The Journal of the Royal Institute of Thailand
Vol. 37 No. 4 Oct-Dec 2012

Preparation and Assessment of Chitosan Encapsulated Menthol Microcapsules for Leave-on Conditioners

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INTRODUCTION

Encapsulation and controlled release of volatile organic compounds from polysaccharide gels have been applied in cosmetics, pharmaceutical, and food technologies for quite a long time.[1-2] Menthol is a naturally occurring compound obtained from a plant origin of peppermint. It is widely used as a flavoring agent for toothpaste, hygiene products, chewing gum and so on.[3] Menthol is generally available in the form of crystals or granules with a melting point at 41-43°C. However, its high volatility and whisker growth are the very important problems concerning its applications and shelf life. The microencapsulation method is an accountable and appropriate technique to solve the mentioned problems.[4]

Chitosan is a β-(1→4)-linked linear polysaccharide composed of 2-amino-2-deoxy-D-glucopyranose, and 2-acetamido-2-deoxy-D-glucopyranose. Chitosan is a cationic biopolymer derivatized by alkaline N-deacetylation of chitin. Chitosan has been used in many applications because of its biocompatibility, biodegradability, non-toxicity and antibacterial activity.[5] Chitosan can be used to form microcapsules, prevent loss of volatile flavors, and enhance stability of the core flavoring materials.[4] The microcapsules are stable during the release time. Kiatkamjornwong et al.[6] successfully prepared the chitosan microcapsules having 0.5-40 μm in diameter by the conventional stirring method in which chitosan is the shell of microcapsules encapsulating the core material of menthol. The size distribution of the conventional method is rather polydisperse and thus a better technique of porous glass membrane emulsification can remedy the large size distribution to a narrow size distribution of microcapsules.[7-10] In this study, the SPG membrane emulsification method was applied to prepare sodium tripolyphosphate crosslinked chitosan shell encapsulating the core material of menthol. Rheological behaviors of the chitosan microcapsules were studied in order to apply them as an active ingredient for a leave-on hair conditioner. The hair treated with the hair conditioner was characterized and analyzed.

MATERIALS AND METHODS

Materials

Chitosan (Sea Fresh Chitosan Lab Co., Ltd., Bangkok, Thailand) with a degree of deacetylation of 95% and a viscosity-average molecular weight ($\overline{M}_V$) of 100,000 g/mol was used as received. Sodium tripolyphosphate (TPP, Merck, Höhenbrunn, Germany) was used as a crosslinker. Light mineral oil (Hopewell International Co., Ltd., Bangkok, Thailand) was used as an oil phase. Menthol with a molecular weight of 156.27 g/mol (Hong Huat Co., Ltd., Bangkok, Thailand) was used as a core material during encapsulation. Poly(oxyethylene-2-stearyl ether) (Brij 72, Greensville Co., Ltd., Bangkok, Thailand) was used as an oil-soluble surfactant. Sodium dodecyl sulfate (SDS, Merck KGaA, Darmstadt, Germany) and cetyl stearyl alcohol (Kao Co., Ltd., Bangkok, Thailand) were used as a surfactant and a co-surfactant, respectively. Poly(vinyl alcohol) (PVA-220, Kuraray Co., Ltd., Tokyo, Japan) with 87-89% of hydrolysis degree and viscosity of 27.0-34.0 mPa s was used as a stabilizer. For the leave-on conditioner preparation, cetearyl alcohol (Kao Industrial, Bangkok, Thailand), and ceteareth-20 (Honghuat, Bangkok, Thailand) were used as a thickening agent. Dipalmitoylethyl hydroxyethylmonium methosulfate (Shanghai Green Leaf Perfumery Co., Ltd., Shanghai, China) was used as a softener. 1,3-Bis(hydroxymethyl)-5,5-dimethylimidazolidine-2,4-dione or glydant (Honghuat, Bangkok, Thailand) was used as a preservative. OriginPro 8 (OriginLab Corporation, MA, USA) was used as a tool for estimating the flow area of each material: microcapsule emulsion, conditioner base and leave-on conditioner.

Reaction Setup

A miniature kit for emulsification with an SPG module was purchased from Kiyomoto Co., Ltd (Japan). A tubular porous glass membrane with a length of 2 cm and 1 cm in diameter was installed in the module. The dispersed phase (an oil phase) was stored in a Teflon vessel (20 cm^3) which was connected to a nitrogen gas inlet, the continuous phase (an aqueous phase) containing a mixture of SDS and PVA in a 250-cm^3 beaker was stirred at 300 rpm with a magnetic bar to prevent creaming of the droplets. With an optimum pressure of nitrogen gas, the dispersed phase can permeate through the uniform pores of the membrane into the continuous phase to form emulsion droplets. The droplets were then stabilized by the PVA and SDS dissolved in the continuous phase.

Preparation of chitosan microcapsules and leave-on conditioners

The preparation of oil-in-water emulsion of chitosan/menthol microcapsules were carried out via a double emulsion method. As mentioned above, the SPG emulsification was used for preparing the primary emulsion, while the high-speed disperser for the secondary emulsion. The final emulsion was obtained by mixing the primary and secondary emulsions.

Preparation of primary emulsion and secondary emulsion

The SPG membrane was pre-wetted in the aqueous phase. A pressure used was slightly above the critical pressure. The ranges of the permeation pressures from 14.1 to 67.0 kPa for the 5.2 μm membrane pore size were applied to the membrane. Menthol was dissolved in a mixture of light mineral oil, and cetyl stearyl alcohol co-surfactant by heating up to 40-43°C and was used as a dispersed phase. The aqueous phase containing 3.0 g PVA and 1.5 g SDS was dissolved in 200 cm^3 of deionized water. The oil phase permeated through the uniform pores of the SPG membrane by the predetermined pressure of nitrogen gas into the aqueous phase to form the o/w primary emulsion.

The secondary emulsion was prepared via a high-speed disperser. The continuous phase was composed of 50.0 g of deionized water, 0.05 g of sodium dodecyl sulfate, and 10 cm^3 of 1% w/v chitosan solution in acetic acid (1% v/v). The oil phase consisting of 5.0 g of mineral oil, the emulsion was then mixed using a high-speed disperser at 14,000 rpm for 90 s. The emulsion was thereafter stirred continuously at 400 rpm to prevent the coagulation.

Preparation of chitosan microcapsules via a double emulsion method

The primary and secondary emulsions at a ratio of 1 : 1 were mixed and stirred gently at 200 rpm for 1 h. Sodium tripolyphosphate (TPP) crosslinking agent with a concentration of 5% was slowly dropped into the mixture of primary and secondary emulsions. The amino group of chitosan-to-TPP ratio was 2 : 1 and the pH was adjusted to 7. Finally, the chitosan/menthol microcapsules were collected and washed repeatedly thrice with petroleum ether and acetone by centrifugation at 3,000 rpm, and then the microcapsules were freeze dried.

Preparation of leave-on hair conditioner

The mixtures of conditioner base listed as the ingredients shown in Table I were mixed with deionized water at 70-80°C, stirred at 400 rpm. The mixture was cooled to 40°C, the glydant as a preservative and chitosan/menthol microcapsule emulsion were then added, and stirred until a homogeneous emulsion was obtained. The conditioner was left for 1 h for further experiments. OriginPro 8 was used to estimate the area of flow of microcapsule emulsion, conditioner base and the leave-on conditioner in order to observe its size with respect to flow.

Table I Composition of leave-on conditioners

Components	Function	Amount (%)
Chitosan microcapsules	Control microcapsules	released 72.0
Deionized water	media	22.5
Cetearyl alcohol Dipalmitoylethyl hydroxyethylmonium methosulfate Ceteareth-20	Conditioner base	5.0
Glydant	Preservative	0.5

Hair treatment with the leave-on hair conditioner

The virgin Asian hairs (2.5 g) were repeatedly cleaned using a 1.25 cm^3 solution containing 14% w/w solution of sodium dodecyl sulfate at room temperature. Then, 0.025 g of the leave-on conditioners was applied onto 0.5 g cleaned hairs and the hairs were air dried at room temperature prior to further analyses.

Characterization

Flow property of the leave-on conditioners by viscosity

The viscosity of chitosan/menthol emulsion, conditioner base and leave-on conditioner was determined by Brookfield viscometer (model LVDV-III Ultra equipped with a needle-shaped no. 64 spindle, U.S.A.). The chitosan/menthol microcapsules (10 cm^3) was transferred to a small sample holder and covered with a lid to prevent water evaporation during the measurement. For the viscosity measurement, the shear rate was scanned from zero shear (0 s^{-1}) to 25.9 s^{-1} while the sample temperature was controlled at 25°C. For the leave-on conditioner, 25 cm^3 of the conditioner paste was transferred to the sample holder and measured by the same method described above.

Stability of the leave-on conditioner by rheometry

Stability of the leave-on conditioner was determined using a controlled stress rheometer (Mohlin gemini HR nano, Malvern Instruments, Malvern Worchester, England) equipped with the cone-plate size of c40/2Ti, 0.07 mm gap geometry, angle of 2°, and shear rate of 1 s^{-1}. The scans were performed in triplicate cycles at temperatures from 4 to $40^{\circ}C$ at a constant shear stress of 1.0 Pa, and a strain frequency of 1.0 Hertz (6.2832 Radian s^{-1}) in a closed system to prevent water evaporation. At the mentioned shear stress and strain frequency, a linear viscoelastic region was established. Storage and loss moduli at varying times (0 to 650 s) were obtained. A triplicate experiment was carried out.

Hair characterization

Cuticle scale patterns of the virgin hair and treated hair were identified based on the material analysis method of the Scientific Working Group, 2011.[11,12] The scale patterns were imprinted on a nail polish. The hair was put on a glass slide, coated with the paddy cure nail polish, and left to dryness in air. The hair was carefully lifted from the cured nail polish thin film. Hair morphology was examined using an optical microscope (model BH2, Olympus Optical Co., Ltd, Tokyo, Japan).

RESULTS AND DISCUSSION

The primary emulsion of menthol with the average droplet size (d_e) of 19.6±2.3 μm and CV of 11.5% was successfully prepared via the SPG membrane average pore size of 5.2 μm (Figure 1). The detailed experimental data and discussion about the synthesis and characterization of the microcapsules are given elsewhere.[13] Chitosan layer of the microcapsules was crosslinked via ionic bonding between the positively charged amino groups of chitosan and the negative charge of TPP. The size of the final microcapsules was 27.6±7.2 µm with CV of 26.3%. Chitosan/menthol microcapsules emulsion was the important ingredient for a leave-on hair conditioner. It mainly composing of the chitosan/menthol microcapsules and a conditioner base was examined for the rheological behavior.

Flow properties

The flow behavior of the chitosan/menthol emulsion, conditioner base, and leave-on conditioner showed that the shear stress linearly increased with shear rate and vice versa for the chitosan/menthol emulsion, conditioner base and leave-on conditioner (Figure 2). They are all non-Newtonian fluid in that the viscosity of non-Newtonian fluids changes with the rate of shear and do not have a constant viscosity value.[14] The thixotropic property was revealed in the chitosan/menthol emulsion that the emulsion exhibited the pseudoplastic property with yield stress [Figure 2 (a)] when one interpolated the shear stress-shear rate curve to the zero shear rate which is attributed to its three-dimensional network structure caused by TPP crosslinking reaction that resisted to flow.[15] This shear thinning behavior is a very valuable role in determining storage stability and a sensory feature of cosmetic product quality in which chitosan is used as a microcapsule shell.

Similarly, the conditioner base and leave-on conditioner can also be observed as the non-Newtonian materials as shown in Figure 2 (b) to (c). Figure 2 exhibits the relationship of shear rate vs. viscosity in that the viscosity of the microcapsules in the emulsion was influenced by its formulation from which the large molecular sized material produced higher viscosity. The viscosity decreased as the shear rate increased and vice versa. Chitosan/menthol microcapsules exhibited a thixotropic behavior of the non-Newtonian fluid when they were subjected to shear rate on the forward direction and decreasing shear rates on the backward direction. It is observed that the flow behavior is influenced by the structure of the microcapsules and conformation of polymer. The structures of the microcapsules in the emulsion and the conditioner base were elongated under high shear and they rebuilt under Brownian motion behavior. The microcapsules were flown and moved thus the viscosity decreased with decreasing shear stress or increasing shear rate. The reversible property occurred as observed in a hysteresis loop created by the area enclosed by the upward and downward curves of shear stress or viscosity. The area within the hysteresis loop from viscosity vs. shear rate curves can be considered as the thixotropic behavior of fluids. The thixotropic property revealed in the chitosan/menthol microcapsules and conditioner base is the pseudoplastic property.[15] The area within the hysteresis loop of chitosan/menthol microcapsules in Figure 2 (a) and Table II was found much smaller (0.15 Pa) than that of conditioner base (17.11 Pa) in Figure 2 (b). The o/w emulsion of chitosan/menthol microcapsules contains a large amount of water than that of the conditioner base which comprises cetearyl alcohol, dipalmitoylethyl hydroxyethylmonium methosulfate, ceteareth-20, that has the higher paste consistency. The flow curves of the leave-on conditioner exhibited the lower viscosity and larger thixotropic loop which may be caused by the effect of chitosan/menthol emulsion. The formulations of the microcapsules containing a large amount of water tend to produce a fluid-like emulsion system that is prone to deform easily under high shear rates than that of the viscous system of a conditioner base. As such, one can observe a larger hysteresis loop of the conditioner base in both the shear stress vs. shear rate and viscosity vs. shear rate.[16,17] Accordingly, one can observe the much larger hysteresis loops of the leave-on conditioner in Figure 2 (c) having a flow area of 15.54 Pa as shown in Table II. It seems to be caused by the dilution effect of the chitosan/menthol microcapsule emulsion in Figure 2 (a) on the conditioner base in Figure 2 (b). When mixing the microcapsule emulsion with the conditioner base, an immiscible composite of leave-on hair conditioner can be expected. At zero shear, the microcapsules had a spherical shape, when it is subjected to a high shear rate, the microcapsules and other particulate molecules were elongated, deformed and broken when passing through a small tubing or slot. When the shear rate was removed or decreased, the molecules relaxed, reformed and restructured to increase their shear stress or viscosity. With the given time frame (not long enough for relaxation time), the downward hysteresis curve could not fully recover to meet the upward hysteresis curve.[15] Based on the observed thixotropic behavior of the three materials, the leave-on conditioner can fully recover its viscosity when a rest time was long enough. Therefore, before using the leave-on conditioner, one needs to break the thixotropic structure by shaking the bottle slightly to make it flow or become more fluidity.

Table 2 area of the flow curves calculated by OriginPro 8

Type of material	Area for flow curve (Pa)*
Microcapsule emulsion	0.15
Conditioner base	17.11
Leave-on conditioner containing chitosan microcapsules	15.54

*Calculated using OriginPro8

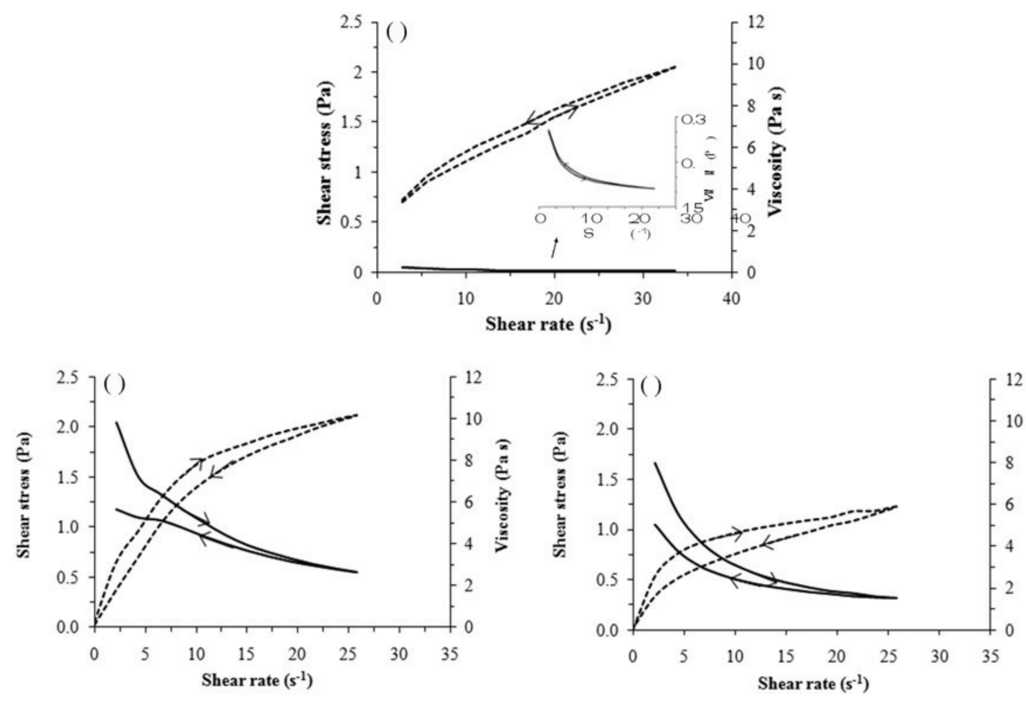

Figure 2

Stability of leave-on conditioner

Storage modulus (G′), loss modulus (G″) and its ratio can be used as an indicator for evaluating the stability of chitosan/menthol microcapsules. Dynamic viscoelastic moduli G′ is always higher than G″ (Pa) as shown in Figure 3a which were monitored as a function of temperature at a time sweep test. When increasing the temperature, the G′ and G″ decreased with the minimal value at 40°C. In contrast, at the low temperature of 4°C, the maximum G′ and G″ were obtained. Likewise, the leave-on conditioner with incorporation of chitosan/menthol microcapsules can resist shear stress because of their dynamic elastic properties as well as structure deformation. A typical increase in the dynamic storage modulus G′ with time is shown in Figure 3a in all the three cycles. The trend of the curves in each cycle is similar during temperature profile. The parameters that quantify the rheological behavior depending on the leave-on conditioner formulation are useful to tailor made of a stable fluid.[18] The stability of leave-on conditioner was contributed by the cetearyl alcohol and dipalmitoylethyl hydroxyethylmonium methosulfate as a thickener pair, which remained unchanged with the addition of the emulsion of chitosan/menthol microcapsules. The loss tangent (tan δ), which is defined to a ratio of G′/G″, as a function of temperature was obtained in Figure 3b. The curves of tan δ beyond room temperature (25°C) revealed the overlapped patterns (lines crossover), and upon increasing temperatures higher than room temperature, the tan δ decreased, i.e., G′ was higher than G″ and more elastic system was obtained besides thermal effect on mobility of molecular chains. In this system, it decreased most at 40°C. Additionally, evaporation of menthol may cause deformation of the microcapsules. Some parts of the polymeric microcapsule wall can force other components out to move or migrate upon applying forces. When the storage modulus G′ increases, the tan δ decreases as governed by the ratio of G′/G″. It can be noted that, the G′ began to decrease rapidly near the temperature of 43°C, the melting point of menthol. The relationship of dynamic viscosity and temperature as shown in Figure 4 gave a unique curve for each cycle. Increasing the temperatures decreased the dynamic viscosity of leave-on conditioner and vice versa. It should be noted that high temperature might accelerate evaporation of the encapsulated menthol in the microcapsules. Deformation of the microcapsule walls occurred and they could be densely combined to become aggregates or agglomerates. The leave-on conditioner having the thixotropic property in the second and third cycles of the test indicated that the viscosity increased with increasing numbers of sweeping cycle as a function of temperature[19] which implied that evaporation of menthol could possibly take place or some kind of a stronger interaction might exist between the conditioner base and the component in the chitosan/menthol emulsion.

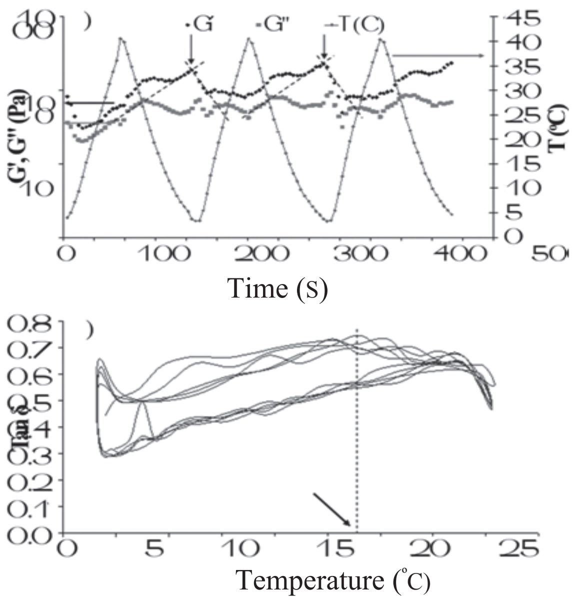

Figure 3

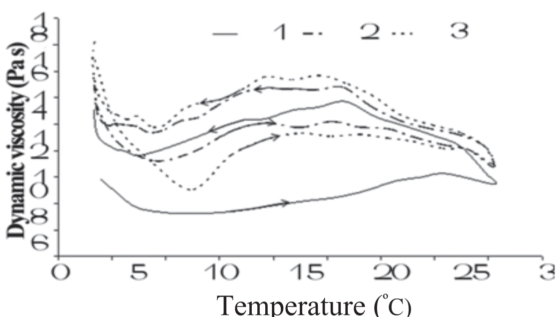

Figure 4

Hair treatment analysis

Optical micrographs in Figure 5 reveal surface morphology of the hair. The hair treated with the leave-on conditioner was smooth compared with the virgin hair. The arrow pointing to the position where the medulla of virgin hair was clearly observed as shown in Figure 5 (a) was compared with the treated hair with the similar angles [Figure 5 (b)]. The scale pattern reveals the hair skin where the cuticle edge of virgin hair is shown in Figure 5 (c). The optical micrograph of the treated hair in Figure 5 (d) shows the substance which stayed near and covered the cuticle edge was probably the conditioner (indicated by the oval line). The conditioner molecules containing cationic surfactants and chitosan molecules provide positively electrical charges to the conditioner.

The negative charge of the hair is attracted to the positively charges conditioner molecules via ionic bonds which allow the conditioner to deposit on the hair. The deposition of the conditioner to the hair results in a reduction of static electricity on the hair surfaces, and consequently increases the hair smoothness. The smooth and soft feels enable easier combing and detangling. The conditioner layer also flattens the cuticle scales against each other, which increases hairs shine, thus the conditioner helps improve the hair quality and appearance.[20]

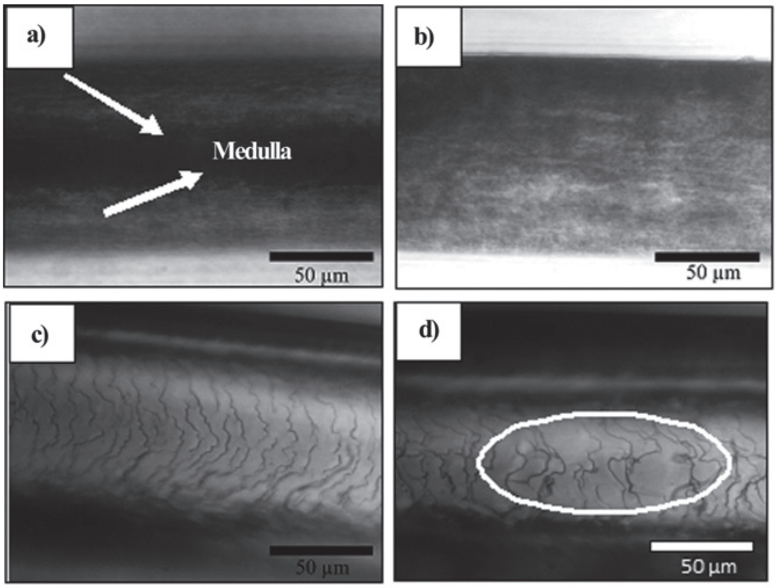

Figure 5

CONCLUSIONS

The flow behavior of chitosan/menthol microcapsule emulsion, conditioner base, and leave-on conditioner is thixotropy in nature. The size of the hysteresis loop was largest in the conditioner base and a bit smaller size than the conditioner base was found in the leave-on conditioner while chitosan/menthol emulsion was the smallest. The stability of the hair conditioner depended on both the rheological behavior of the conditioner base and the emulsion of chitosan/menthol emulsion. The leave-on hair conditioner of chitosan/menthol emulsion base was stable as evaluated by rheological measurement. The hair conditioner rendered hair with softness and easy combing as indicated by the flattened cuticle scales and improved hair shinning.

ACKNOWLEDGEMENTS

The authors thank the Thailand Research Fund for funding the research under the Senior Scholarly Consolidation Grant no. RTA5080004. Many thanks go to the Department of Imaging and Printing Technology, and the Department of Environmental Science, both of the Faculty of Science for providing research facilities.

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Abstract

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The chitosan/menthol emulsion was prepared via Shirasu Porous Glass (SPG) membrane with an average pore size of 5.2 µm to give the average emulsion drop size of 19.6 ± 2.3 µm with a coefficient of variation (CV) of 11.5%. Another set of 1.5% w/w chitosan emulsion was prepared by dispersing at 14,000 rpm for 90 s. The two emulsions were mixed and the tripolyphosphate-crosslinked chitosan gave the microcapsule size of 27.6 ± 7.2 µm with 26.3% CV. A leave-on hair conditioner was prepared and its rheological property was investigated for application on hair. The chitosan microcapsules, the conditioner base and the leave-on conditioner exhibited a pseudoplastic behavior with a thixotropic hysteresis. Tan delta (G''/G') as a function of applied temperature of less than 1 indicated that the conditioner was elastic and stable which could be used up to 40˚C. The hair conditioner exhibited a much larger thixotropic hysteresis for at least three cycles.

Keywords: SPG membrane, emulsion, conditioner base, leave-on conditioner, viscosity