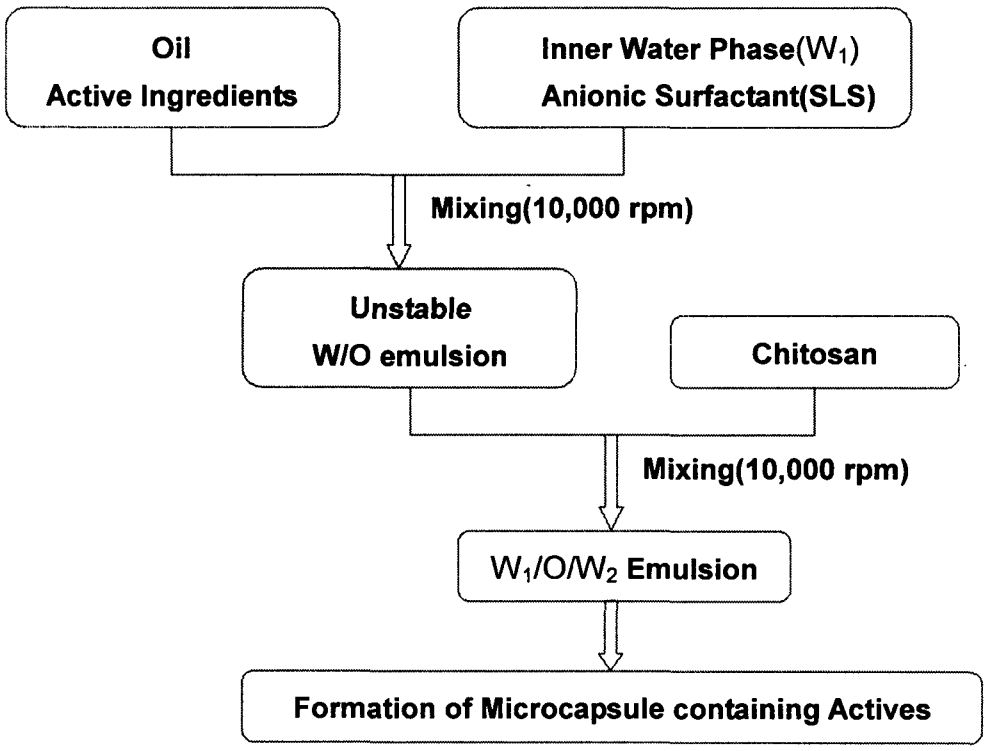

Figure 3. Preparation of microcapsule containing active ingredients.

Results and Discussion

Photomicroscopy and size of chitosan microcapsule

This study was conducted in order to prepare the chitosan microcapsule by interacting the chitosan with a negatively charged surfactant(SLS) and $W_1/O/W_2$ emulsification method. The structure of chitosan microcapsules was easily observed by a light microscope (Figure 4) and was stable at room temperature, without any change in the mean size of the capsules for at least 12 months. Firstly prepared chitosan microcapsule has a multinuclear(W_1) structure($W_1/O/W_2$) but inner water phase(W_1) disappeared with the lapse of time. Finally, chitosan microcapsule looks like oil droplets of a normal O/W emulsion. The size distribution of chitosan microcapsules are between 0.5~10 ㎛.

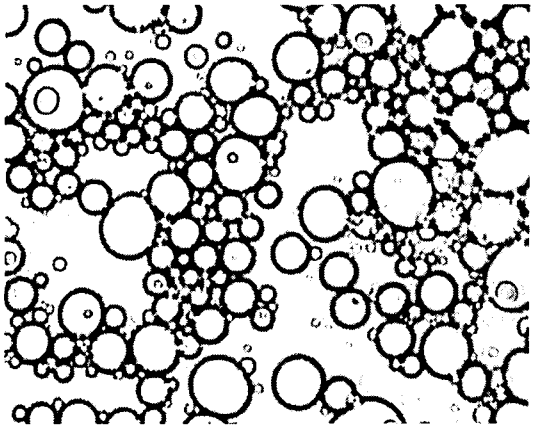

Figure 4. Photomicroscopy of chitosan Microcapsule(X 400)

Zeta potential of chitosan microcapsule dispersion.

In order to determine whether the chitosan is indeed adsorbed onto the oil droplets and not simply increases the emulsion stability by increasing the viscosity of the continuous phase, the zeta potential of systems was measured at various SLS concentrations and pH 4.8 buffer solution. [11] As seen in figure 5, at pH 4.85, a slightly positively-charged emulsion(+1.3mV) is obtained, when no SLS is present, as expected due to the presence of the positively charged chitosan. When adding 0.015%(w/w) SLS, the Zeta potential of droplets become +14mV. At higher SLS concentrations, above 0.03%(w/w), the Zeta potential is about +26mV. These results clearly indicate that the chitosan molecules are absorbed to the oil-water interface by interacting the chitosan with a negatively charged SLS. Oil separation was not observed even after several months at 45℃, in the system was contained 0.4%(w/w) chitosan and more than 0.03%(w/w) SLS.

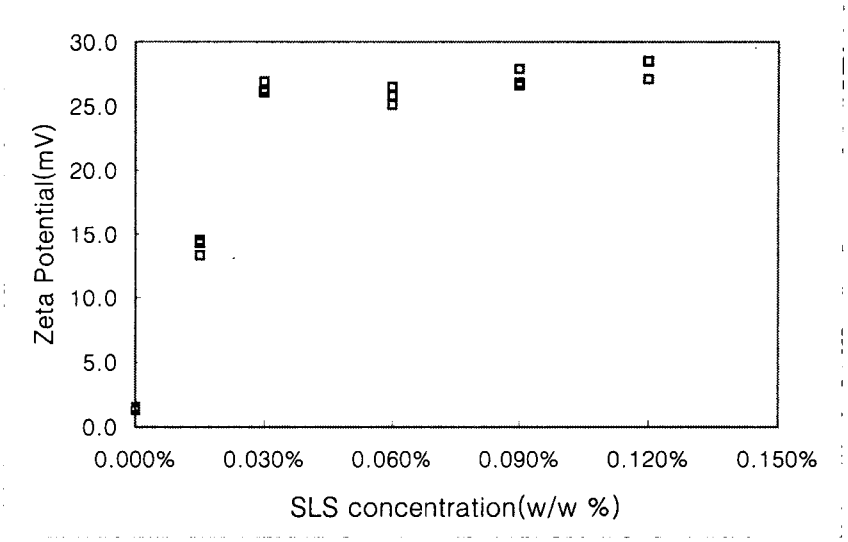

Fig 5. Zeta potential of chitosan microcapsule dispersion having various SLS concentrations, and constant chitosan concentration(0.4%) at pH 4.85

Retinol stability in CMR and CMR added O/W cream

Retinol is the most bioactive form of vitamin A and contains all of the *trans* double bonds in the isoprenoid side chain. Vitamin A is important in a wide variety of biological functions. However, all of the members of this group readily undergo degradative reactions that are characteristic of conjugated double-bond systems. In this study, retinol was incorporated into chitosan microcapsule and the kinetics of retinol degradation investigated. The chitosan microcapsules containing retinol(CMR) were stored different temperatures(20℃, 45℃) and pH values(pH = 4.0 and 6.0). The time course of the retinol degradation[12,13,14] was monitored for 8 weeks. The results are plotted as % of the remaining retinol versus time.(Figure 6.) The remaining percentages of retinol at 45℃ for 8 weeks were 15.6%(pH 4.0) and 59.8%(pH 6.0), respectively. The degradation of retinol in CMR was significantly slower pH 6.0 than pH4.0. These results indicate that the encapsulated retinol is also sensitive to the pH of systems. It is important that chitosan has a good solubility at wide range of pH values. The effect of antioxidants on retinol stability in CMR was investigated with oil-soluble antioxidants at pH 6.0 condition and the results are shown in Figure 7. The addition of tocopherol and dimethylmethoxy chromanol stabilizes retinol effectively. Addition of 0.03% dimethylmethoxy chromanol and 0.1% tocopherol in CMR improved the stability of retinol up to 65%, 62% at 45℃ after 8weeks, respectively. The results are shown Figure 7.

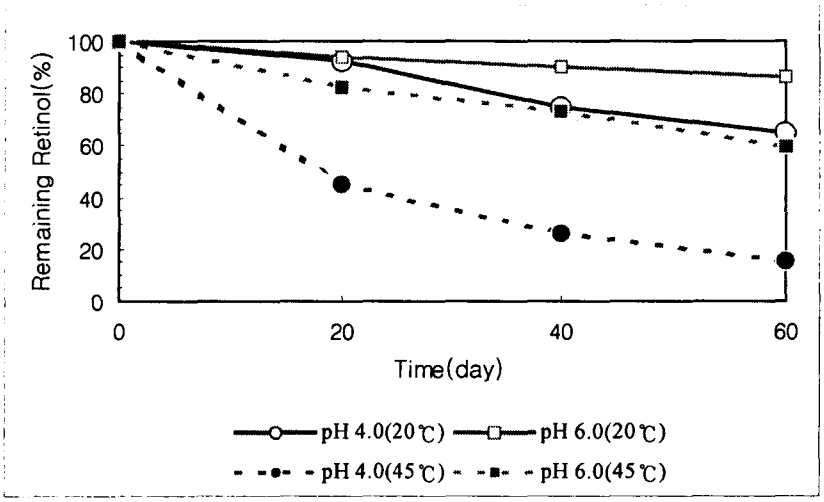

Figure 6. Retinol stability in CMR at various conditions

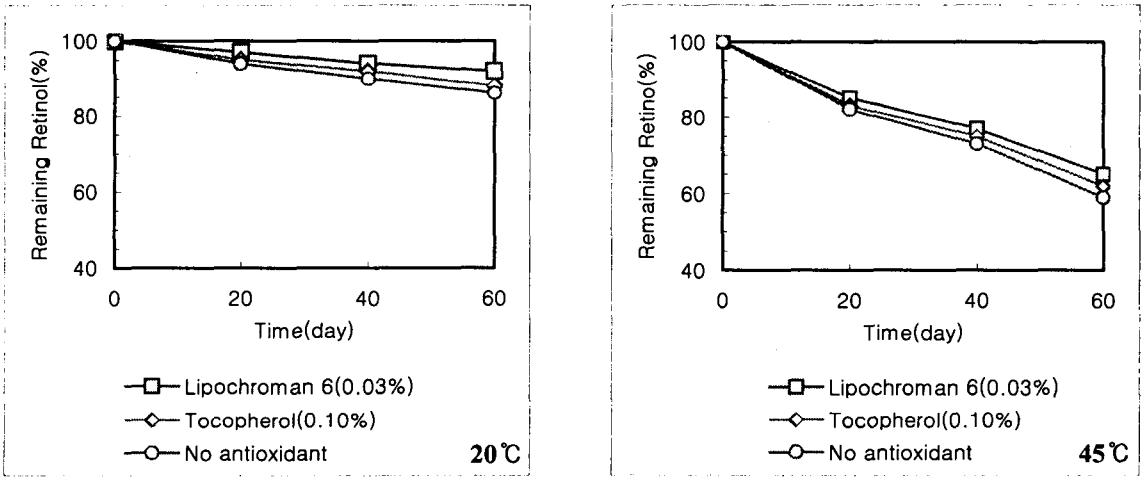

Figure 7. Effect of antioxidants on retinol stability in CMR at pH 6.0

Next, we checked the retinol stability in CMR and pure retinol added O/W cream. The percentages of remaining retinol in each cream are 70.5%, 12.7% , respectively, at 45℃ after 8 weeks. The retinol stability in CMR added O/W cream was better than CMR suspension.

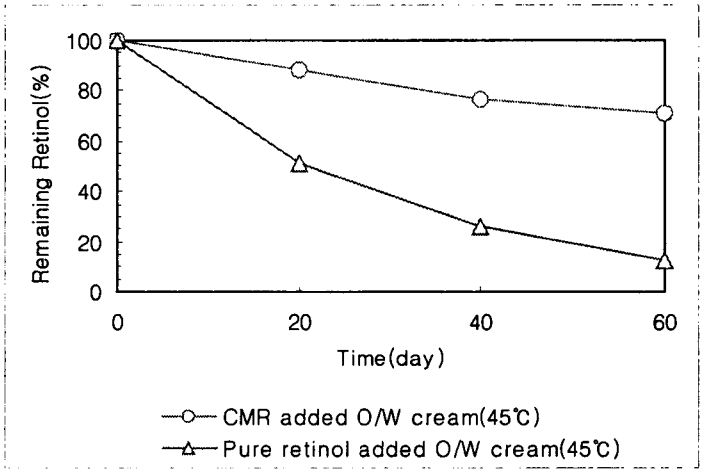

Figure 8. Retinol stability in cream containing chitosan CMR and O/W emulsion added pure retinol(45℃)

These results could be obtained by the second complex coacervation of the positively charged CMR with negatively charged polymer, added as a viscosity increasing agent, in cream. However, the second coacervation decreased the viscosity of the systems.

Conclusion

This study provided the chitosan microcapsule preparation method using the coacervation of the cationic chitosan with anionic SLS and the $W_1/O/W_2$ emulsification techniques. The results of measuring the zeta-potential of chitosan microcapsules showed that the water insoluble membrane was effectively formed to the oil-water interface. The percentages of remaining retinol in CMR added cream are 70.5% at 45℃ after 8 weeks; therefore chitosan treated microcapsule may be used for a potential encapsulation method of unstable active ingredients. The studies on the skin permeation of actives and the second coacervation for more stable capsule wall formation are currently under investigation.

Reference

1. M. Magill, Microencapsulation ofr the personal care industry, *Cosmet. Toiletr.*, **105**(6) 59-62(1990)

2. P. B. Deasy, "Microencapsulation and Relared Drug Processes," in *Drugs and the Pharmaceutical Science*, Volume 20 (Marcel Dekker, New York).

3. J. Rozenblat, S. Magdassi, and N. Garti, Effect of electrolytes, stirring and surfactants in the coacervation and microencapsulation process in presence of gelatin, *J. Microencap.*, **6**, 515-526(1989)

4. Simon Benita "Microencapsulation Methods and Industrial Applications," in *Drugs and the Pharmaceutical Science*, Volume 73 (Marcel Dekker, New York).

5. K. Miyazawa, I. Yajima, I. Kaneda, T. Yanaki, Preparation of a new soft capsule for cosmetics, *J. Cosmet. Sci.*, **51**, 239-252(2000)

6. Gary J. Dee, Oliver Rhode, Rolf Wachter, Chitosan-Multi-Functional Marine Polymer, *Cosmet. Toiletr.*, **116**(2) 39-44(2001)

7. F. Shahidi, J. K. V. Arachchi, Y. J. Jeon, Food applications of chitin and chitosans, *Trends in Food Science & Technology*, **10**, 37-51(1999)

8. Agerkvist, I. Eriksson, B., Selective flocculation with chitosan in E. Coly disintegrates: Effects of ionic strength and multivalent metal ions. *Journal of Dispersion Science and Technology*, **14**, 269-293 (1993)

9. Ping He, Stanley S. Davis, Lisbeth Illum, Chitosan microspheres prepared by spray drying, *International Journal of Pharmaceutics*, **187**, 53-65(1999)

10. Lina Zhang, Yong Jin, Haiqing Liu, Yuming Du, Strcture and control release of Chitosan/Carboxymethyl Cellulose Microcapsules, *Journal of Applied Polymer Science*, Vol **82**, 584-592(2001)

11. S. Magdassi, U. Bach, K. Y. Mumcuoglu, *J. Microencapsulation*, **14**(2), 189-195(1997)

12. S. C. Lee, H. G. Yuk, D. H. Lee, K. E. Lee, Y. I. Hwang, R. D. Ludescher, Stabilization of Retinol through Incorporation into Liposomes, *Journal of Biochemistry and Molecular Biology*, **35**(4) 358-263)

13. T. Tsunoda, K. Takabayashi, Stability of all-trans-retinol in cream, *J. Soc. Cosmet. Chem*, **46**, 191-198(1995)

14. K. Yoshida, T. Sekine, F. Matsuzaki, T. Yanaki, M. Yamaguchi, Stability of Vitamin A in Oil-in-Water-Oil-Type Multiple Emulsions, *JAOCS*, **76**(2), 195-200(1999)