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PREPARATION OF MICROEMULSION OF CHITOSAN AND GREEN TEA
EXTRACT

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ฉัฏฐา ลีปิยะรักษา : การเตรียมไมโครอิมัลชันของไคโตซานและสารสกัดชาเขียว (PREPARATION OF MICROEMULSION OF CHITOSAN AND GREEN TEA EXTRACT) อ. ที่ปรึกษาวิทยานิพนธ์หลัก: ดร. สิริวรรณ พัฒนาฤดี, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม: ศ. ดร. สุดา เกียรติกำจรวงศ์, 57 หน้า.

เตรียมอิมัลชันแบบน้ำในน้ำมันของสารสกัดชาเขียวกระจายใน mineral oil โดยใช้สารลดแรงตึงผิวผสมสองชนิดที่มีสัดส่วนของ Span 80 ต่อ Tween 80 เป็น 9:1 ซึ่งมีค่า HLB = 5.3 ปั่นกวนอิมัลชันนี้ด้วยเครื่องโฮโมจีไนเซอร์ที่ความเร็วรอบ 13,000 รอบต่อนาที เป็นเวลา 5 นาที ได้อิมัลชันที่เสถียร นำอิมัลชันที่เตรียมได้มาผสมกับสารละลายลดแรงตึงผิว Tween 80 และสารละลายไคโตซาน ตามลำดับ สารละลายไคโตซานเตรียมจากไคโตซานน้ำหนักโมเลกุล $1.2 \times 10^5, 3.7 \times 10^5$ หรือ 8.5×10^5 ดอลตัน, deacetylation ร้อยละ 87-88 ที่ความเข้มข้นร้อยละ 1, 2 และ 3 (น้ำหนักต่อปริมาตร) ทำการปั่นกวนเป็นเวลา 60 นาที พบว่า อิมัลชันของไคโตซานมีความหนืดเพิ่มขึ้น เมื่อความเข้มข้นและน้ำหนักโมเลกุลของไคโตซานเพิ่มขึ้น จากการวิเคราะห์ขนาด พบว่า อิมัลชันของไคโตซานมีขนาดอยู่ในช่วง 124-275 นาโนเมตร และศักย์ซีตา (zeta potential) มีค่าอยู่ในช่วง 16-51 mV เมื่อนำอิมัลชันของไคโตซานไปเคลือบเส้นผมพบว่า อิมัลชันของไคโตซานที่ความเข้มข้นร้อยละ 3 (น้ำหนักต่อปริมาตร) และน้ำหนักโมเลกุลที่ 3.7×10^5 ดอลตัน เคลือบบนเส้นผมได้มากกว่าที่ความเข้มข้นร้อยละ 1 และ 2 (น้ำหนักต่อปริมาตร) เมื่อนำเส้นผมหลังเคลือบด้วยไคโตซานไปทดสอบด้วยเครื่องวัดความลื่นและเครื่องหวีผม พบว่า เส้นผมหลังเคลือบด้วยอิมัลชันของไคโตซานให้ความลื่นและหวีง่ายกว่า ในขณะที่เมื่อเพิ่มน้ำหนักโมเลกุลของไคโตซาน

สาขาวิชา ปิโตรเคมีและวิทยาศาสตร์พอลิเมอร์   ลายมือชื่อนิสิต ....

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NUTTA LEEPIYARAKSA: PREPARATION OF MICROEMULSION OF CHITOSAN AND GREEN TEA EXTRACT. ADVISOR: SIRIWAN PHATTANARUDEE, Ph.D., CO-ADVISOR: PROF. SUDA KIATKAMJORNWONG, Ph.D., 57 pp.

The water-in-oil (w/o) emulsion of green tea extract was prepared by dispersing the extract in mineral oil mixed with a mixture of two surfactants at the ratio of Span 80 to Tween 80 of 9:1 having an HLB value of 5.3, using a homogenizer with a stirring speed of 13,000 rpm, homogenizing time of 5 min to form a stable emulsion which was later stabilized by Tween 80. The emulsion of green tea extract was mixed with chitosan solution (MW 1.2×10^5, 3.7×10^5 or 8.5×10^5 Dalton at 87-88% deacetylation) at 1, 2 and 3% w/v. The mixture was mechanically stirred for 60 min. It was found that the viscosity of the chitosan emulsion increased as a function of chitosan concentration and molecular weight. The average size and zeta potential were found in the range of 124-275 nm and 16-51 mV, respectively. The hair surface was coated with chitosan emulsion. It was shown that the emulsion of green tea extract with chitosan at 3% w/v (MW 3.7×10^5 Dalton) could coat hair surface as smoother as those obtained from the concentration of 1% and 2% w/v. The property of hair slip and ease of combing was determined by Texture Analyzer (TA) and Instron, respectively. It was found that 3% w/v (MW 3.7×10^5 Da) chitosan emulsion was the best condition for hair coating which yielded the highest slip and the easiest combing on hair surface. Besides, the higher the molecular weight of chitosan is, the lower slippery, feel, and ease of combing on hair surface.

Field of Study: <u>Petrochemistry and Polymer Science</u>   Student's Signature…………..

Academic Year: 2008   Advisor's Signature…………..

                                                   Co-Advisor's Signature………

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CHAPTER I

INTRODUCTION

1.1 General Introduction

In recent years, the application of green tea extract has expanded greatly in personal care products i.e. face care product, body care product, and hair product. The major composition of green tea is catechin which belongs to the family of flavonoids. Catechin is polyphenolic antioxidant plant metabolites that can help reducing skin irritation from sunlight.

Although hair is lifeless and needs no protection from ultraviolet (UV) radiation because hair shaft carcinogenesis is not feasible; and although damaged hair can be removed and replaced by new growth; hair photoprotection plays a significant role in preserving hair shaft for the sake of cosmetic worth, especially appearance problems.

Much like natural fiber, natural unprocessed human hair undergoes photoyellowing, a chemical process by which wool, cotton, or silk, as well as human hair discolor after sunlight exposure.

Human hair contains three melanin pigments. The first two, eumelanin and pheomelanin, accounts for the brown and red hues found in hair, respectively. The third pigment, oxymelanin, is found after unprocessed hair has been exposed to sunlight. This melanin reduces the cosmetic value of the hair as well as affecting hair dye and perm.

UV radiation also affects hair lipids, making photodamaged hair dull and dry. Hair lipids wrap the hair shaft conferring gloss and manageability, but if they are absent, hair is vulnerable to static electricity, breaks with combing abrasion, and looks frizzy. As regards topical exogenous hair photo-protection, it has not been different from skin photo-protection. However, this topical approach failed in creating an even film that covers the whole surface area of every hair on the head. Moreover, it is impossible because the total surface area of hair on a human head is enormous. As well as hair dyes, there are other hair products that can act as hair photo-protection agents, among which we found shampoos, instant conditioners, deep conditioners, and

hair styling products. Some shampoos intended for dyed hair include sunscreens. They are designed to extend the color of dyed hair. However, UV protection from shampoos is rather challenging since the surfactant should be washed completely before styling.

In this study, green tea extract is prepared in a form of water-in-oil-in-water emulsion. The emulsion of green tea extract is mixed with positively charged biopolymer which is chitosan. The chitosan emulsions are prepared from various molecular weights $1x10^5$, $3x10^5$, and $8x10^5$ Dalton and concentrations. Colloidal properties of emulsion such as particle size, viscosity, zeta potential, and morphology are characterized. The emulsion prepared are also applied on a group of hair and later characterized in terms of slippery feel and ease of combing on hair surfaces.

1.2 Objectives

1.2.1   Prepare the microemulsion of chitosan containing green tea extract**.**

1.2.2   Measure colloidal and physical properties of the emulsion.

1.3 Scope of research

1.3.1   Research and gather related information.

1.3.2   Prepare the emulsion of green tea extract.

1.3.3   Study stability of the emulsion of green tea extract.

1.3.4   Prepare the microemulsion of chitosan containing green tea extract.

1.3.5   Study colloidal and physical properties of emulsion.

1.3.6   Compare hair's healthy appearance and hair's performance between the untreated hair and the chitosan emulsion treated hair.

1.4 Expected benefit

To obtain the microemulsion of chitosan and green tea extract for an application of hair care product.

CHAPTER II

THEORY AND LITERTURE REVIEW

2.1 Green Tea Extract

Tea is the common name for the family Theaceae, comprising a group of mostly woody flowering plants, and for one of its important genera, *Camellia*. Tea is distributed through out tropical and subtropical areas, but most species occur in eastern Asia and South America. Green tea is a type of made solely with the leaves of *Camellia sinensis* that has undergone minimal oxidation during processing. Green tea originates from China and has become associated with many cultures in Asia from Japan. It is a small, shrub-like, evergreen tree that belongs to the family Theaceae and seeds contain a volatile oil and its leaves contain the chemicals caffeine and tannin. Green tea extract is a bioflavonoid rich. It contains a high content of polyphenols as shown in Figure 2.1.
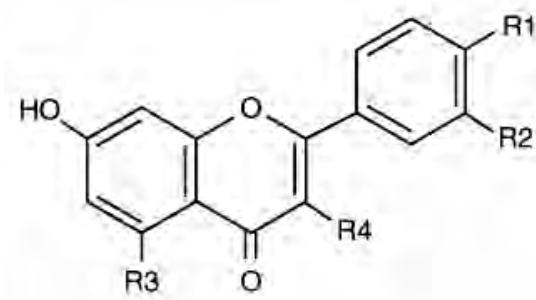
Figure 2.1 Structure of falvonoid.

Tea polyphenols is a natural compound extracted from tea. It is composed of more than 30 substances containing phenolic group, its chemical structure can be sorted into four types: catechin type, flavone and flavonol type, anthocyanidin and procrypsis anthocyanidin type, phenolic acid type and catechin type is the most occupying 60%-80% of total phenols. Green tea is composed of catechin (C), epicatechin (EC), epigallocatechin (EGC), epicatechin gallate (ECG), epigallocatechin gallate (EGCG) and gallocatechin gallate (GCG) as seen in Figure 2.2.
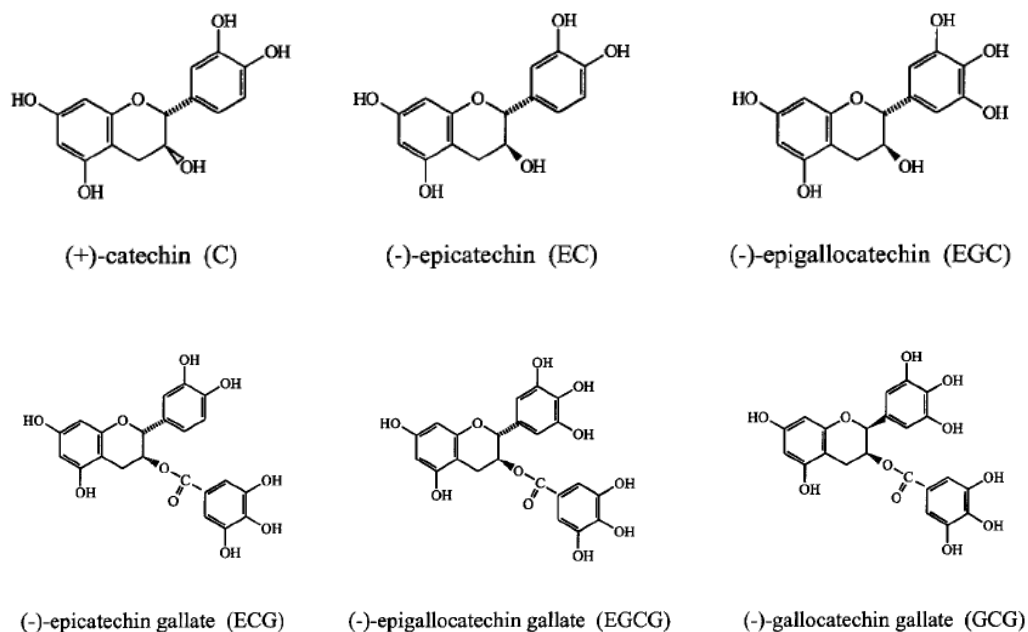
Figure 2.2 Structures of components in green tea.

Green tea is a popular ingredient found in many beauty products including moisturizers, cleanser, bath and shower gels, toothpastes, depilatories, shampoos, leave on, and perfumes. Its inclusion in such products is because of the proposed antioxidant effects of polyphenols found to be naturally occurring in the green tea leaf [1].

Green tea is made from unfermented leaves and reportedly contains the highest concentration of powerful antioxidants called polyphenols. Antioxidants are substances that scavenge free radicals. Free radicals occur naturally in the body that alter cells, but environmental toxins (including ultraviolet rays from the sun, radicals, cigarette smoke, and air pollution). This would exert a proper UV protection property. Recent study [2] found that the green tea polyphenols, especially (-)-epigallocatechin gallate (EGCG), can protect human cellular DNA from UV and visible radiations induced damages, is cultured in human cells as well as in human peripheral blood cells after green tea ingestion. Katiyar et al. [3] studied the relationship between the tea consumption and the various types of cancer, and found that the skin cancer induced by UVR was inhibited better than other types of cancer. Beside UV-protective effect, green tea extract has been extensively studied because of its

powerful anti-carcinogenic, anti-aggregant, anti-allergic, anti-bacterial, anti-mutagenic, and anti-oxidant activities.

2.2 Chitin and Chitosan

Chitin, a linear polymer of β-(1-4)-linked N-acetyl-D-glucosamines is a polysaccharide of major importance, first identified in 1884 (Figure 2.3).

Chitosan is a natural aminocopolysaccharide, similar in structure to cellulose. Chitosan is a copolymer of 2-amino-2-deoxy-β-D-glucan combined with glucosidic linkages. The only difference between chitosan and cellulose is the amino (-NH$_2$) group in the position C-2 of chitosan instead of the hydroxyl (-OH) group found in cellulose. Chitosan is obtained from the deacetylation of chitin, a component of protective cuticles of crustaceans such as crabs, shrimps, prawns, lobsters and insects as well as in cell wall of yeasts and fungi. Acetamide group of chitin can be converted into amino group to give chitosan. Chitosan represents long-chain polymers having molecular mass up to several million Daltons. It has an average molecular weight ranging between 3,800 and 2,000,000 Daltons with 66% to 95% deacetylation.

Chitosan is a cationic polysaccharide in acidic condition. The polymer contains free amino groups and hence, is insoluble in water and organic solvents, however, it is soluble in dilute aqueous acidic solution (pH<6.5). In acidic pH, amino groups can undergo protonation thus, making it soluble. Solubility of chitosan depends upon the distribution of free amino and N-acetyl groups. Usually 1-3% aqueous acetic acid solutions are used to dissolve chitosan. Chitosan is biocompatible with living tissues since it does not cause allergic reactions and rejection. It breaks down slowly to amino sugars, harmless products, which are completely absorbed by the human body [4].
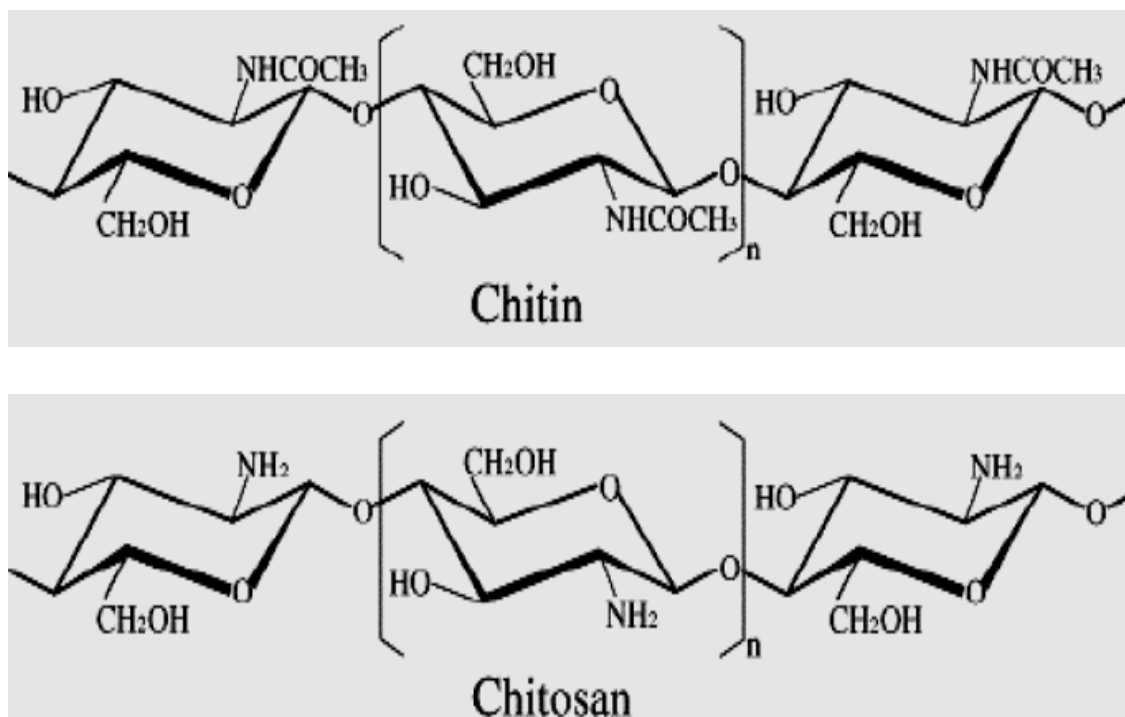
Figure 2.3 Structures of chitin and chitosan [5].

Chitosan is a useful emulsifier that yields stable water-in-oil-in-water (w/o/w) multiple emulsions [6].

Applications of chitosan

The poor solubility of chitin is the major limiting factor in its utilization. Chitosan is considered as a potential polysaccharide because of its free amino groups that contribute polycationic, chelating, and dispersion forming properties along with ready solubility in dilute acetic acid.

Wastewater Treatment

The prime commercial application for chitosan currently is in industrial wastewater treatment since chitosan carries a partial positive charge and binds to metal ions, thus makes the metal ions removal from waste streams or contamination sites easier.

Food Industry

The food processing industry extensively uses polysaccharides in food product development and processing for the purpose of imparting desirable functional properties such as thickening, gelling, emulsifying, and whipping.

Cosmetics

Chitosan has been used extensively in hair care, especially commercial shampoos and conditioners as the main ingredient because of several advantages. Among these advantages, chitosan is physiologically safe as it contains no harmful monomer from any polymerization step. Another one is the ability to form film with protein which is more stable at high humidity, less statically charged during brushing and combing than other traditional hair treated fixatives.

2.3 Colloid

A colloid is a type of chemical mixture in which one substance is dispersed evenly throughout another. The particles of the dispersed substance are only suspended in the mixture, unlike a solution, where they are completely dissolved within a solvent. This occurs because the particles in a colloid are larger than in a solution-small enough to be dispersed evenly and maintain a homogenous appearance, but large enough to scatter light and not dissolve. Because of this dispersion, some colloids have the appearance of solution. A colloidal system consists of two separated phases: a dispersed phase (or internal phase) and a continuous phase (or dispersion medium). A colloidal system may be solid, liquid, or gaseous. Many familiar substances are colloids.

Emulsion

In the classic type of emulsion, the two immiscible liquids involved are water and oil, the latter being either of vegetable or mineral origin. Depending on which is the disperse phase, emulsions of different physical characteristics are obtained. When the water is dispersed in the oil, we obtain a water-in-oil emulsion (w/o) in the opposite case, we obtain an oil-in-water emulsion (o/w). The most striking difference between these two types of emulsion is in their appearance and feel. An o/w emulsion

has a creamy texture, while a w/o emulsion feels greasy [7] as shown in Figure 2.4.
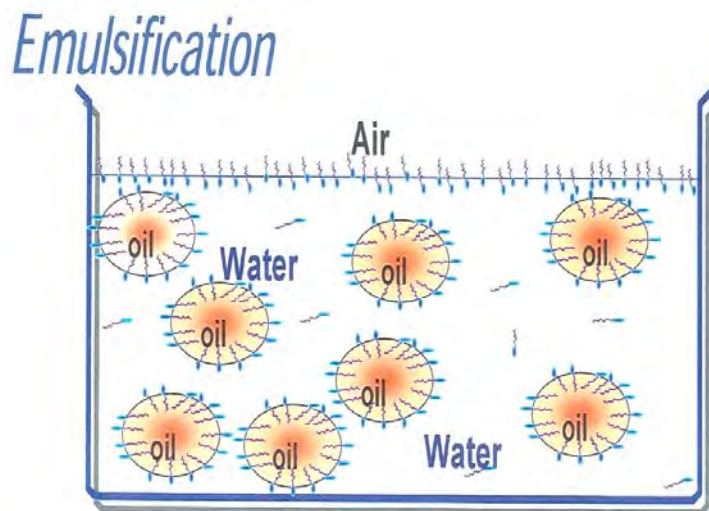
Figure 2.4 Surfactants facilitate formation of an emulsion.

Emulsification is the process by which emulsions are prepared. Emulsion is also a term used in the oil fieldT as untreated well production that consists primarily of crude oil and water. Emulsions tend to have a cloudy appearance, because many interfaces scatter light that passes through the emulsion. Emulsions are unstable and thus do not form spontaneously. Energy input through shaking, stirring, homogenizing, or spraying processes are needed to form an emulsion. Over time, emulsions tend to revert to the stable state of the phases comprising the emulsion. Surface active substances (surfactants) can increase the kinetic stability of emulsions greatly so that, once formed, the emulsion does not change significantly over years of storage.

There are three types of emulsion instability: flocculation, where the particles form clumps; creaming, where the particles concentrate towards the surface (or bottom, depending on the relative density of the two phases) of the mixture while staying separated; and coalescence where the particles coalesce and form a layer of liquid.
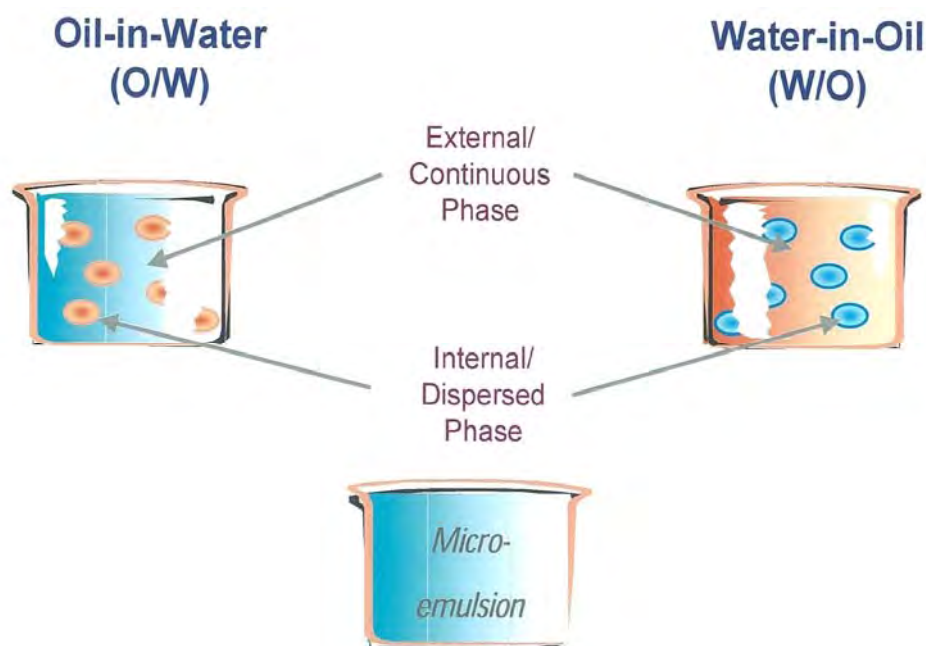
Figure 2.5 Emulsion types.

Multiple emulsions are emulsions having ternary, quaternary, or more complex structures. The simplest multiple emulsions, sometimes called "double emulsions" are in fact ternary systems, having either a water-in-oil-in water or oil-in-water-in-oil structures (Figure 2.5), whereby the dispersed droplets contain smaller droplets of a different phase. Multiple emulsions have a number of potential applications in pharmaceutical, cosmetic, food and separation sciences.

Multiple emulsions are complex systems where both water-in-oil (W/O) and oil-in-water (O/W) emulsion types exist simultaneously. In the case of water-in-oil-in-water multiple emulsions, the oil droplets have smaller water droplets within them, and the oil droplets themselves are dispersed in a continuous water phase, Oil-in-water-in-oil multiple emulsions, on the other hand, consist of tiny oil droplets entrapped within larger water droplets, which in turn are dispersed in a continuous oil phase [8] as seen in Figure 2.6.

Multiple emulsions have been prepared by both one-step emulsification and two-step emulsifications [9]. The most common and better controlled preparation of double emulsions is based on the two-step emulsification process, where a

hydrophobic emulsifier is used for the primary w/o emulsification, and hydrophilic emulsifier is used for preparation of the o/w emulsions [10, 11].
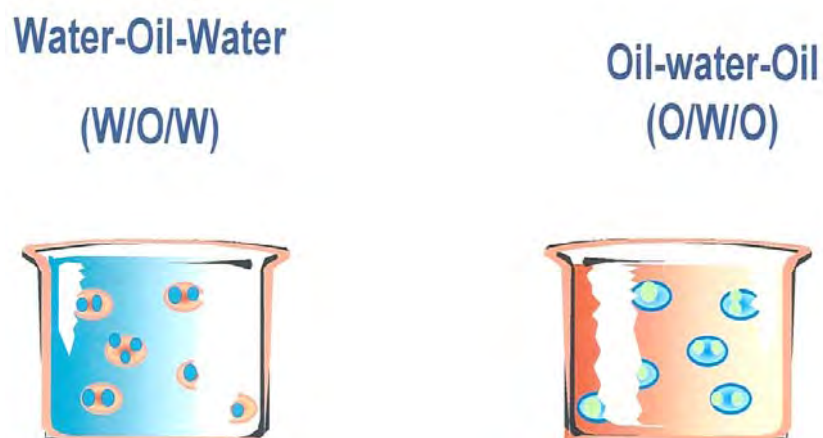
Figure 2.6 Multiple emulsions.

2.6 Encapsulation

Encapsulation is a process by which small particles or droplets are surrounded by a coating to produce encapsulated spheres or capsules. If the small particles are dispersed uniformly within the polymer matrix either at the macroscopic or molecular levels, these are called encapsulated spheres. If the capsules or spheres are in the range smaller than 1 micron, they are called nanocapsules or nanospheres. In the capsules, the material inside the capsule is referred to as the core, internal phase or fill, whereas the wall is sometimes called a shell, coating or membrane. The configuration of the core can be a spherical or irregular particle, liquid-phase suspended solid, solid matrix, dispersed solid and aggregates of solid or liquid forms. The core is the component requiring protection that may be composed of one or more [12]. This technology is used in food and beverages to control the release of active ingredients, protect ingredients from the environment, lower flavor loss during the product shelf-life, extend the flavor perception and mouthfeel over a longer period of time, and enhance the ingredient bioavailability and efficacy [13, 14].

2.5 Human Hair

Human hair is a keratin containing appendage that grows from large cavities or sacs called follicles. The follicle is embedded in the skin and contains the hair root, the part of the hair projecting from the skin is the shaft (Figure 2.7). Hair covers a large percentage of the human body and serves several functions. On the scalp, it provides protective properties, such as protecting against sunburn and acting as a thermal insulator. It also has sensory properties which can assist in the hair's protective action. The hair also fulfils attractiveness and adornment functions. Although we are primarily concerned with scalp hair, on other body areas the hair serves similar and related functions.
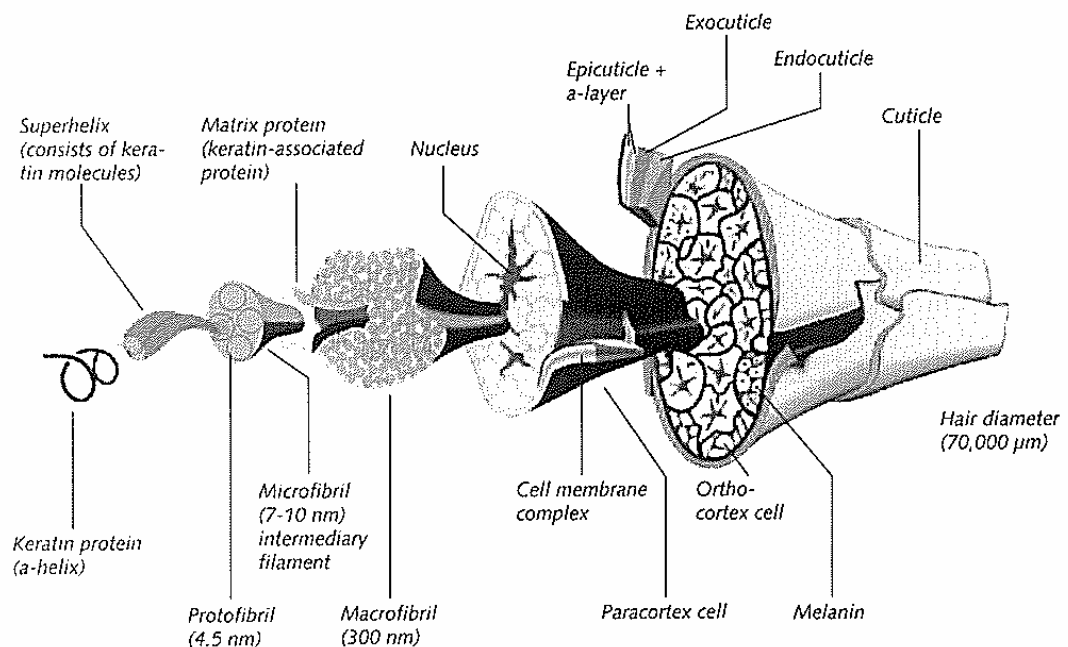
Figure 2.7 Structure of hair

Hair composition

Protein (60-90%): The main component of hair is keratin. This is a complex fibrous protein consisting of over 20 amino acids. In human hair, it can be found at very high levels and it is typically found in a helical arrangement (α-keratin). It is this protein that provides the main mechanical strength of the hair. The protein also forms

a natural protein which may sometimes be removed and this will leave the hair more likely to tangle.

Lipids (up to 9%): Both free and structural lipids can be found in human hair. Some examples of these lipids are fatty acid, ceramides, cholesterol, cholesterol sulphates and fatty alcohols.

Water (up to 30%): Water is a fundamental component of hair and it exists as a continuous three dimensional network inside the α-keratin structure. It is linked to the physical and cosmetic properties such as, dryness (moisturisation). If hair is well moisturised it appears to be more healthy.

Pigment (2%): The pigment present in hair is called melanin and is responsible for the colour and different shades of hair. Two types can be found: Eumelanin -yellow or black and Phaomelanin – yellow or reddish.

Damage to hair structure

Damage to hair is usually measured by looking at the surface condition of the hair fiber. In general, the surface condition of hair varies from head to head, between hair fibers and according to the follicle is susceptible to damage from various sources such as, weathering, chemical and mechanical damage.

Near the root where the hair is recently produced, the cuticle structure is in a perfect condition. A few millimeters from the root, the hair is showing the first indication of 'wear and tear', and the cuticle edges are becoming chipped and jagged. A few centimeters along the fiber, there is even more damage to be seen, the cuticle is now lifting away from the fiber and being lost. Further along the fiber, the cuticle scales have nearly all been removed, exposing the fibrous interior. Finally, the end of the fiber which is the oldest part of the hair, all the cuticle has gone and the fiber has broken apart producing a classical split end.

Weathering

Weathering of the hair encompasses all the damage caused to the hair fiber every day, such as, friction (combing and brushing), wetting, UV radiation, and cosmetic procedures. This damage is usually most prominent near the tip of the fiber. Most of the structural changes which are not as prominent as the spilt ends are only visible microscopically.

Chemical Damage

Chemical damage is caused by treatments such as perming, dyeing and bleaching. During these processes, the cuticle becomes raised and softened, making it more susceptible to cuticle loss during mechanical abrasion and washing. The loss of cuticle can then lead to the formation of split ends [15].

2.6 Literature Reviews

In 2002, Rodriguez et al. [16] studied emulsification of sunflower oil by chitosan solutions with deacetylation degrees (DD) between 75 and 95%. Chitosan solutions that allowed a wide range of viscosity were prepared in 0.2, 1.0, 2.0% concentration in 0.1 M HCl. It was observed that the droplet distribution was unimodal at low DD (75%) and at high DD (95%), and more concentrated emulsion (2.0% chitosan) had good stability.

In 2004, Jiao and Diane [17] studied a long-term stability of water-in-oil-in-water (W/O/W) multiple emulsion with respect to the concentration of Span 83 and Tween 80. It was observed that the optimal surfactant concentrations for w/o/w emulsion long-term stability were 20% w/v Span 83 in the oil phase and 0.1% w/v Tween 80 in the continuous phase. Higher concentrations of Tween 80 had a destructive effect on w/o/w emulsion stability, which correlated with observation that interfacial film strength at the oil/water interface decreased as the Tween 80 concentration increased. High Span 83 concentrations increased the storage modulus G' (Solid like) values and hence enhanced multiple emulsion stability.

In 2004, Porras et al. [18] studied the formulation of water-in-oil nano-emulsion in water/mixed nonionic surfactant/oil system by a concentration method. Several mixtures of Span 20, Span 80, Tween 20 and Tween 80 were studied. It has been proved that mixtures of surfactants can provide better performance than pure surfactants. The emulsions with mean droplet sizes between 30 and 120 nm were obtained with higher size and the higher water quantity. The nano-emulsions prepared presented good stability without phase separation, sedimentation or creaming, during weeks.

In 2004, Fernandez et al. [19] studied emulsion consisting of water, paraffin oil and a mixture of non-ionic surfactant. It appeared that the higher the surfactant

concentration, the smaller the droplets can be obtained. This size was 0.3 μm for 10% w/v surfactant.

In 2006, Wang et al. [20] studied a method to prepare uniform-sized microsphere and solve problems by combining a special membrane emulsification technique and a step-wise crosslinking method. The chitosan/acetic acid solution was pressed through the uniform pore of a porous glass membrane into a paraffin/petroleum ether mixture containing PO-500 emulsifier, to form a W/O emulsion with uniform droplet size. The suitable preparative conditions were determined as follows: pH value of aqueous phase and TPP solution of 3.5-4.0, the molar ratio of aminogroup of chitosan to aldehyde group of glutaraldehyde of 1:1 and the crosslinking time of glutaraldehyde was 60 min.

In 2006, Kim [21] studied green tea extract which was used as a dyeing stock for cotton. The UV protection property of the dyed cotton fabric was examined. Chitosan mordanted green tea dyed cotton showed better dyeing and higher UV protection property compared with the unmordanted green tea dyed cotton. As the chitosan concentration in mordanting increased, the dyeing efficiency and the UV protection property increased. Therefore, adapting chitosan mordanting in green tea dyeing can increased the UV protection property of cotton fabrics to some extent.

In 2007, Klaypradit and Huang [22] studied chitosan encapsulation process of fish oil using an ultrasonic atomizer. Emulsion preparation variables such as concentration of wall materials [chitosan (CS), maltodextrin (MD) and whey protein isolate (WPI) and tuna oil were optimized. At 20g/100g tuna oil, the optimum ratios of CS to MD (maltodextrin) and of CS to WPI were 1:10 and 1:1, respectively. The combination of CS and MD giving the smallest particle size had the highest emulsion stability.

In 2007, Andres et al. [23] analyzed the microstructure, stability, and rheology of model emulsions prepared with distilled water, refined sunflower oil, and different Spans (20, 40, 60, and 80) as emulsifiers. W/o emulsions were formed with percentages of water between 10 and 20%, whereas at higher percentages, w/o/w emulsions were obtained. Sizes of oil droplets in w/o/w emulsions decreased with increasing Span 60 concentrations.

CHAPTER III

EXPERIMENTAL

3.1 Materials

3.1.1 Surfactant

Span 80 (sorbitan monoolate, HLB=4.3) and Tween 80 poly (oxyethylene sorbitan monooleate, HLB=15) were obtained from East Asiatic Public Company, Thailand. The structures of both surfactants are shown in Figures 3.1-3.2, respectively.
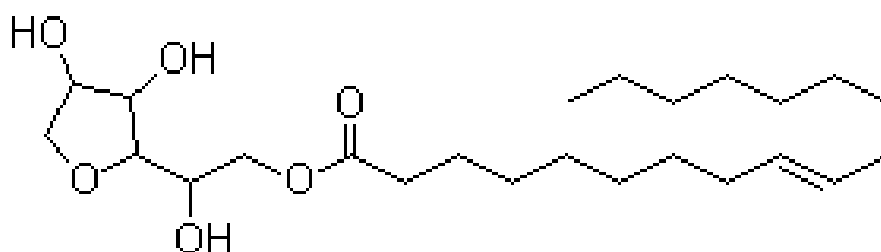

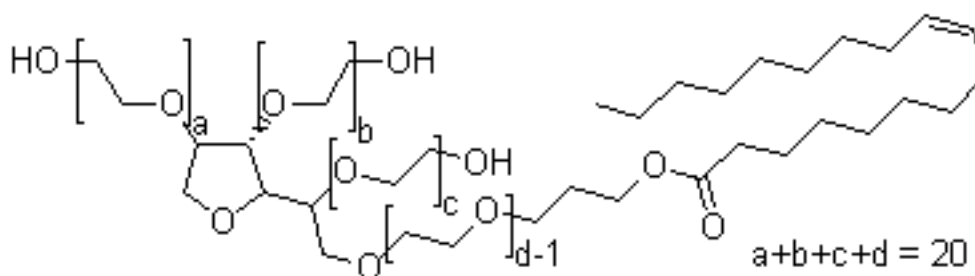**Figure 3.1** Chemical structures of Span 80 [24].

Figure 3.2 Chemical structures of Tween 80 [24].

3.1.2 Chitosan

Poly (beta-(1, 4)-2-amino-2-deoxy-D-glucose), $(C_6H_{11}NO_4)_n$, molecular weights 1.2×10^5, 3.7×10^5, and 8.5×10^5 Dalton, 87-88% degree of deacetylation was obtained from Koyo Chemical Company, Japan.

3.1.3 Green Tea Extract

Green tea extract was obtained from Berli Jucker Specialties Limited, Thailand (solids content, 5.9%).

3.1.4 Other Materials

1. Mineral oil was provided by Hong Huat Company, Thailand.

2. Acetic acid (CH_3COOH) was purchased from Merck, Frankfurt, Germany.

3. Hair switch (Thai female hair, 25-35 year old provided by Unilever Thai Trading)

3.2 Instruments

1. Laser light scattering particle size analyzer, Model Mastersizer 2000, Malvern, Worcestershire, UK.

2. Homogenizer, Model Ultra Turrax T25, IKA Labotechnik, Staufen, Germany.

3. Stirrer, Model Voriomag Poly50, Komet, Göttingen, Germany.

4. Rheometer, Model Haake RheoStress 1, Haake, Karlsruhe, Germany.

5. Light Microscope, Model AxioCam MRc5, ZEISS, Göttingen, Germany.

6. pH meter, Model CG840, SCHOTT, Mainz, Germany.

7. Balance, Model CP224S and LP6200S, Sartorius, Göttingen, Germany.

8. Texture analyser, Model TA.XT Plus, Stable Micro Systems, Surrey, UK.

9. Tensile tester, Model 5564, Instron, Massachusetts, USA.

10. Zetasizer Nano Series, Malvern Instrument, UK.

11. Transmission electron microscopy, JEOL JEM-2100, Japan.

12. Scanning electron microscopy, JSM-5410LV.

3.3 Methods

3.3.1 Water-in-oil emulsion preparation

The water-in-oil emulsion was prepared using 10% w/w green tea extract and 20% w/w water phase and 60% w/w oil phase containing 10% w/w of the surfactant mixture (9% w/w of Span 80 and 1% w/w of Tween 80, $HLB_{mixture} = 5.3$) and stirred at 400 rpm at 75 °C and subsequently homogenized at 13,000 rpm for 1, 3, and 5 min. The samples were cooled at room temperature under moderate stirring at 300 rpm.

3.3.2 Preparation of chitosan solution

The solutions of 1, 2 and 3% w/v chitosan in 1% v/v acetic acid were prepared.

3.3.3 Preparation of chitosan emulsion

The w/o emulsion prepared from 3.3.1 was re-emulsified in Tween 80 solutions at 1, 3, 6, and 10% w/v. Then, the emulsion was poured into the chitosan solution and stirred at 350 rpm for 60 min.

3.3.4 Particle size and zeta potential measurements

The average particle sizes of the particles were measured by dynamic light scattering (DLS), and zeta potential was obtained with zetasizer nano series instrument (Malvern Instrument, United Kingdom). The same sample was repeatedly, measured for 3 replicates.

3.3.5 Scanning electron microscopy

SEM photographs were taken using a scanning microscopy (JEOL JSM-6400, Japan). The sample was placed on a metal stub and coated with gold. For each sample, the micrographs were randomly taken from 5 different areas.

3.3.6 Transmission electron microscopy

The shape and surface morphology of the particles were investigated by transmission electron microscopy (JEOL JEM-2100, Japan). The sample was stained with a small drop of 2% phosphotungstic acid before the microscopic analysis.

3.3.7 Rheological Property Measurement

Rheotogical measurement of the emulsions was performed using Rheometer at $25°$ C. Shear stress was varied from 0.01-400 Pa.

3.3.8 Measurement of hair smoothness by texture analysis

Hair cleaning: 5 switches are washed twice by 2.5 gram of 14% sodium lauryl ether sulphate (SLES), agitated for 30 seconds, followed by rinsing for 30 seconds

under tap water. After cleaning, 0.15 ml of chitosan emulsion was applied onto a damp switch using a syringe and manually worked through from root to tip. This technique is used for measuring the friction force of hair surface that is directly related to the smoothness or slippery feel.

3.3.9 Measurement of hair detangling and ease of combing by Instron combing

Hair cleaning: 5 switches are washed twice by 2.5 gram of 14%SLES, agitated for 30 seconds, followed by rinsing for 30 seconds under tap water. After cleaning, 0.15 ml of chitosan emulsion was applied onto a damp switch using a syringe and manually worked through from root to tip. The ease of combing can be measured by monitoring the frictional forces which result as the hair passes through the comb. These combing forces are measured as a function of the distance traveled along the tress. Parameters such as the maximum combing force, the average combing force and/or the combing can then be used to evaluate performance.

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Stability of the water-in-oil emulsion

The water-in-oil emulsion was prepared using 10% w/w green tea extract and 20% w/w water phase in 60% w/w oil phase having 10% w/w of the surfactant mixture (by mixing 9% w/w of Span 80 and 1% w/w of Tween 80 to give an HLB value of 5.3) and stirred at 400 rpm at 75°C and subsequently homogenized at 13,000 rpm for 1, 3, and 5 min.

The homogenization for 5 min gives a stable emulsion, i.e., no phase separation. When the homogenization time was less than 5 min, the emulsion was separated into two phases (the water phase and oil phase). Figure 4.1 shows that Sample A and B are less stable and phase separation began in less than 2 weeks at room temperature but Sample C is a stable emulsion. Figure 4.2 also shows the appearance of the emulsion prepared with and without the surfactants (Span 80 and Tween 80) prepared at 5 min homogenization time. Without the surfactants, the emulsion cannot maintain stability as seen as formation of large droplet agglomerates widely spread in the emulsion in Figure 4.3 (A). The minute-sized droplets in an average size of 97 nm are obtained when the mixed surfactant is added as shown in Figure 4.3 (B). Since the surfactant molecules can stabilize the minute-sized droplets, therefore, the emulsion is then stable when Span 80 and Tween 80 mixture is added [18]. The size of droplets in the water-in-oil emulsion depends on the homogenization time in which the optimum time was obtained at 5 min at HLB 5.3.
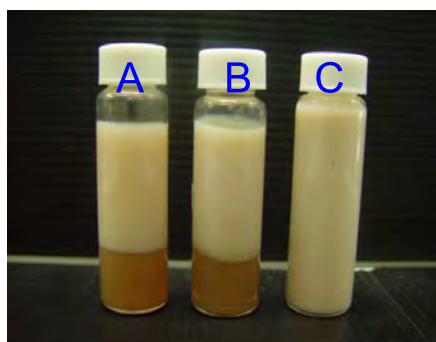

Figure 4.1 Photomicrographs of the water-in-oil emulsions prepared with different homogenization times at (A) 1 min, (B) 3 min and (C) 5 min.
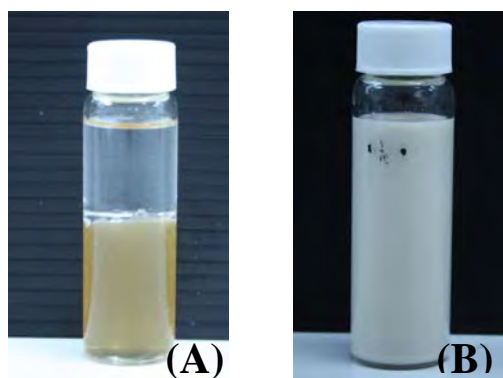
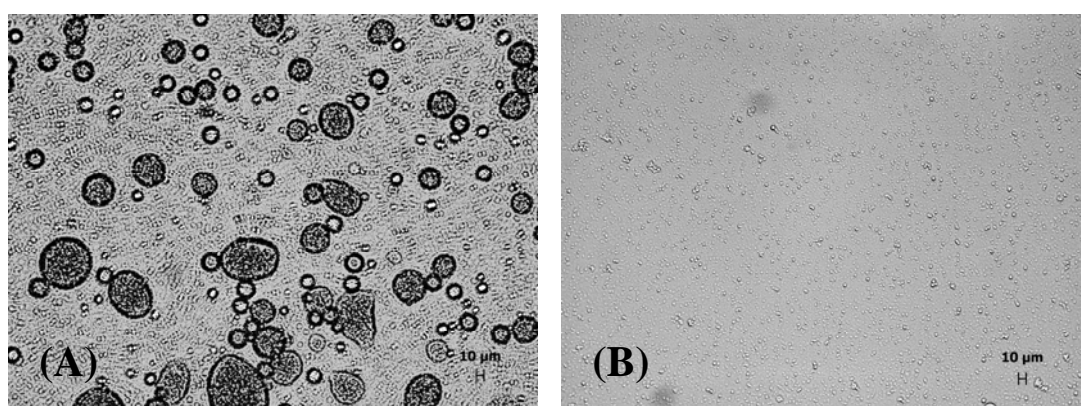**Figure 4.2** Photomicrographs of the emulsions (A) without the surfactant, and (B) with the surfactant.

Figure 4.3 Photomicrographs of the droplet size of emulsion (A) without the surfactant, and emulsion (B) with surfactant.

4.2 Effect of surfactant concentration on stability of chitosan emulsions

The effect of the surfactant (Tween 80) concentration on chitosan emulsions is studied at 1, 3, 6 and 10% w/w. At low concentration (1% w/w), a phase separation occurs within a short period of time (1 day) as shown in Figure 4.4 (A). When the concentration was increased to 10% w/w, the smaller chitosan emulsion droplets are obtained with good stability (Figure 4.4 (D)). The average droplet size is ranged from 0.9-0.1 μm when the surfactant concentration is varied from 1-10% w/w (Figure 4.5). Therefore, the stability of the emulsion increases as Tween 80 concentration increases in the formulation because of the stable interfacial area. The chitosan emulsion prepared with 10% w/w Tween 80 gives the longest stabilized time, i.e., after 6 months of storage at room temperature (Figure 4.6).
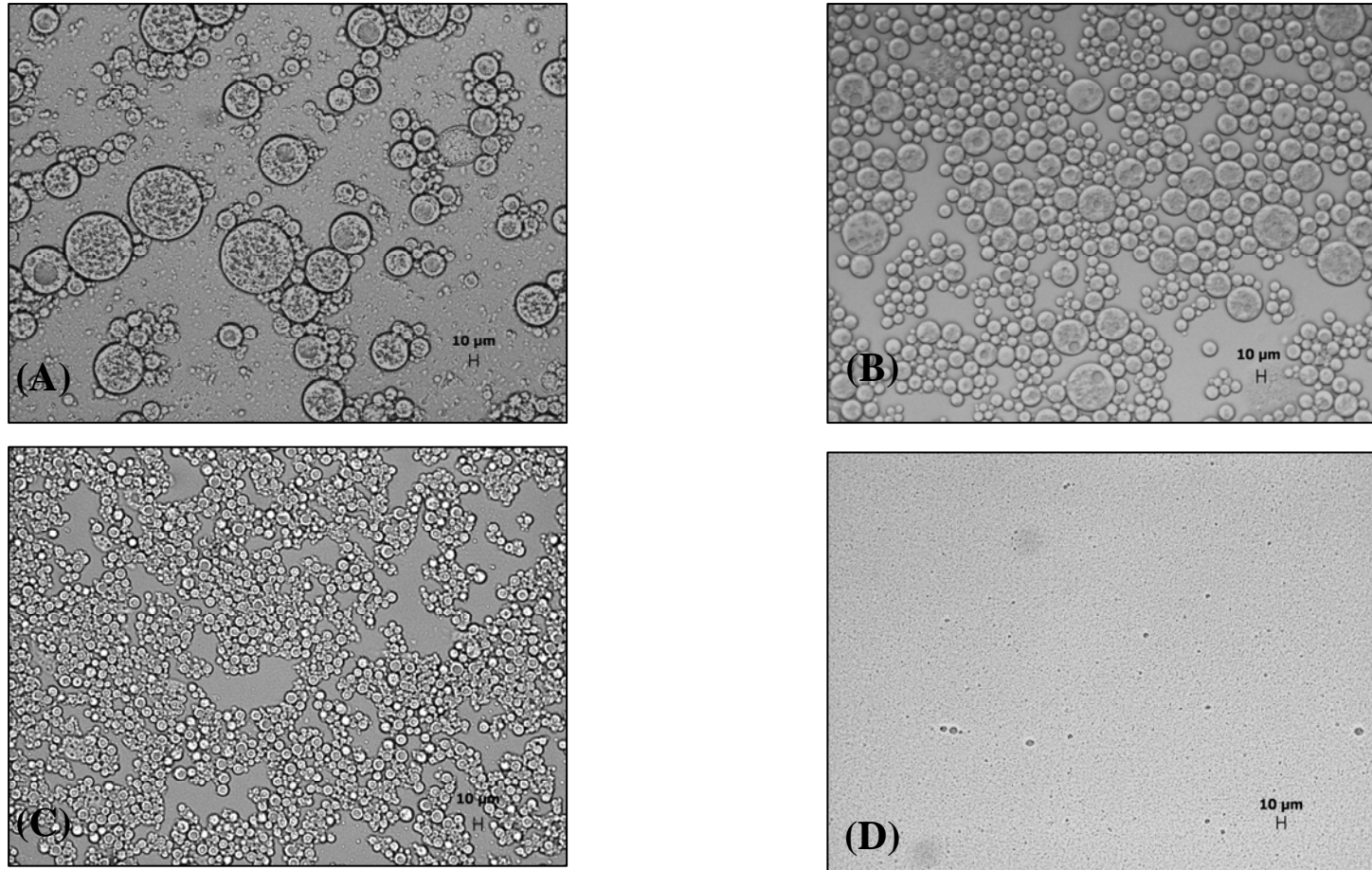
Figure 4.4 Photomicrographs of chitosan emulsions prepared with Tween 80 (w/w) (A) 1%, (B) 3%, (C) 6%, and (D) 10%.
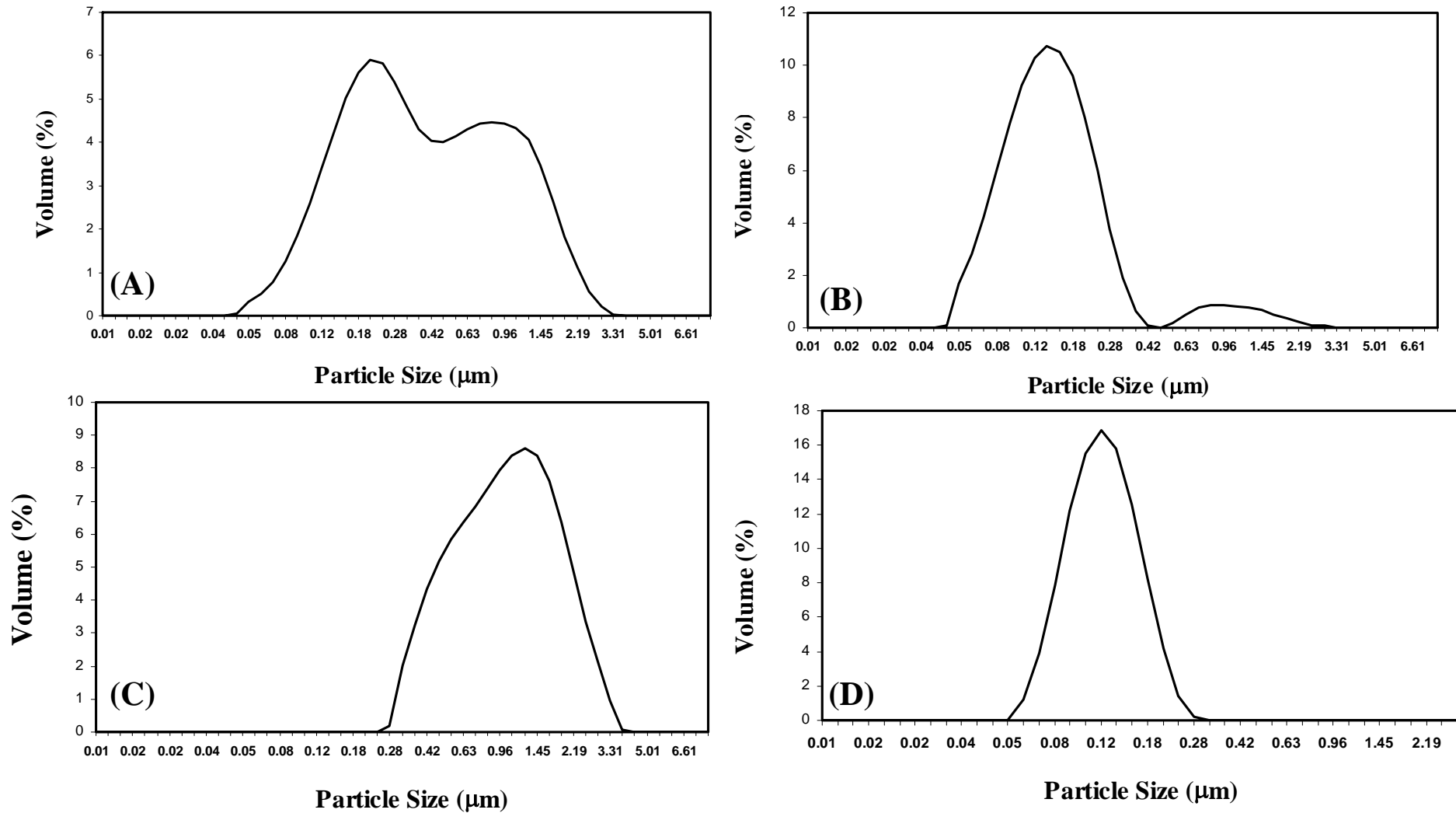
Figure 4.5 Particle size of chitosan emulsions prepared with Tween 80 (w/w) (A) 1%, (B) 3%, (C) 6%, and (D) 10%.
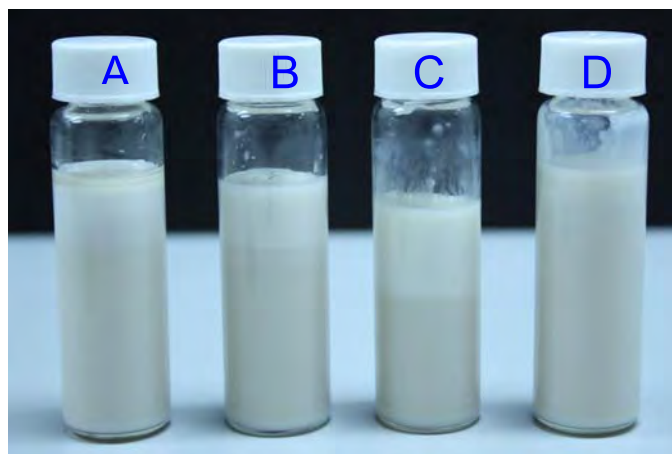
Figure 4.6 Effect of the Tween 80 surfactant concentration on stability of chitosan emulsions prepared with Tween 80 (A) 1% (w/w), (B) 3% (w/w), (C) 6% (w/w), and (D) 10% (w/w).

4.3 Particles size and zeta potential of the chitosan emulsions

It is observed that the high solid content emulsion is successfully prepared. The average particle size and zeta potential of the particles depend on chitosan concentration and molecular weight. When the concentration and molecular weight of chitosan increases, the particle size and zeta potential of the particles also increase. The particle size increases from 150 to 275 nm with the chitosan addition, whereas the zeta potentials from 10-51mV are obtained. It has been known that zeta potential technique measures a net charge density at the particle surface. This surface charge directly controls the distribution of ions in the surrounding interfacial region and can affect their capacity for aggregation. The zeta potential of the particle becomes positive charge as a result of the protonated amino group in the chitosan structure. The results from Table 4.1 shows that the particles prepared with 2% w/v of the highest molecular weight (8.5×10^5 Da) has a higher zeta potential and size larger than those prepared with 1% w/v of the lowest molecular weight (1.2×10^5 Da). The particle size distributions of the emulsions are shown in Figures 4.7-4.12 and the particle sizes are summarized in Table 4.1

The results suggest that adding the w/o emulsion into the chitosan solution can create a chitosan layer on the particle surface (as seen in Figure 4.13). This is probably due to the reason that the chitosan molecule, having some hydrophobicity,

can be potentially adsorbed onto the oil droplets in the w/o emulsion more than those partitioning in the aqueous phase. Therefore, the higher concentration and molecular weight of chitosan tend to increase the particle size. The zeta potential of the particles also obviously increases as a function of molecular weight and concentration of the different amounts of protonated amino group.

Table 4.1 average particle size and zeta potential of the particles prepared by various types and concentration of chitosan**.**

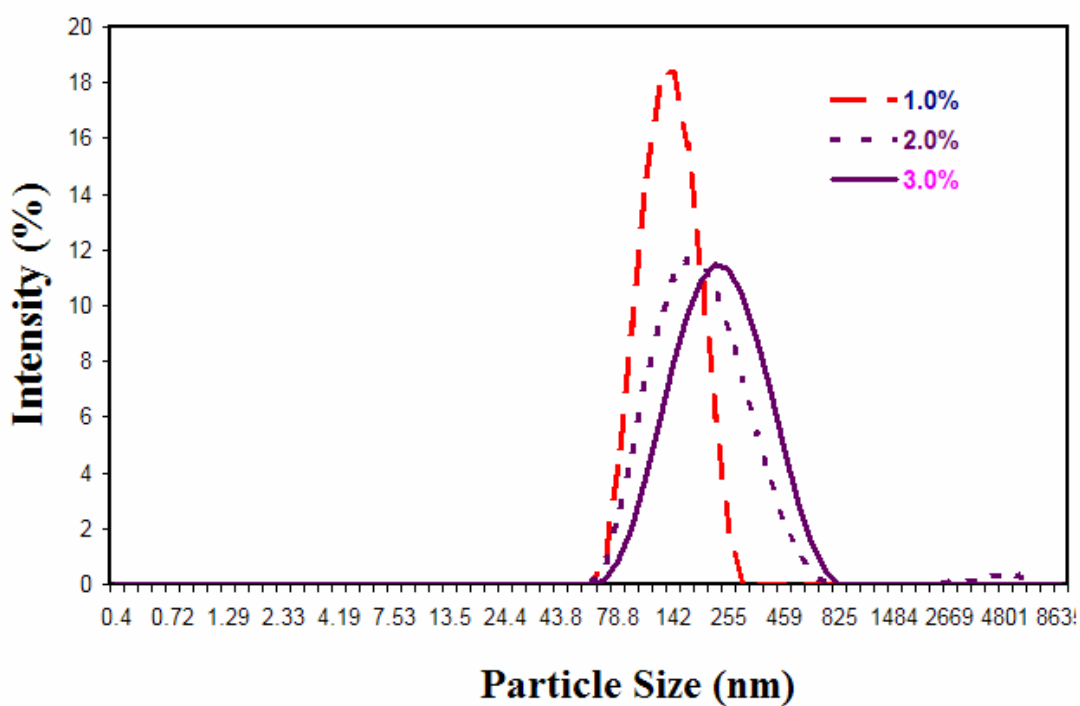| Chitosan (Dalton)		% Solids	Viscosity at 25°C (cP)	Particle size (nm)	Zeta potential (mV)
Without Chitosan		49.7	40	97±0.5	-16±1.3
1.2×10^5	1%	41.4	60	124±0.4	+10±1.4
	2%	42.6	100	167±5.1	+26±0.3
	3%	42.7	340	182±3.6	+39±1.3
3.7×10^5	1%	40.7	80	153±1.3	+16±0.8
	2%	39.5	680	203±4.7	+38±1.6
	3%	42.5	5600	275±6.1	+45±1.1
8.5×10^5	1%	44.4	700	173±1.2	+30±0.3
	2%	45.5	2100	215±1.2	+51±1.3

Figure 4.7 Particle size of emulsions prepared with chitosan 120kDa) at various concentrations.
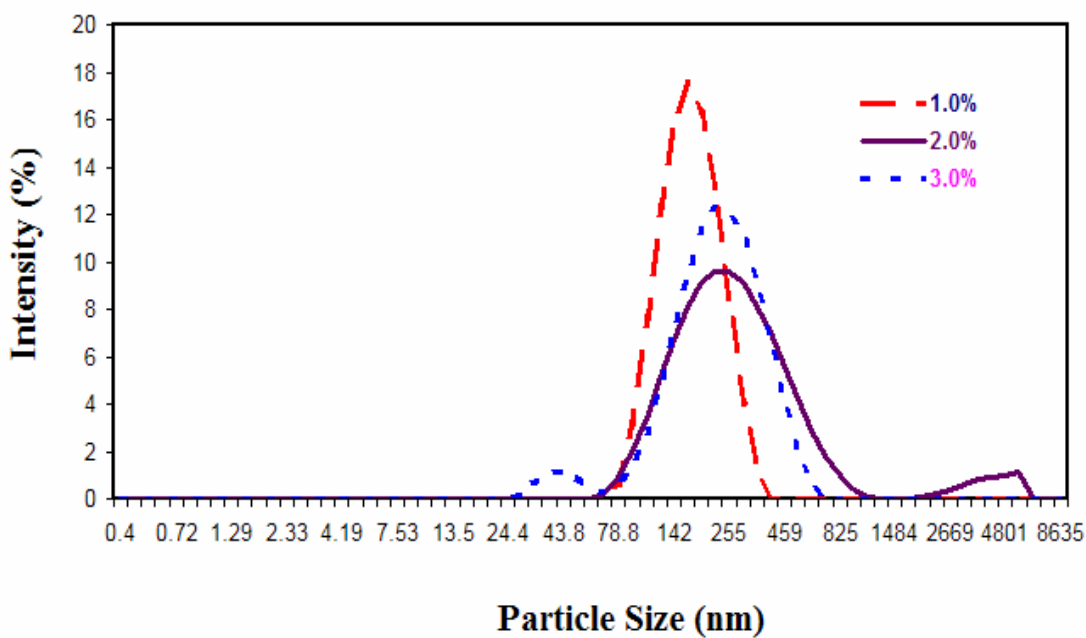

Figure 4.8 Particle size of emulsions prepared with chitosan (370kDa) at various concentrations.
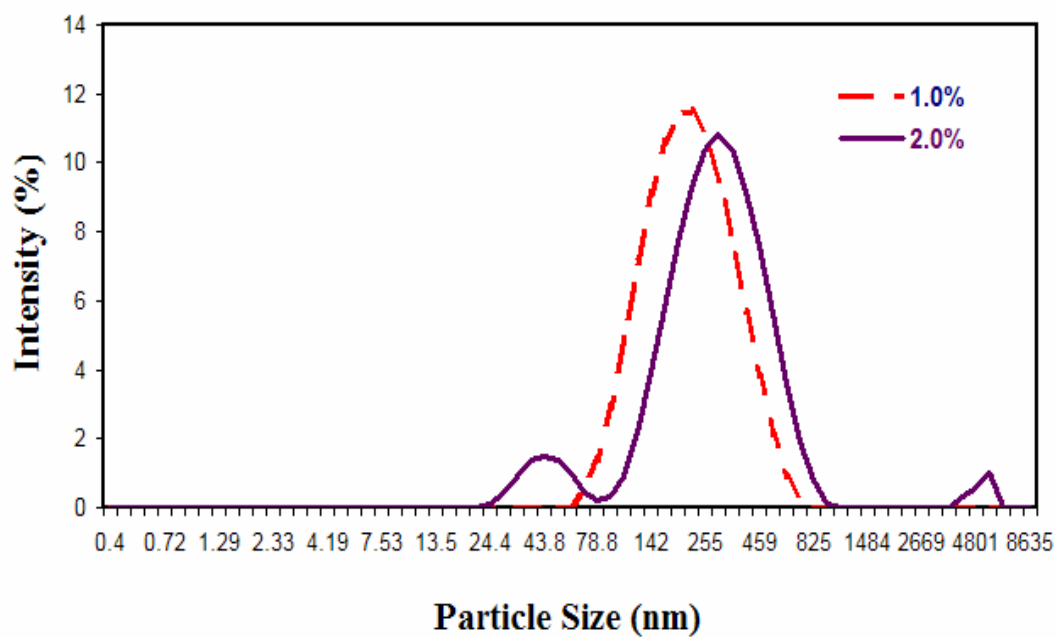
Figure 4.9 Particle size of emulsions prepared with chitosan (850kDa) at various
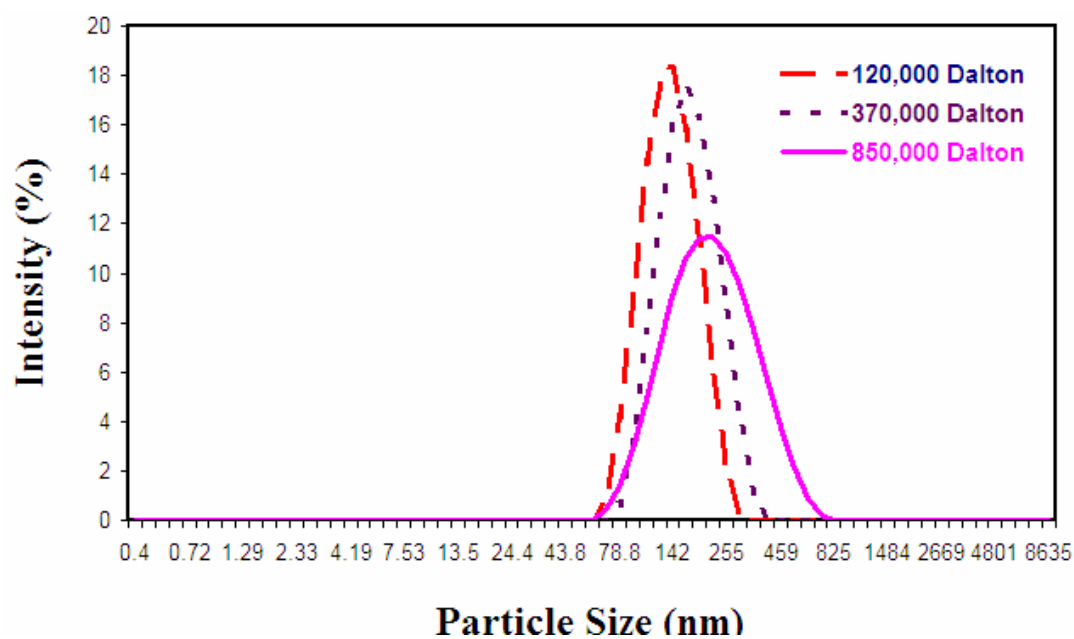concentrations.

Figure 4.10 Particle size of emulsions prepared with 1% w/v chitosan
concentration of various molecular weights.
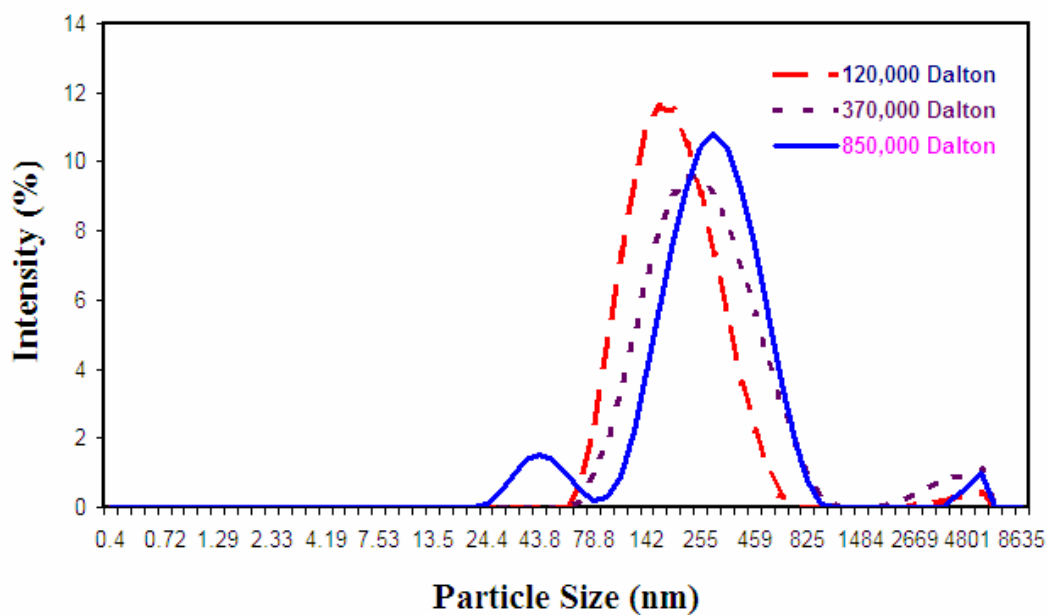
Figure 4.11 Particle size of emulsions prepared with 2% w/v chitosan concentration of various molecular weights.
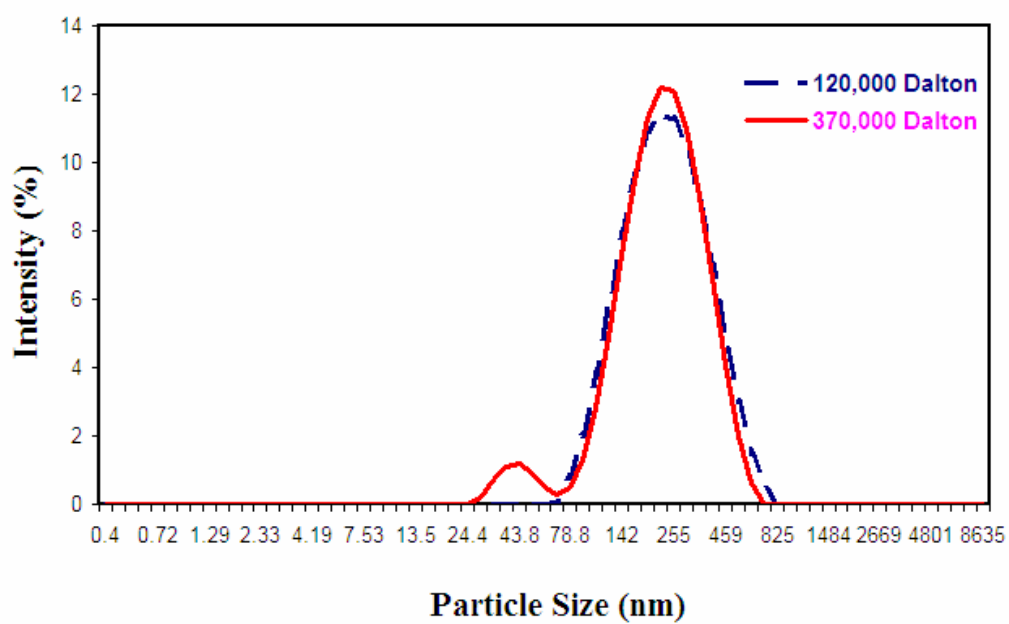

Figure 4.12 Particle size of emulsions prepared with 3% w/v chitosan concentration of various molecular weights.

4.4 Morphology of chitosan emulsion by transmission electron microscopy

TEM images of green tea emulsions (w/o and w/o/w) and chitosan emulsions are shown in Figures 4.13-4.14. Green tea emulsion (w/o) appears to be a spherical particle, however, it is difficult to find a regular spherical particle in the w/o/w emulsion. With chitosan addition, the encapsulating layer of chitosan is visible on the particle surface. At 2% w/v of chitosan solution in Figure 4.13, the layer appears prominently.
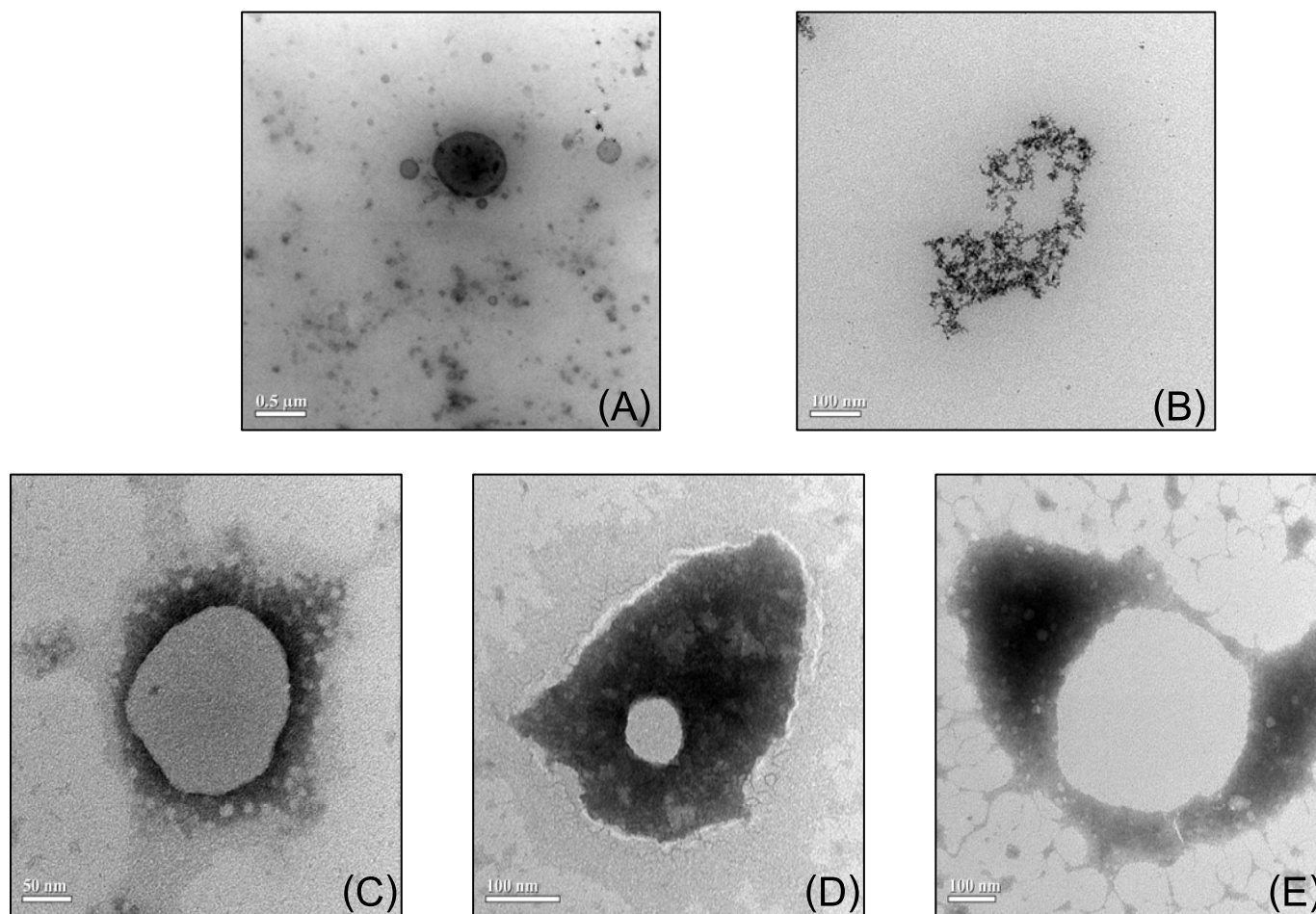
Figure 4.13 TEM micrographs of chitosan emulsions prepared with chitosan (A) Green tea emulsion (w/o), (B) w/o/w emulsion, (C) 2% w/v of 120kDa, (D) 1% w/v of 370kDa, and (E) 2% w/v of 370kDa.
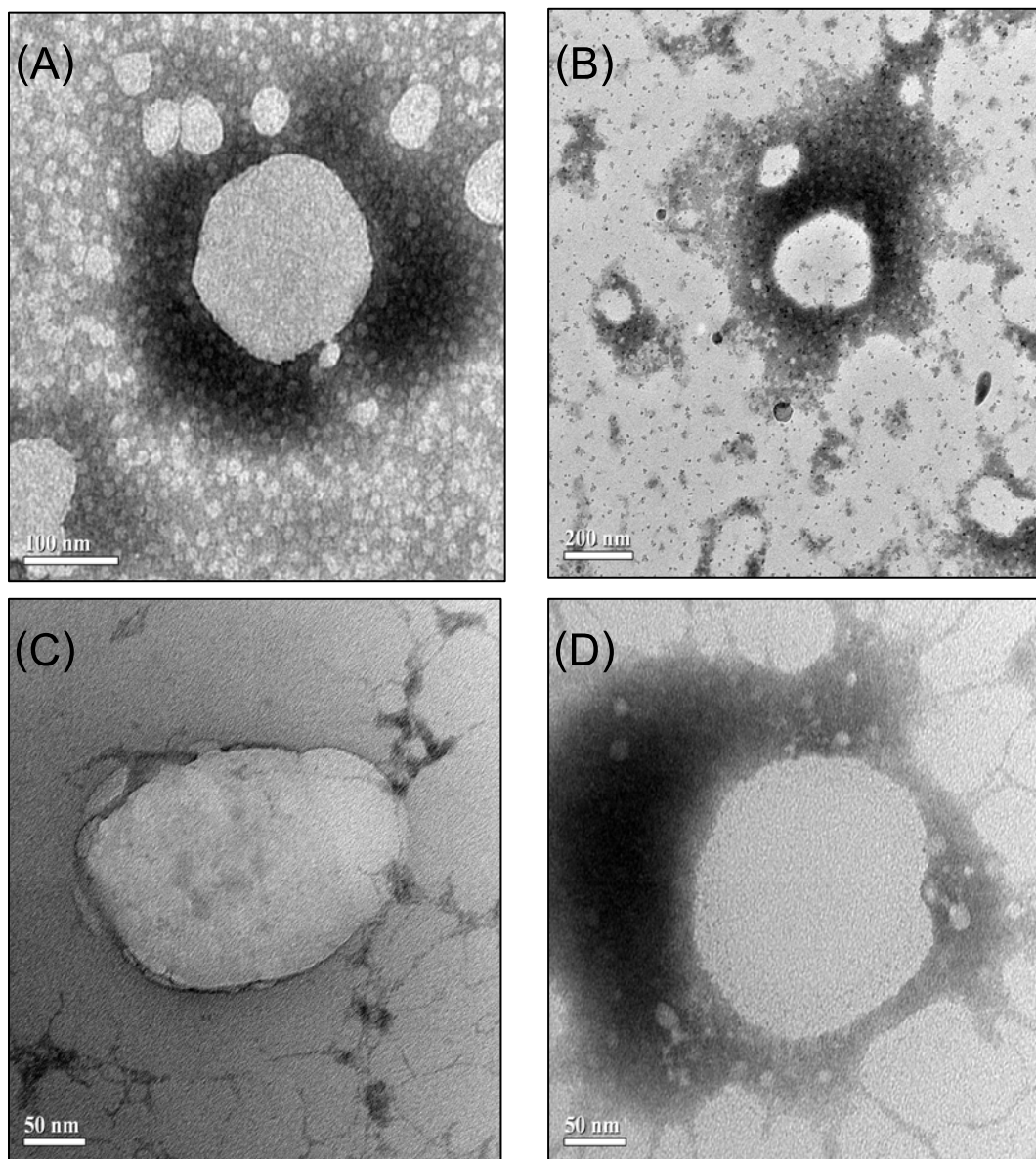
Figure 4.14 TEM micrographs of chitosan emulsions prepared with chitosan (A) 2% w/v of 120kDa, (B) 2% w/v 120kDa, (C) 1% w/v of 120kDa, and (D) 2% w/v of 370kDa.

4.5 Rheological properties of the chitosan emulsion

Rheological behavior of the water-in-oil emulsion (w/o) is characterized in terms of viscosity versus shear stress (τ). The result is shown in Figure 4.14. The emulsion exhibits non-Newtonian behavior in which the viscosity of emulsion decreases with an increase in shear stress.
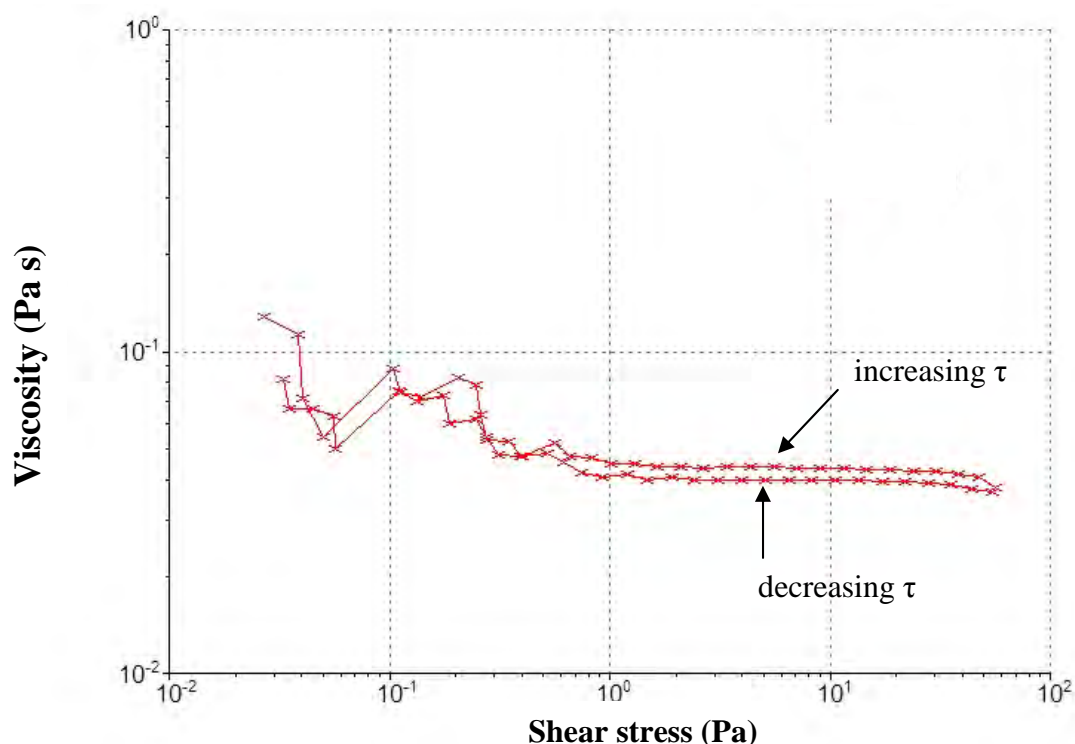

Figure 4.15 Viscosity versus shear stress for the water-in-oil emulsion.

The chitosan emulsions also behave like a non-Newtonian shear-thinning fluid. The viscosity of chitosan emulsion decreases with an increase in shear stress.

By nature of the product that viscosity decrement upon high shear increasing is named "Pseudoplastic". The behavior represents the shear-thinning. This is the nature of natural polymer and long chain polymer like chitosan. The viscosities of chitosan emulsions with three molecular weights at various concentrations are shown in Figures 4.15-4.21. It is observed that without the chitosan addition, the emulsion has the lowest viscosity compared with the ones with chitosan (Figure 4.21). When the concentration of chitosan is increased, the viscosity of the emulsion also increases

accordingly. Therefore, the highest concentration of chitosan produces the highest viscosity of emulsion. Besides, the molecular weight of chitosan generates the similar effect as well. This is probably caused by the influence of particle size on the emulsion viscosity. At low concentrations when the shear stress is gradually varied, the flow of emulsion for both the upward and backward directions is identical which indicates that the emulsion is stability under shear. When the backward direction the viscosity is not identical to the upward direction, it indeed represents the "Thixotropy behavior". The breadth of increasing and decreasing shear rates depend on the molecular weights and the corresponding concentrations. Figure 4.21 summarizes the effect of shear stress of the emulsion on various chitosan molecular weights and concentrations where at 3% w/v of medium molecular weight ($3.7x10^5$ Da) and 2% w/v of the highest molecular weight ($8.5x10^5$ Da) a loop is observed. This behavior relates to microstructure of product in both continuous phase and dispersed phase. In general, microstructure is the site that functional ingredients are assembled and finally reside in the dispersion. As for the network structure, chitosan particles have loose contact among them. However, once a shear force is applied, the force breaks down the network. After the product is allowed to rest or relax for a specific time, the structure of the emulsion can recover to its original stage. This is another evidence to confirm the "Thixotropy behavior". Therefore, the flow behavior can be used to represent the product stability.
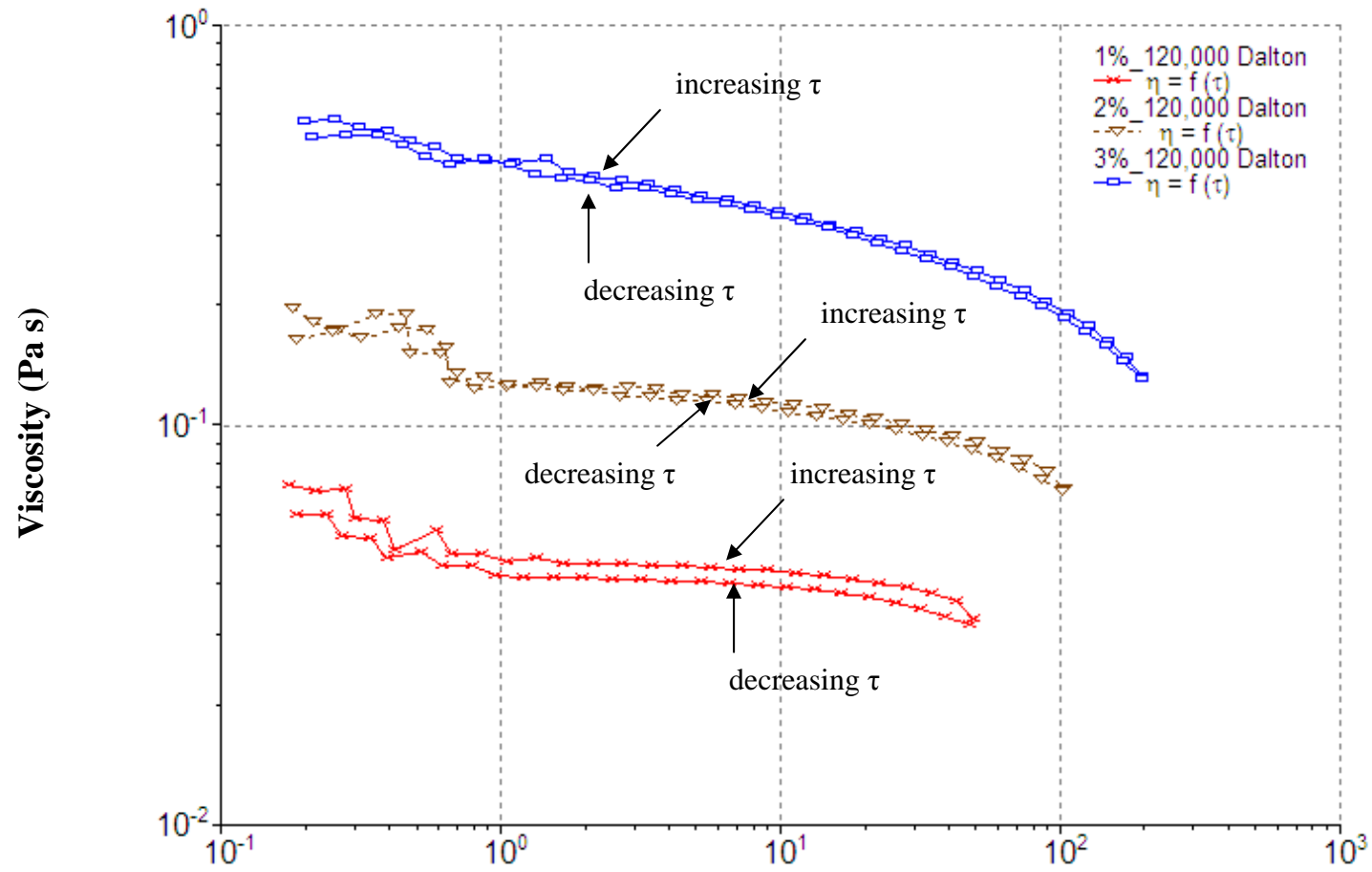
Figure 4.16 Viscosity as a function of shear str **Shear stress (Pa)** pared with 120kDa chitosan at various concentrations.

Figure 4.17 Viscosity as a function of shear stress of the emulsion prepared with 370kDa chitosan at various concentrations.
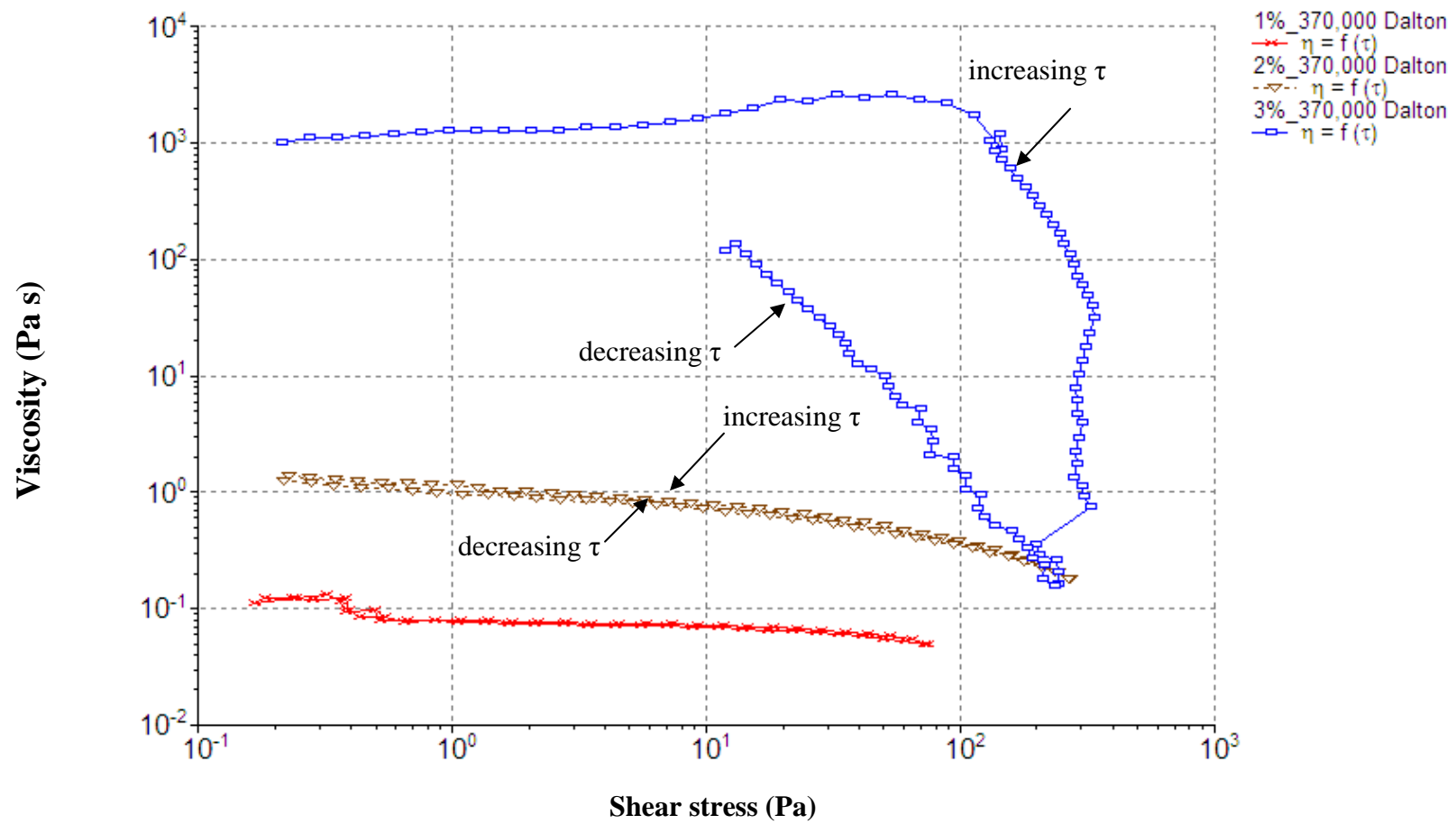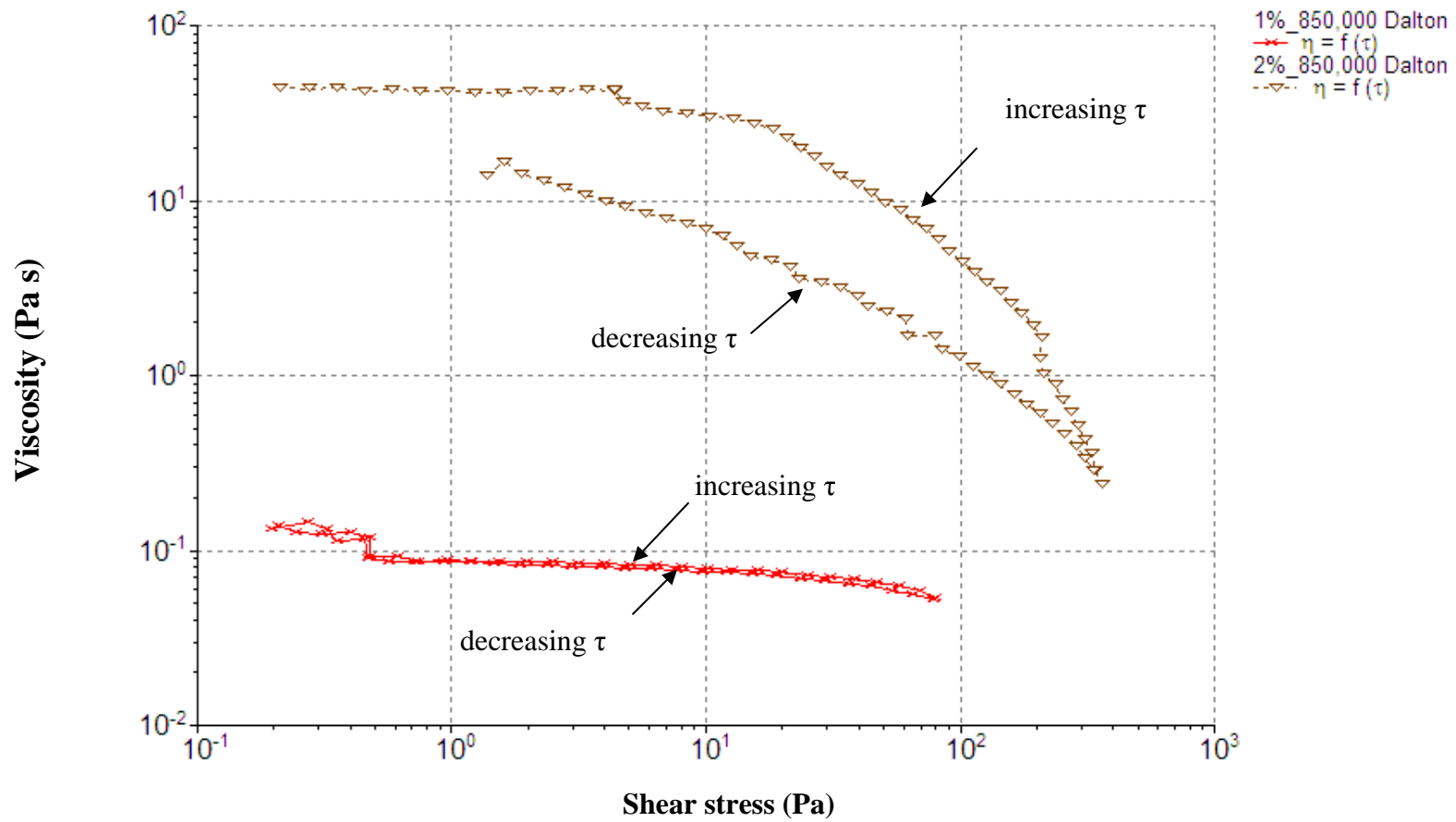
Figure 4.18 Viscosity as a function of shear stress of the emulsion prepared with 850kDa chitosan at various concentrations.
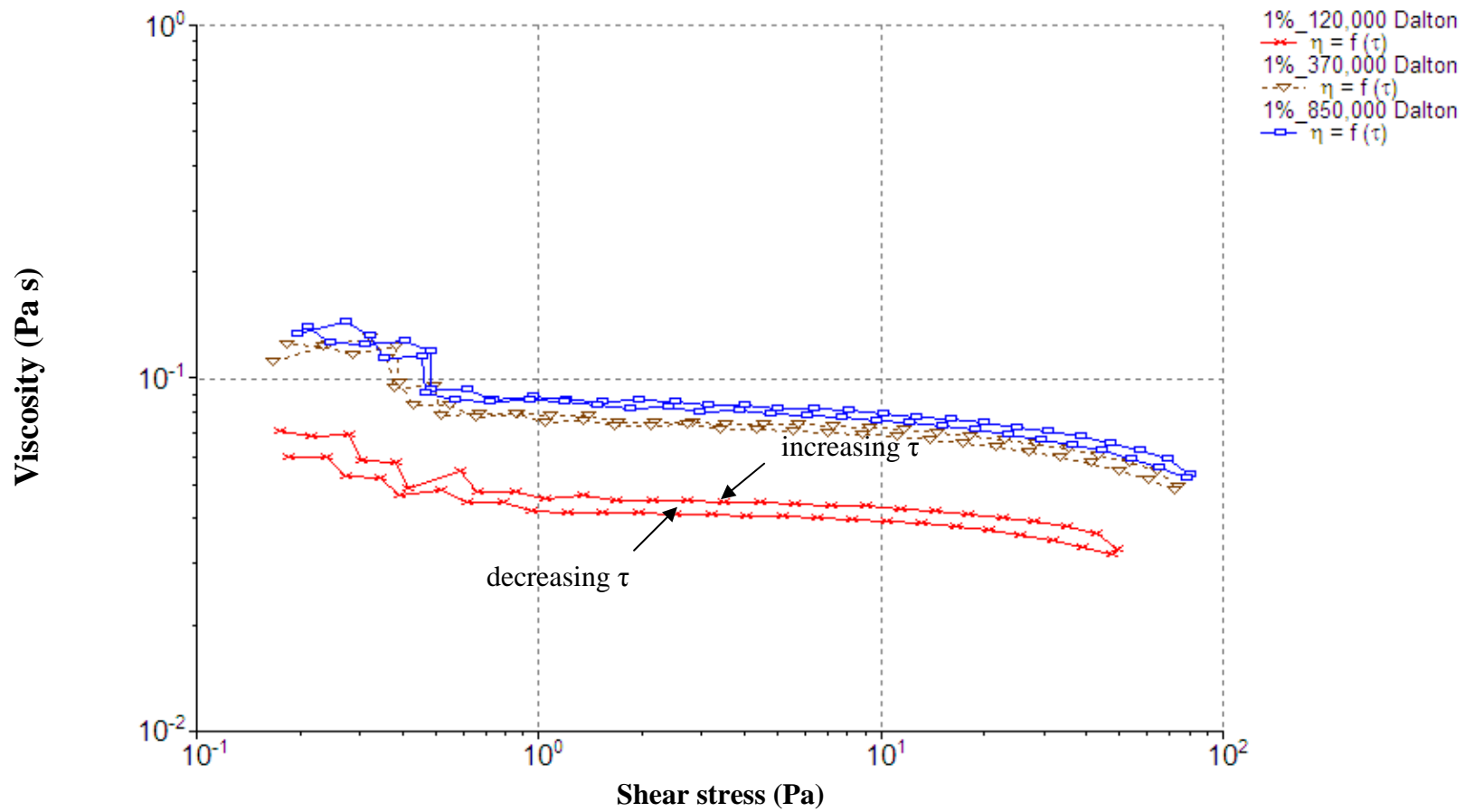
Figure 4.19 Viscosity as a function of shear stress of the emulsion prepared with 1% **w**/v chitosan concentration with various molecular weights.

Figure 4.20 Viscosity as a function of shear stress of the emulsion prepared with 2% w/v chitosan concentration with various molecular weights.

Figure 4.21 Viscosity as a function of shear stress of the emulsion prepared with 3% w/v chitosan concentration with various molecular weights.

Figure 4.22 Viscosity as a function of shear stress of the emulsions prepared with various chitosan concentrations and molecular weights.
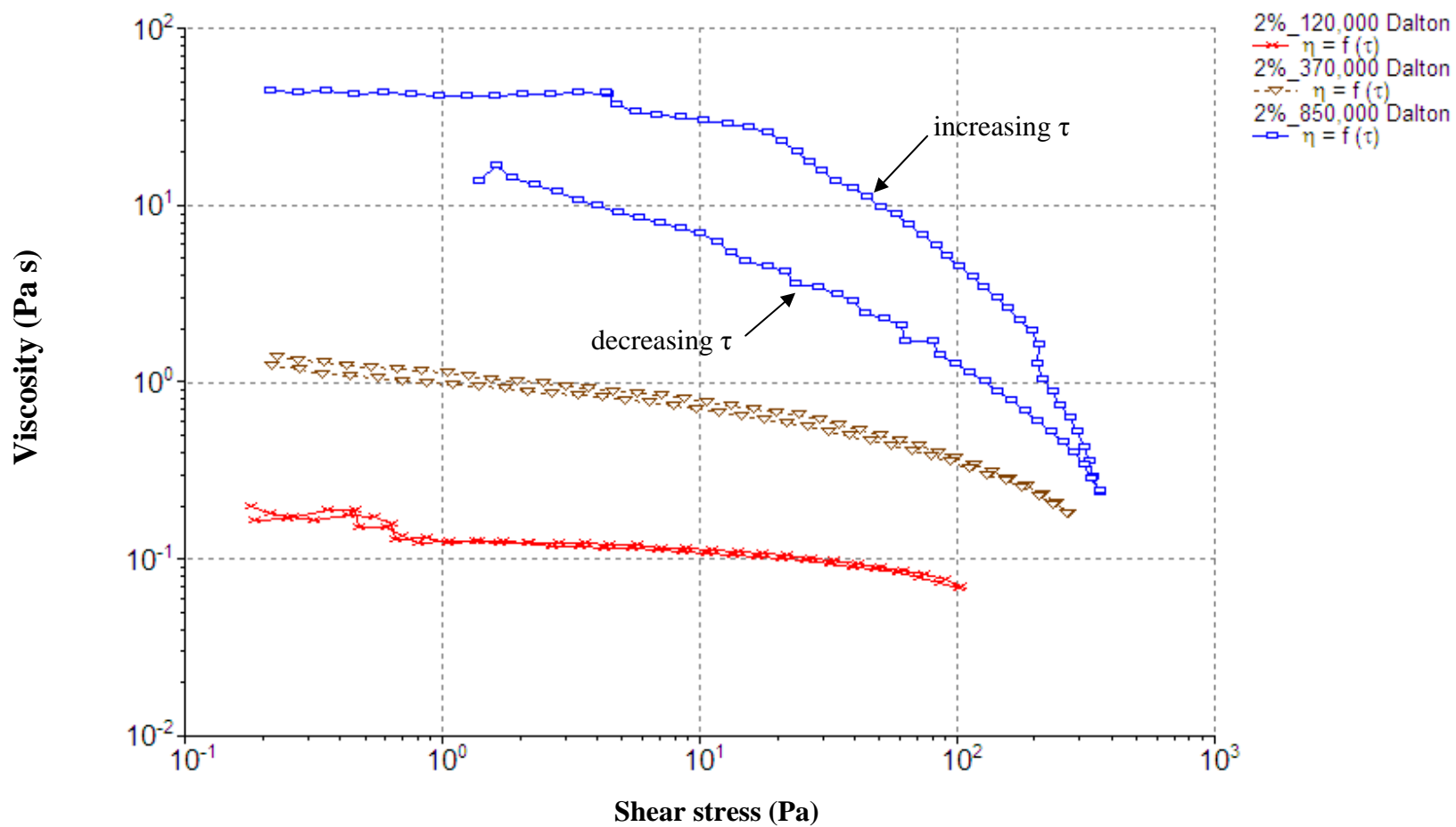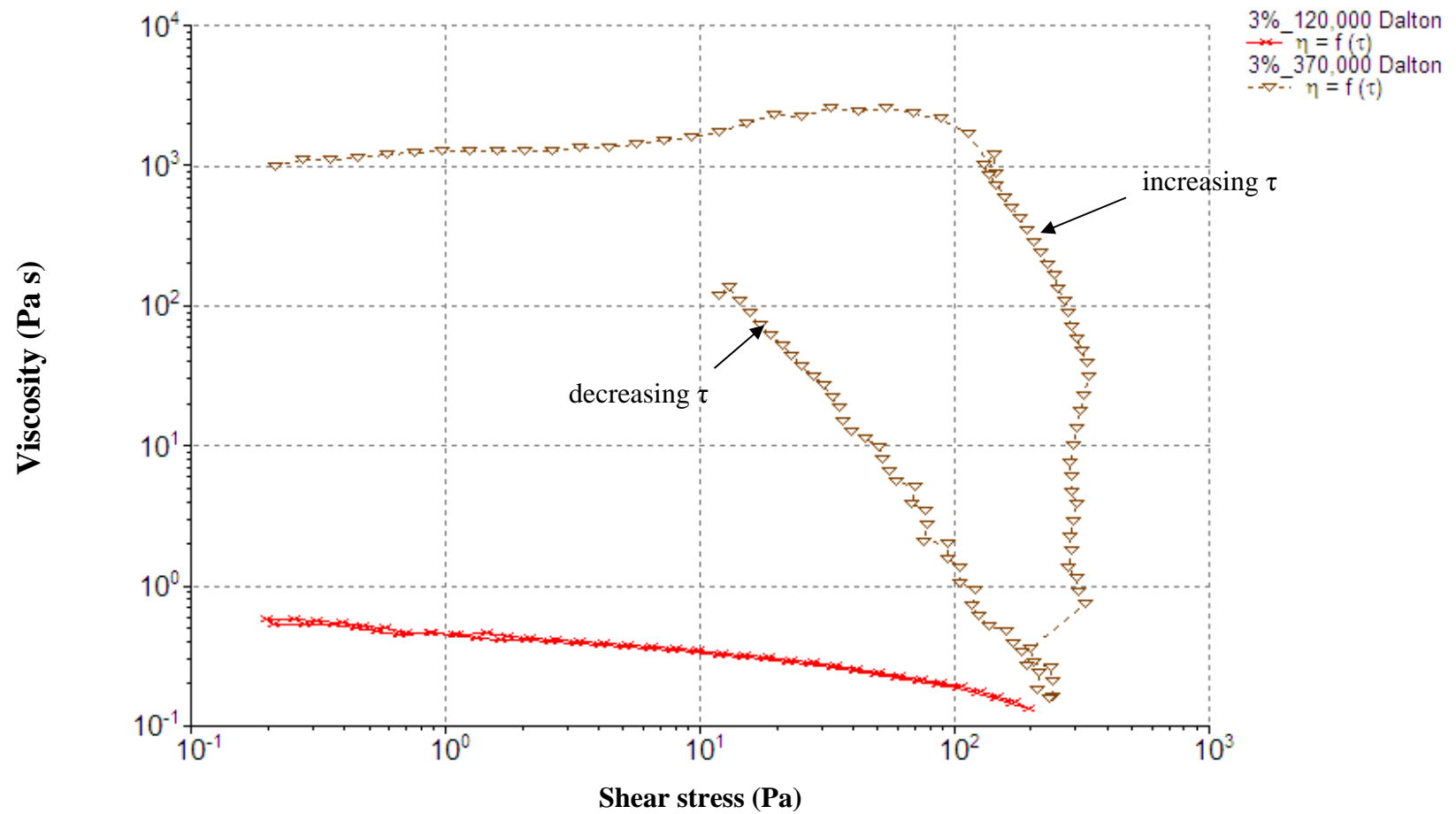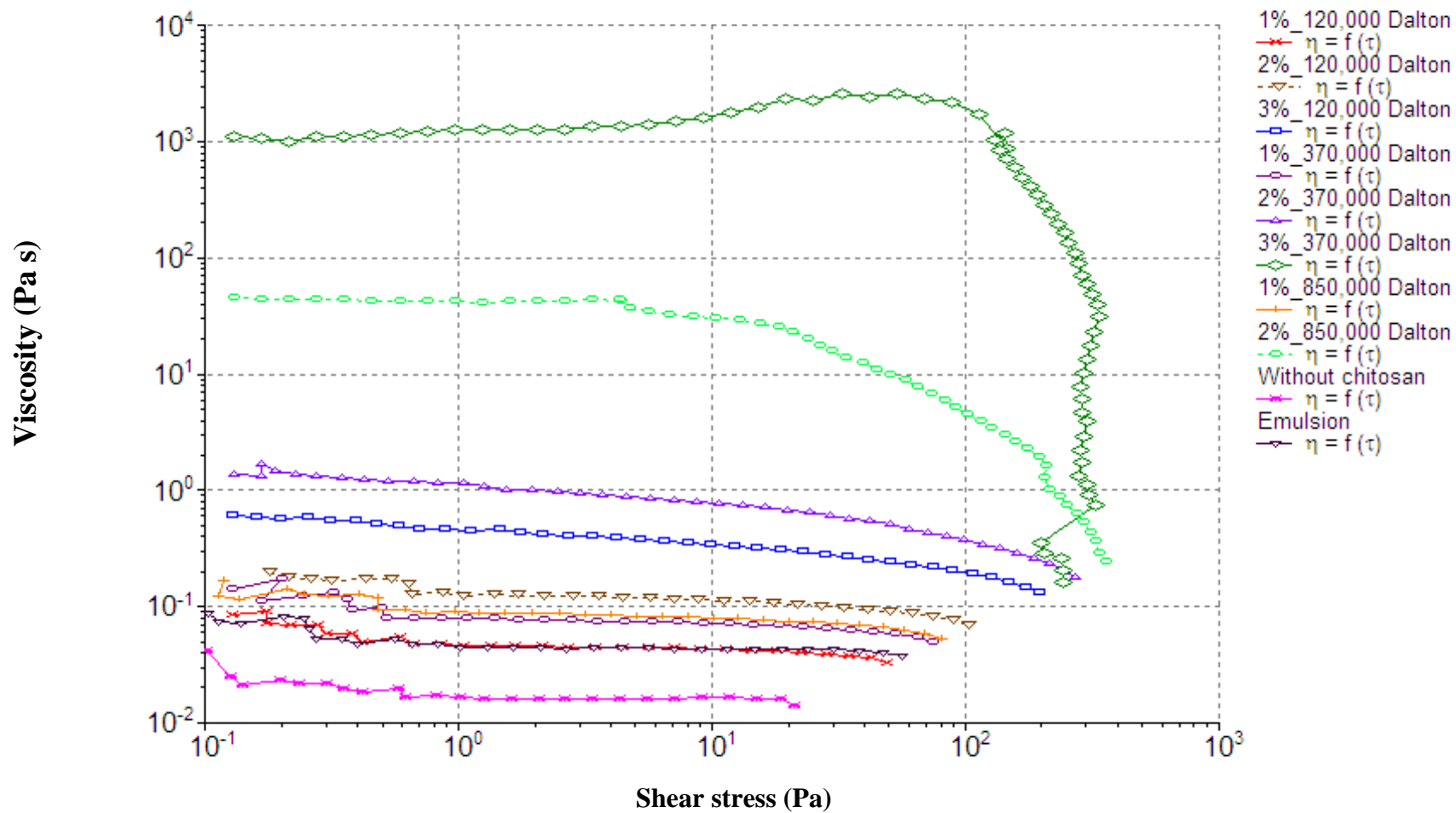
4.6 Appearance of hair coated with chitosan emulsion by SEM

SEM micrographs of virgin hair and emulsion without chitosan treated hair are presented and compared in Figure 4.23

After applying the chitosan emulsion on hair switches, it is noted that the hair surface is gradually covered as the chitosan concentration increases (Figure 4.23). Some small specks and light coating are deposited and observed on the hair surface, which make the hair cuticle shallower. The highest coverage is obtained from chitosan having 3% w/v of the medium molecular weight and 1% w/v of the highest molecular weight. However, when the 2% w/v of chitosan with 8.5×10^5 Da was applied to hair switches, uneven coating on the hair is observed as shown in Figure 4.23 (J) because the emulsion has too high viscosity and cannot be applied easily.

Having green tea extract in the chitosan emulsions is expected to protect hair from UV radiation. Since green tea extract is associated with several health benefits. These benefits include potential cancer-fighting properties, and a strong antioxidant effect that protects the body from the damaging effect of free radicals. The antioxidative effect is originated from the principal polyphenolic components extracted from green tea leaves, i.e. (-)-epicatechin (EC), (-)-epicatechin gallate (ECG), (-)-epigallocatechin gallate (EGCG), (-)-epigallocatechin (EGC), and gallic acid (GA).
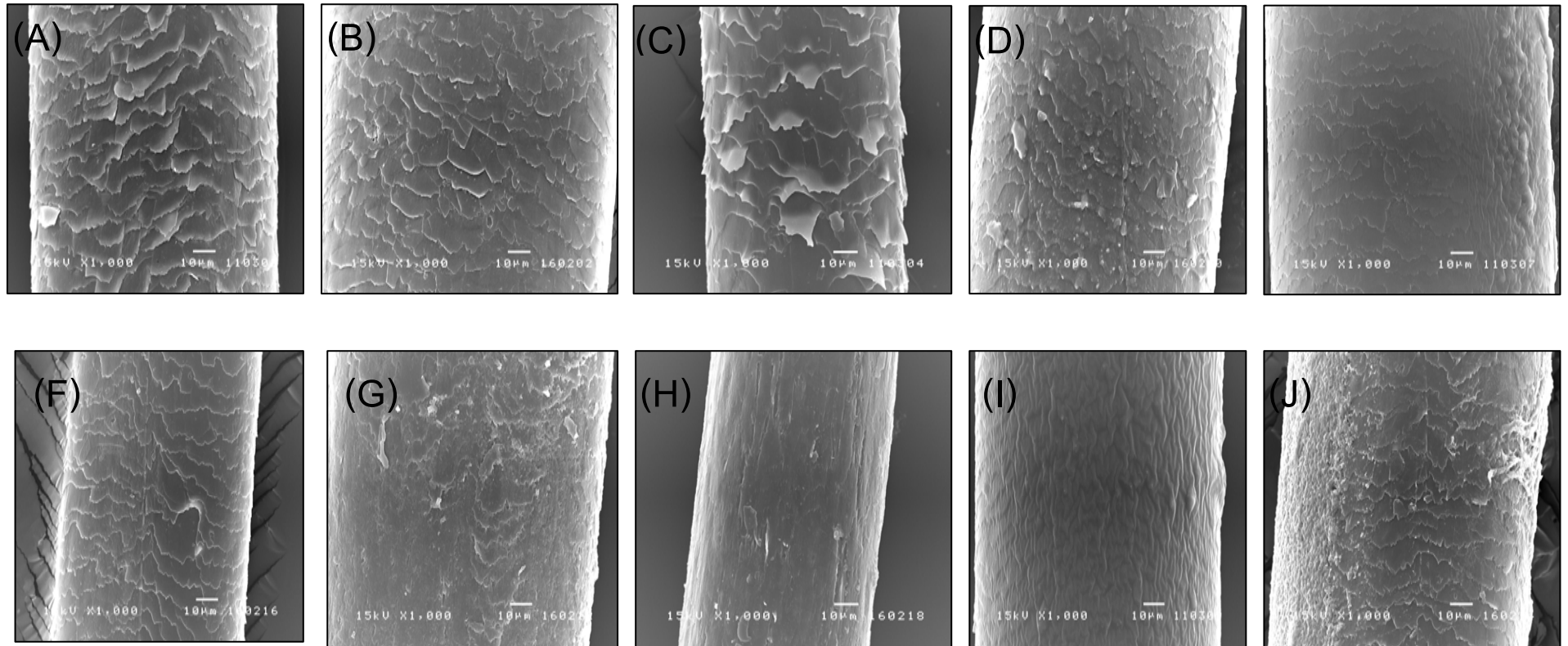
Figure 4.23 SEM micrographs of chitosan emulsion treated hair at (A) Virgin hair, (B) without chitosan, (C) 1% w/v 120kDa, (D) **2%** w/v 120kDa, (E) 3% w/v 120kDa, (F) 1% w/v 370kDa, (G) 2% w/v 370kDa, (H) 3% w/v 370kDa, (I) 1% w/v 850kDa, and (J) 2% w/v 850kDa(x1000).
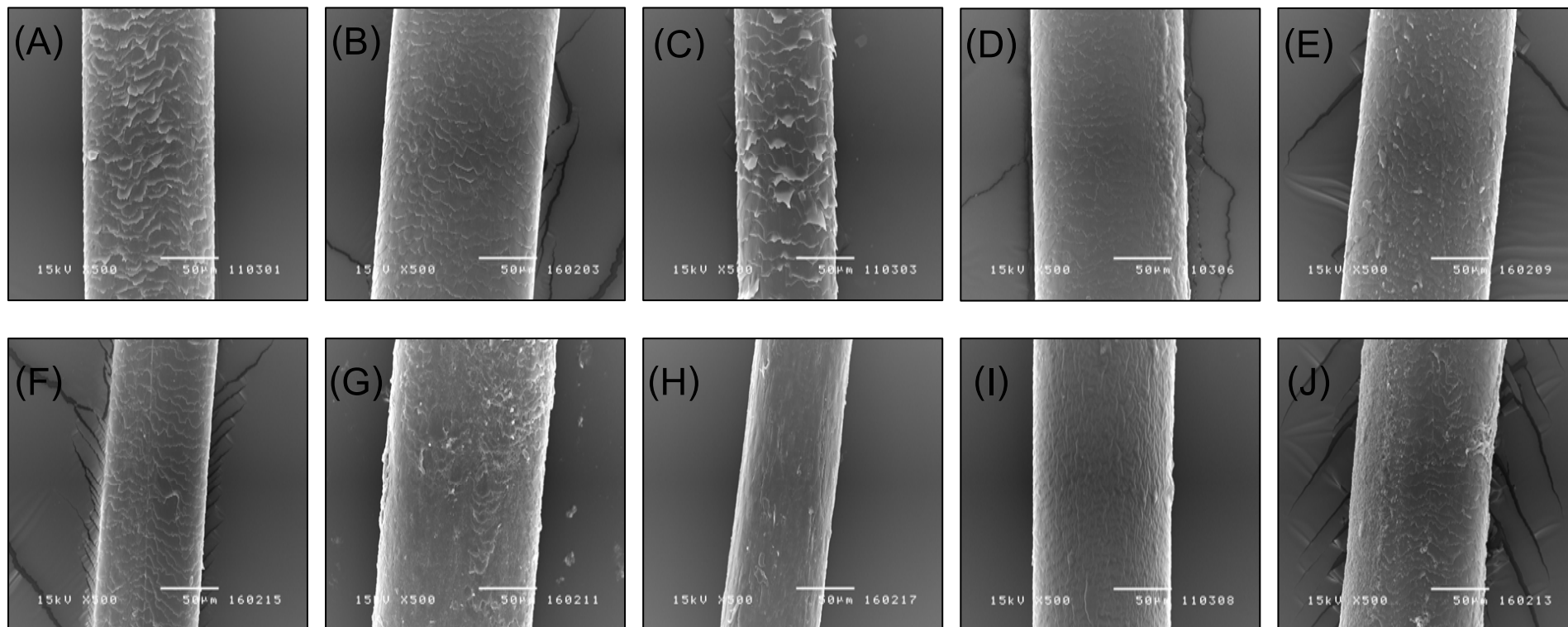
Figure 4.24 SEM micrographs showing appearance of (A) virgin hair; (B) without chitosan treatment; the hair treated with chitosan emulsion having: (C) 1% w/v, 120kDa; (D) 2**%** w/v, 120kDa; (E) 3% w/v, 120kDa; (F) 1% w/v, 370kDa; (G) 2% w/, 370kDa; (H) 3% w/v, 370kDa; (I) 1% w/v, 850kDa; and (J) 2% w/v, 850kDa (x500).

4.7 Texture Analysis

After applying the chitosan emulsion (1.2×10^5 Da) to 2.5 gram of hair switches (6 inches long and 1.5 inches wide), the friction force of hair is decreased compared to the virgin hair, i.e., more slippery on hair surface is obtained (Figure 4.25). Similar trends are also observed with other molecular weights ($3.7 \times 10^5$ Da and 8.5×10^5 Da) as seen in Figures 4.26-4.27. The higher the concentration, the greater decrease in friction force of the hair becomes. This is attributed to the reason that the hair surface gains more coverage from chitosan emulsions which provide the hair surface with viscous film as seen previously in SEM micrographs. Likewise, friction force of the hair increases with increases in the molecular weight of chitosan, especially at the highest molecular weight (Figure 4.28). This increase in friction force is probably due to the result of uneven coating and rough surface obtained from highly viscous chitosan emulsions. The lowest friction force or the highest slip on hair surface is found at 3% w/v of 3.7×10^5 Da chitosan as evidenced by the SEM in Figure 4.23 (H).

4.8 Instron Combing

After applying the chitosan emulsion to 2.5 gram of hair switches (6 inches long and 1.5 inches wide), it is much easier to comb the coated hair. This can be seen in Figure 4.29 as a decrease in maximum load of the hair treated with the chitosan emulsion compared to the untreated hair analyzed by Instron combing. The higher the chitosan concentration, the lower the maximum load is needed to apply to the switched hair. However, the maximum load of the hair is increased with increasing molecular weight of chitosan (Figure 4.30-4.31). A similar reason as mentioned in texture analysis can be applied to the Instron combing.

Figure 4.32 summarizes all the maximum Load results. It ca be seen that the virgin hair and hair treated the chitosan-free emulsion tend to give the higher maximum load. The minimum load is obtained at 3% w/v of 3.7×10^5 Da chitosan, which indicates that the hair is easily combed. On the other hand, the highest molecular weight (8.5×10^5 Da) and increasing increase the maximum load of hair switches. The latter condition caused by the viscous property, resulting in an uneven and rough surface on the hair switches, which in turn increases maximum load.
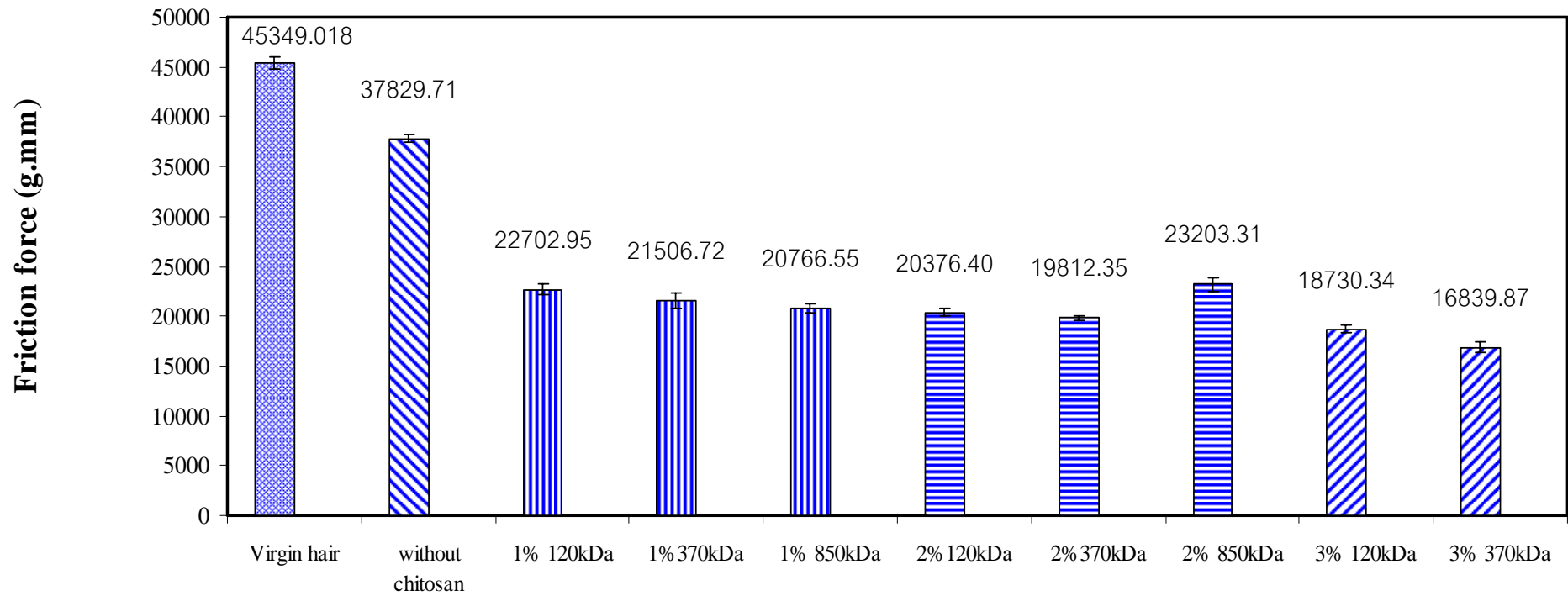
Figure 4.25 Friction force of hair surface treated with chitosan emulsions prepared at various molecular weights and concentrations.

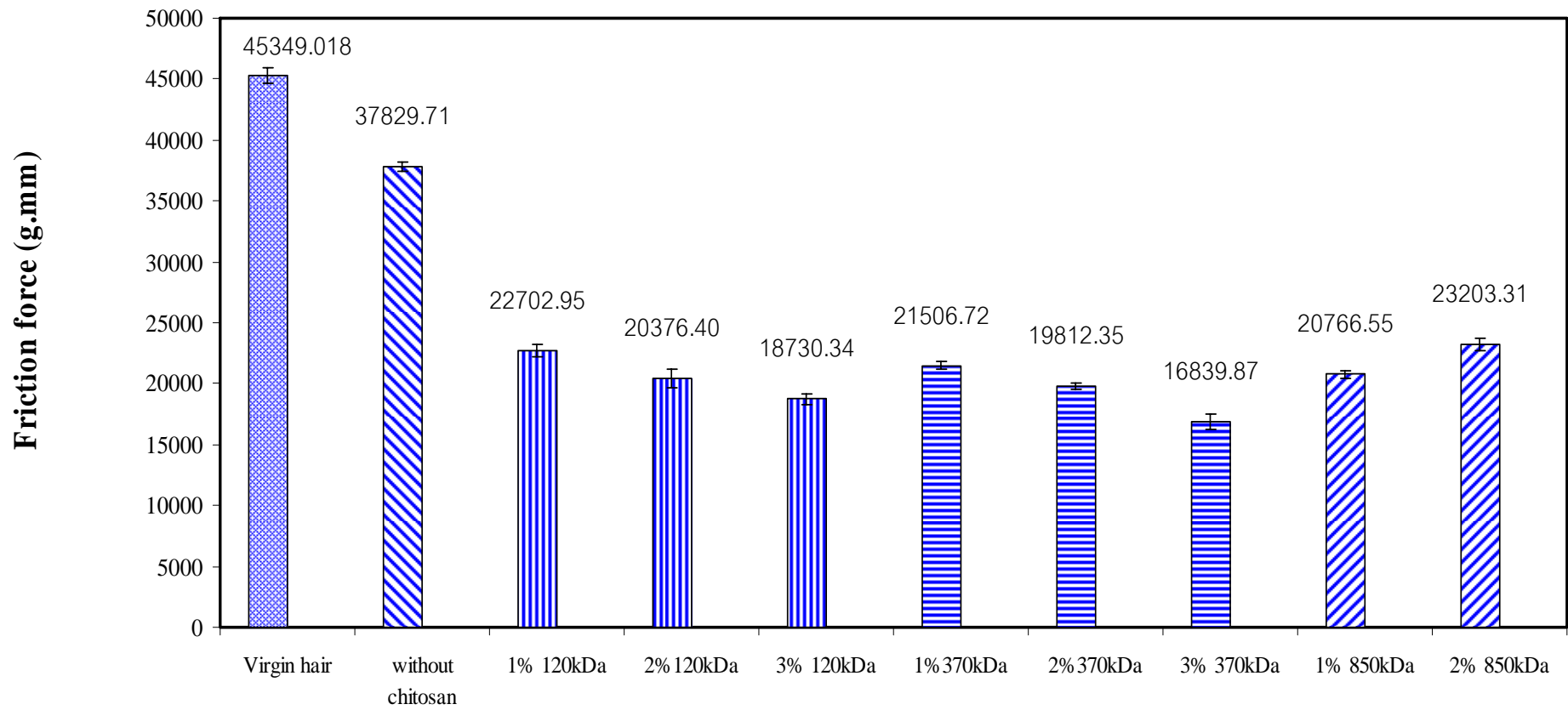**Figure 4.26** Friction force of hair surface treated with chitosan emulsions prepared at various concentrations and molecular weights.
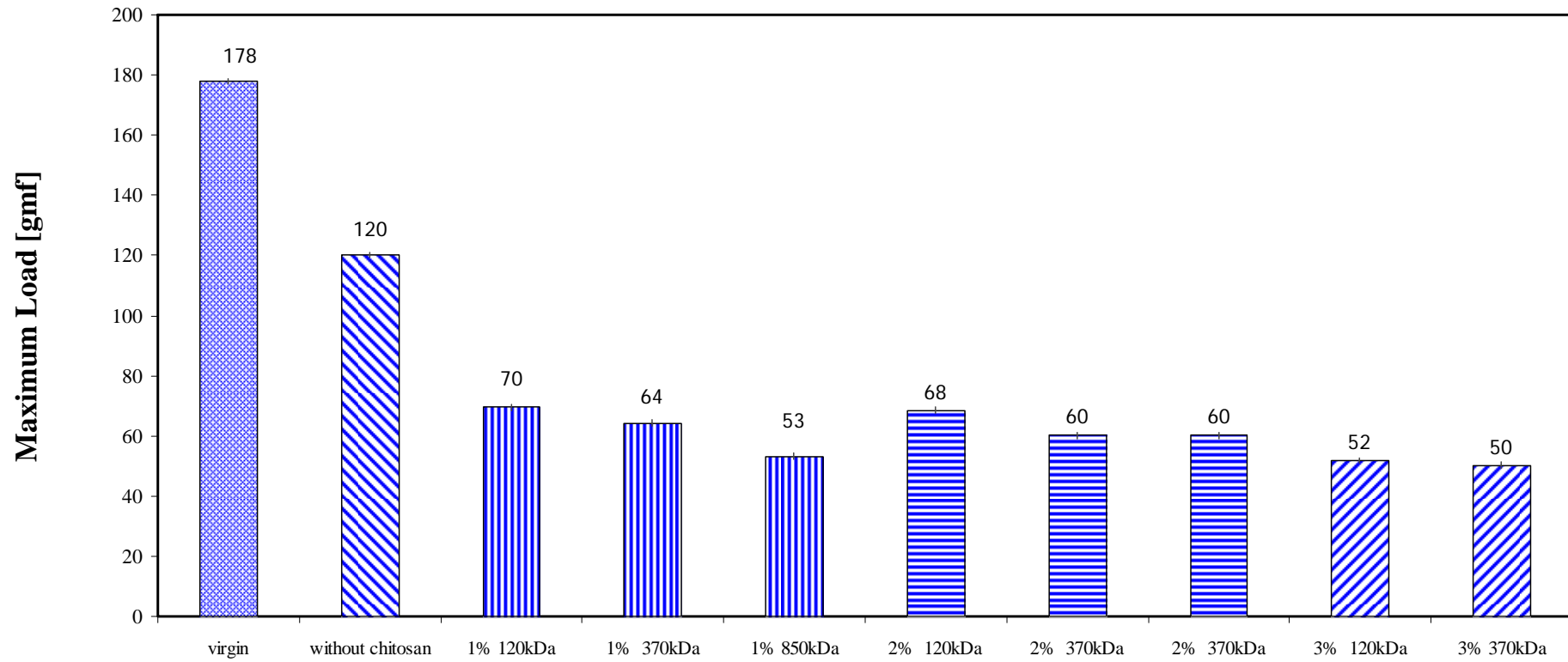
Figure 4.27 Maximum Load of hair treated with chitosan emulsions at various molecular weights and concentrations.
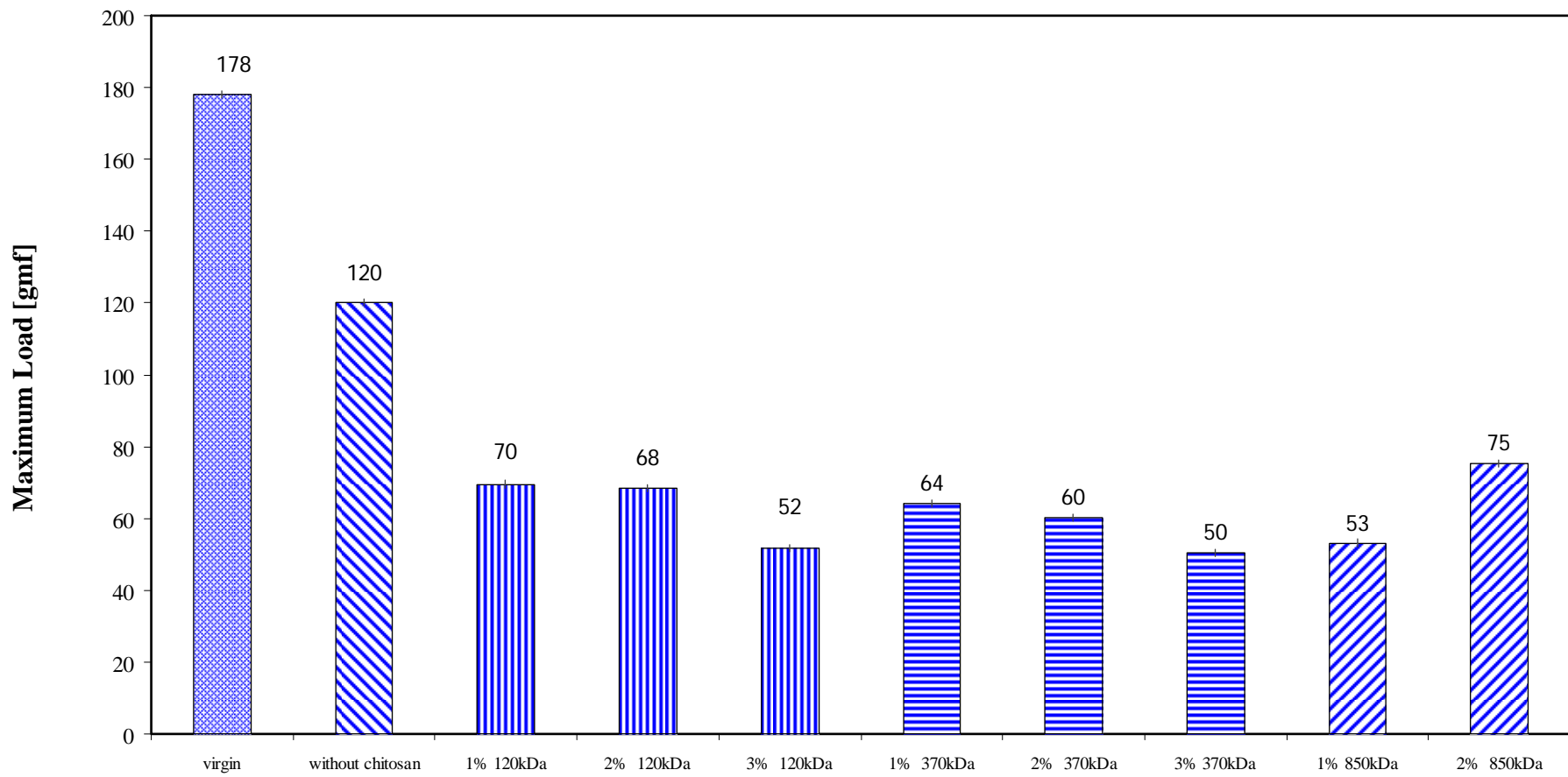
Figure 4.28 Maximum Load of hair treated with chitosan emulsions at various concentrations and molecular weights.

CHAPTER V

CONCLUSIONS AND SUGGESTIONS

Chitosan containing green tea extract

The water-in-oil emulsion of chitosan containing green tea extract at an HLB of 5.3 has good stability without phase separation. TEM micrographs indicate the encapsulating layer of chitosan on the emulsion droplet. The size and zeta potential of chitosan emulsions are in the range of 124-275 nm and 16-51 mV, respectively. The particle size and zeta potential of the chitosan emulsions increase with increasing chitosan concentrations and molecular weights. The viscosity of the chitosan emulsion is increased when increasing chitosan concentrations.

When the concentration of chitosan is increased, the viscosity of the emulsion also increases. Therefore, the highest concentration of chitosan yields the highest viscosity of emulsion. Besides, the molecular weight of chitosan has also a similar effect. At low concentrations when the shear stress is gradually increased, the flow of emulsion for both upward direction (increased shear stress) and backward direction (decreased shear stress) is identical suggesting that the chitosan emulsion is stable under shear.

The treated hair and its operational properties

The hair treated with chitosan emulsions look much smoother. The light coating on the surface makes the hair cuticle shallower. When applying the high concentration of chitosan emulsion, the decrease in friction force of the hair is observed. The friction force of the hair is increased with increases in the molecular weight of chitosan, especially at the highest molecular weight. The lowest friction force or the highest slip on hair surface is found at 3% w/v of 3.7×10^5 Da chitosan.

The virgin hair and emulsion without chitosan treated hair tend to have higher maximum load. The minimum load, indicating an easy combing, is obtained at 3% w/v of 3.7×10^5 Da chitosan, On the other hand, the highest molecular weight (8.5×10^5 Da) and increasing chitosan concentration increase the maximum load of hair

switches. The higher the chitosan emulsion concentration, the lower the maximum load needed to apply to the switches hair was found.

Suggestions

1. To further study the UV protection of the microemulsion of chitosan and green tea extract on hair coating.

2. To apply the microemulsion of chitosan and green tea extract in leave on and sunscreen product.

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APPENDICES

APPENDIX A

1. The HLB system

The HLB system basically consists of a means of ranking surfactants relative to a primary reference pair. Amphipathy is described by an HLB number, then the oil to be emulsified is assigned a required HLB. In simple terms, it only remains to match the HLB of the emulsifier blend, to that required by the oil.

The HLB of an emulsifier blend is calculated from:

$$HLB_{mix} = \frac{[W_A HLB_A + W_B HLB_B]}{[W_A + W_B]}$$

$$HLB_{mix} = \frac{[9.0 \times 4.3 + 1.0 \times 15]}{[9.0 + 1.0]} = 5.37$$

W_A is percent w/w of Span 80

W_B is percent w/w of Tween 80

HLB_A is HLB of Span 80

HLB_B is HLB of Tween 80

High HLB materials tend to promote o/w emulsions whilst low HLB materials tend to promote the w/o emulsions. The oil will exhibit the two required HLB is values, the HLB required to form an o/w emulsion and the HLB required to form a emulsion.

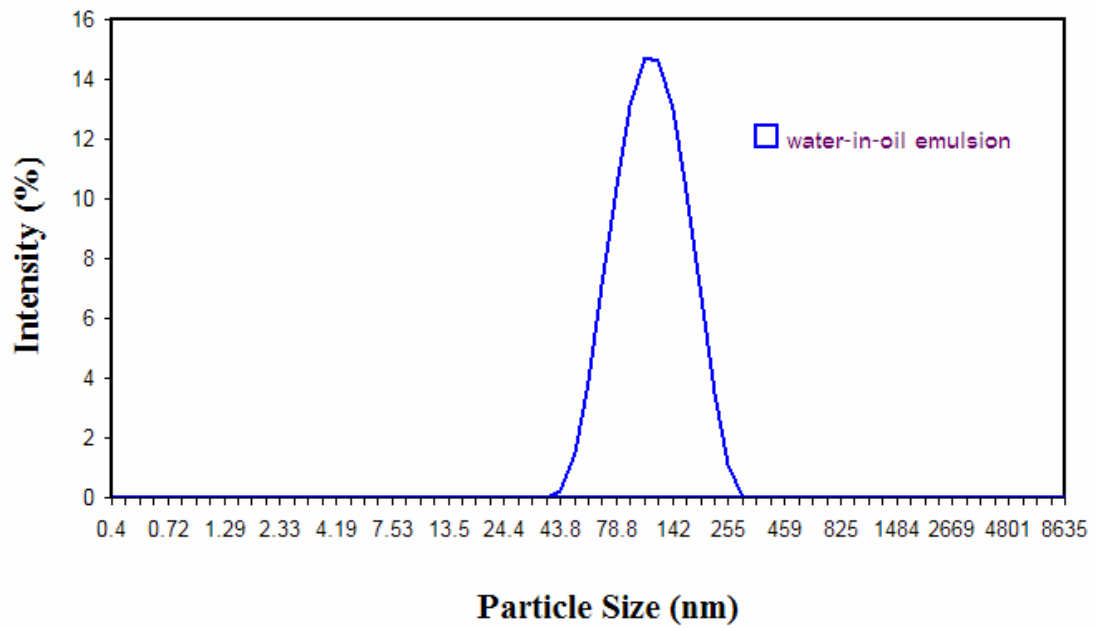## 2. Droplet diameter distribution of water-in-oil emulsion

Figure 1 Particle size of water-in-oil emulsion.

3. Miniature Tensile Tester (MTT)

This technique uses for measuring the tensile property of hair that is related to the hair strength

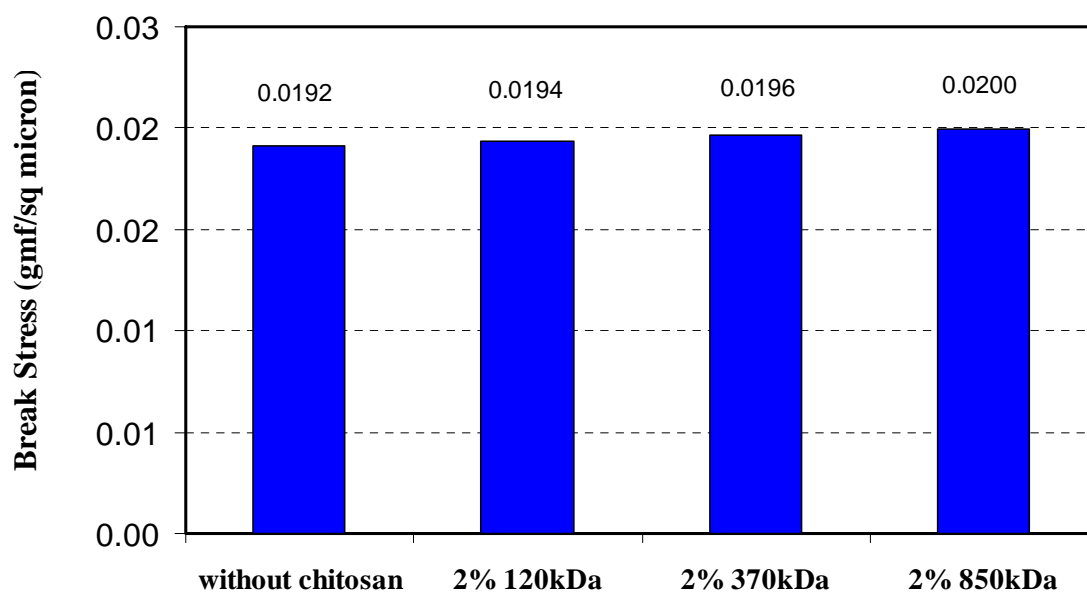| Break Stress (gmf/sq micron)		
Emulsion	**Mean**	**Std. Deviation**
without chitosan	0.0192	0.0187
2% 1.2×10^5 Da	0.0194	0.0197
2% 3.7×10^5 Da	0.0195	0.0182
2% 8.5×10^5 Da	0.0200	0.0227

Figure 2 Tensile strength of hair treated with and without chitosan emulsions at different concentrations and molecular weights.

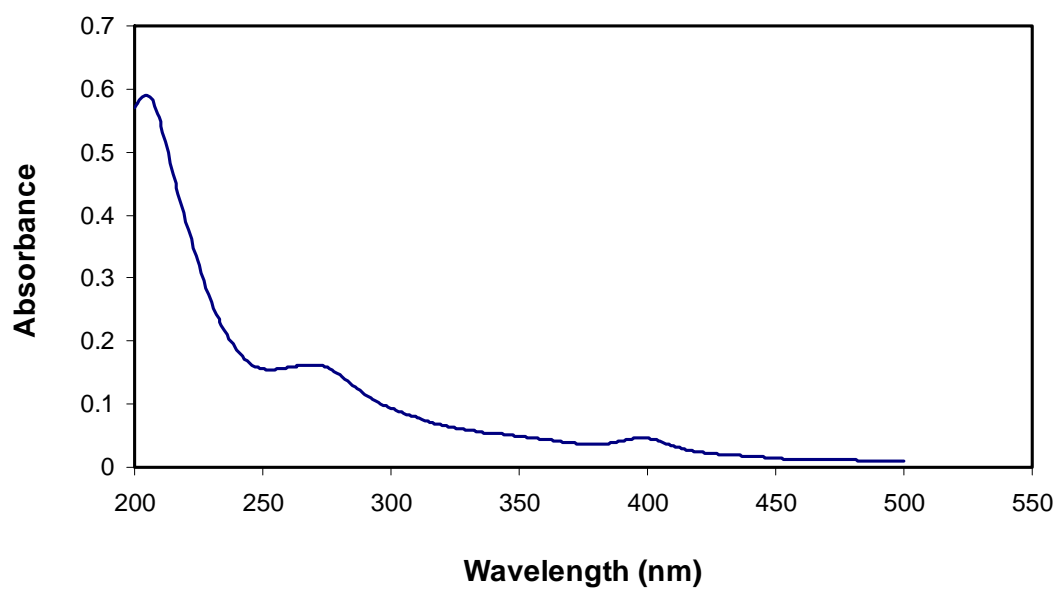## 4. UV absorption of green tea extract

Figure 3 UV absorption of 0.01 % green tea solution.
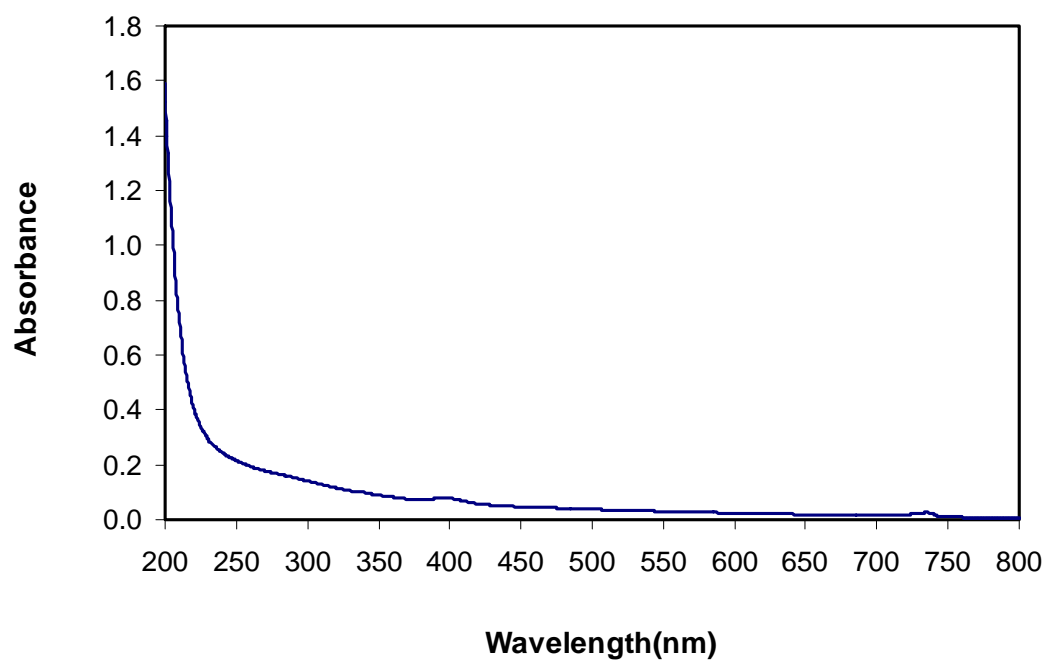
5. UV absorption of chitosan

Figure 4 UV absorption of 0.01 % chitosan solution (370kDa).

VITA

Miss Nutta Leepiyaraksa was born on March 6, 1975 in Bangkok. She received a Bachelor's Degree of Science in Chemistry from Ramkhamhaeng University in 2000. After graduation, she worked as a research at Unilever Thai Trading for 3 years before entering the Master's degree in the Program of Petrochemistry and Polymer Science, Faculty of Science, Chulalongkorn University.

She has presented this work "Preparation of microemulsion of chitosan and green tea extract" at the 12th National Graduate Research Conference, Khon Kaen University, February, 12-13, 2009.