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STABILITY OF ROSELLE, LAC AND GARDENIA COLORS IN MICELLAR SYSTEMS

Miss Piyarat Ketmaro

A Thesis Submitted in Partial Fulfillment of the Requirements
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สีธรรมชาติถูกนำมาทดแทนการใช้สีสังเคราะห์เนื่องจากมีความเป็นพิษต่ำ อย่างไรก็ตามการนำสีจากธรรมชาติมาใช้ยังคงมีข้อจำกัดในเรื่องความคงตัว งานวิจัยนี้จึงมุ่งศึกษาการเปลี่ยนแปลงและความคงตัวของสีที่สามารถละลายน้ำได้จากธรรมชาติ 3 แหล่งคือ สีจากกระเจี๊ยบ ครั่ง และลูกพุด เมื่ออยู่ในระบบไมเซลล์ที่เตรียมจากสารลดแรงตึงผิวที่มีประจุต่างกัน 3 ชนิด ได้แก่ ซีติลไตรเมทิลแอมโมเนียมโบรไมด์ (สารลดแรงตึงผิวประจุบวก) โซเดียมโดเดซิลซัลเฟต (สารลดแรงตึงผิวประจุลบ) และทวีน 80 (สารลดแรงตึงผิวที่ไม่มีประจุ) ที่ความเข้มข้น 1 5 10 และ 15 เท่าของความเข้มข้นวิกฤตไมเซลล์ คุณลักษณะทางเคมีฟิสิกส์ของสีที่สกัดได้ถูกประเมินด้วยวิธีต่างๆ คือ การดูดกลืนแสง ค่าคงที่การแตกตัว โครมาโตรกราฟีชนิดของเหลวสมรรถนะสูง และโครมาโตรกราฟีชนิดของเหลว-สเปกโตรเมตรีชนิดมวล ความคงตัวเบื้องต้นภายใต้ 6 สภาวะ (น้ำ อุณหภูมิ กรดด่าง ออกซิเดชัน และแสงยูวี) และความคงตัวของสีในระบบไมเซลล์ ถูกตรวจสอบโดยใช้การวิเคราะห์ด้วยเทคนิคสเปกโทรสโกปีในรูปแบบของค่าความเข้มของสี ความหนาแน่นของสีและร้อยละของสีที่คงเหลืออยู่ที่เวลาต่างๆ ร่วมกับการวัดสีด้วยระบบซีไออีแอลเอบี ค่าความยาวคลื่นที่สารสามารถดูดกลืนแสงสูงสุดของสีจากกระเจี๊ยบ ครั่ง และพุดมีค่าเท่ากับ 520 490 และ 443 นาโนเมตร ซึ่งสอดคล้องกับสีที่เห็นด้วยตาคือ สีแดง สีส้ม และสีเหลือง ค่าคงที่การแตกตัวปรากฏของสีกระเจี๊ยบและครั่งมีค่าเท่ากับ 3.00 ± 0.08 และ 5.96 ± 0.15 ตามลำดับ ผลโครมาโตรกราฟีชนิดของเหลวสมรรถนะสูงและโครมาโตรกราฟีชนิดของเหลว-สเปกโตรเมตรีชนิดมวลแสดงว่าสารสกัดสีกระเจี๊ยบประกอบด้วยเดลฟินิดีน-3-แซมบูไบโอไซด์และไซยานิดิน-3-แซมบูไบโอไซด์เป็นสารให้สีหลัก สีของครั่งเป็นสารผสมของแลคคาอิคแอซิดชนิด เอ บี และซี ในขณะที่สารประกอบหลักในสีของพุดคือโครซิน สารประกอบเอสเทอร์โครซิติน โมโนเจนติโอไบโอไซด์ โมโนกลูโคไซด์ และสารประกอบเอสเทอร์ของโครซิติน โมโนเจนติโอไบโอไซด์ สีของกระเจี๊ยบไม่คงตัวในสภาวะด่าง ภายใต้ความร้อน ภายใต้ออกซิเดชัน และภายใต้แสงยูวี สีของครั่งไม่คงตัวในสภาวะด่าง ภายใต้ออกซิเดชัน และภายใต้แสงยูวี ในขณะที่สีของพุดไม่คงตัวในทุกสภาวะ นอกจากนั้นลำดับความคงตัวของสีจากกระเจี๊ยบและครั่งในระบบไมเซลล์คือในบัฟเฟอร์มากกว่าไมเซลล์ของทวีน 80มากกว่าไมเซลล์ของเอสดีเอส ในขณะที่ลำดับความคงตัวของสีจากพุดคือในไมเซลล์ของเอสดีเอสมากกว่าบัฟเฟอร์มากกว่าไมเซลล์ของทวีน 80 การประเมินสีและการเปลี่ยนแปลงของสีโดยใช้เทคนิคยูวี-วิซิเบิล สเปกโทรสโกปีและระบบซีไออีแอลเอบีให้ผลที่สอดคล้องซึ่งกันและกัน วิซิเบิลสเปกโทรสโกปีแสดงอันตรกิริยาที่เกิดขึ้นระหว่างสีและไมเซลล์ได้อย่างเด่นชัด แต่วิธีนี้ไม่สามารถใช้ระบุรายละเอียดของสีที่มองเห็นได้ ระบบซีไออีแอลเอบีเป็นวิธีที่ดีกว่าในการระบุคุณภาพของสี แต่ไม่สามารถแสดงถึงอันตรกิริยาระหว่างสีและไมเซลล์

ภาควิชา วิทยาการเกษัชกรรมและเกษัชอุตสาหกรรม ลายมือชื่อนิสิต.....

สาขาวิชา เกษัชกรรม ลายมือชื่ออ.ที่ปรึกษาวิทยานิพนธ์หลัก.....

ปีการศึกษา 2552 ลายมือชื่ออ.ที่ปรึกษาวิทยานิพนธ์ร่วม.....

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PIYARAT KETMARO : STABILITY OF ROSELLE, LAC AND GARDENIA COLORS IN MICELLAR SYSTEMS. THESIS ADVISOR: ASST. PROF. WALAISIRI MUANGSIRI, THESIS CO-ADVISOR: ASST. PROF. PORNPEN WERAWATGANONE., 120 pp.

Natural colors were used in place of synthetic colors because of low toxicity. However, limitation of using natural colors is due to their stabilities. This research intends to study changes and stabilities of three natural water-soluble colors; i.e. roselle petal, lac stick, and gardenia fruit in 3 micellar systems; i.e. sodium dodecylsulphate or SDS (anionic surfactant), cetyltrimetylammonium bromide or CTAB (cationic surfactant), and Tween80 (non-ionic surfactant) at concentration of 1, 5, 10, and 15 folds of their CMC values. Physicochemical characteristics of the extracted dyes were evaluated using several techniques including visible spectroscopy, pK_a determination, HPLC, and LC-MS. Preliminary stability under six conditions (water, temperature, acid, base, oxidation, and UV light) and color stability in micellar systems were monitored by spectroscopy technique in terms of color intensity, color density, and percentage of color remaining at various times and CIELab system. The maximum wavelengths (λ_{max}) of roselle, lac, and gardenia colors were 528, 490, and 443 nm corresponding to visualized colors of red, orange, and yellow, respectively. The apparent pK_a of roselle and lac dyes were 3.00 ± 0.08 and 5.96 ± 0.15, respectively. HPLC and LC-MS showed that roselle dye composed of delphinidin-3-sambubioside and cyanidin-3-sambubioside as major coloring. Lac dye was a mixtures of laccaic acid A, B, and C, while the major components of gardenia dye were crocin, crocetin monogentiobiosyl monoglucosyl ester, and crocetin monogentiobiosyl ester. Roselle dye was unstable in alkaline, at elevated temperature, under oxidation, and under UV light. Lac color was unstable in alkaline, under oxidation and under UV light, while gardenia color was unstable in all conditions. Stabilities of roselle and lac dyes in micellar systems were in an order of in buffer > Tween80 micelles > SDS micelles while the color stability of gardenia was in an order of in SDS micelles > buffer > Tween80 micelles. Evaluation of color and color changes using UV-visible spectroscopy and CIELab system gave consistent results to one another. Visible spectroscopy clearly shows interaction between dyes and micelles but cannot describe observed color in details. CIELab system is a better way in describing color quality but cannot demonstrate dye and micelle interactions.

Department : Pharmaceutics and Industrial Pharmacy Student's Signature .....

Field of Study : Pharmaceutics Advisor's Signature .....

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CMC	=	Critical micelle concentration
ANOVA	=	Analysis of variance
mg	=	Milligram
g	=	Gram
ml	=	Milliliter
nm	=	Nanometer
SDS	=	Sodium dodecylsulphate
SLS	=	Sodium laurylsulphate
CTAB	=	Cetyltrimetylammonium bromide
HLB	=	Hydrophile-lipophile balance
max	=	Maximum
CIE	=	Commision Internationale de l’ Eclairage
C	=	Chroma
h	=	Hue
rpm	=	Rounds per minute
µm	=	Micrometer
N	=	Normality
M	=	Molarity
mM	=	Millimolarity
UV	=	Ultraviolet
A	=	Absorbance
HPLC	=	High Performance Liquid Chromatography
LC-MS	=	Liquid Chromatography-Mass Spectrometry
V	=	Voltage
psi	=	Pound per square inch
min	=	Minute
HCl	=	Hydrochloric acid
NaOH	=	Sodium hydroxide
H_2O_2	=	Hydrogen peroxide

CI	=	Color intensity
CD	=	Color density
amu	=	Atomic mass unit
K_a	=	Acid dissociation constant

CHAPTER I

INTRODUCTION

Colors play an important role in daily life. The color is employed to decorate many products such as food, beverage, cosmetic, medicine, etc. Since product appearance does not only catch consumer attraction but also increases consumer appreciation towards product quality. Moreover, color indicates product properties. In other words, color change implies a quality change.

The colors are divided into 2 groups synthetic colors and natural colors. Synthetic colors are man-made colors such as erythrosine, tratarzine, etc, while the natural colors are pigments or dyes made by living-organism such as chlorophyll from plants or resin from insect secretion, etc. Natural colors are gained popularity among consumers over years due to their safty. However, instability of natural colors limits their applications resulting in dull shades, rapid fading and color change.

Prevalence and popular natural colors in Thailand are red from roselle petal *(Hibiscus sabdariffa* Linn.) and lac resin *(Laccifer lacca* Kerr.) and yellow from gardenia fruit *(Gardenia jasminoides* Ellis. or cape jasmine). Major coloring compounds in roselle, lac, and gardenia are anthocyanins, laccaic acids, and crocin, respectively. Roselle and lac colors are very water soluble and their color shades in solution are pH-dependence. The main anthocyanins in roselle petals are delphinidin-3-sambubioside and cyanidin-3-sambubioside (Pouget, Vennet and Lejeune, 1990). Major compounds in lac color are laccaic acids which are in anthraquinone dye family. Crocin is classified in the group of carotenoid dyes. Crocin is a digentiobioside ester of crocetin, a dicarboxylic carotenoid. The gentiobiose, a β-1,6-diglucoside make this molecule water soluble (Hendry and Houghton, 1992).

Dyes and surfactants are always co-existed in several pharmaceutical, cosmetic, and food products. The surfactants are classified into three different groups according to charge on its hydrophilic headgroup; cationic, anionic, and non-ionic surfactant. It has been shown that charge and concentration of surfactant affected

color stability. Anthocyanins in sodium dodecylsulphate micellar system was more stable than anthocyanins in aqueous solution (Mulinacci et al., 2001). On the contrary, absorbances at absorption maxima ($A_{\lambda max}$) of Rose Bengal and Crystal Violet colors in Triton-X 100 micellar system were decreased over time (Kundu et al., 2006). Although natural colors from roselle, lac, and gardenia have been used for a long time, detailed studies on these dyes are few. This research focused on physicochemical properties of dyes including UV characteristics, pK_a values, and their color stabilities in micellar systems.

The objectives of this study were as followed;

To study

1. Change and stability of roselle, lac, and gardenia colors in micellar systems, which were prepared from three surfactants (cationic, anionic, and non-ionic surfactant).
2. Change and stability of roselle, lac, and gardenia colors in micellar systems, which were prepared from three surfactants at micellar concentrations.

CHAPTER II

LITERATURE REVIEWS

A. Background

Nowadays, the color of products has a strong influence on our perception of acceptability and palatability. Color is important as means to identify product, judge product quality, and evaluate basic esthetic value (Marmion, 1991). Consequently, for many years, colors play a predominant role in human products such as food, beverage, medicine, etc.

Natural colors are preferred to synthetic ones due to their safety; therefore, the use of natural colors has been considered (Francis F. J., 1989). However, natural colors have limitation on stability, which affects consumer utilization. Thais have been using natural water-soluble colors for centuries. Ones of the most popular natural colors in Thailand are red from roselle petal *(Hibiscus sabdariffa* Linn.) and lac resin *(Laccifer lacca* Kerr.) and yellow from gardenia fruit *(Gardenia jasminoides* Ellis. or cape jasmine). Major coloring compounds in roselle, lac, and gardenia are anthocyanins (Francis, 1989), anthraquinones (Watanabe and Terabe, 2000), and carotenoides (Pouget, 1990), respectively. Anthocyanins and anthraquinones in roselle and lac colors are very water soluble and their colors are pH dependent (Wrolstad, Durst and Lee, 2005). The main anthocyanin constituents in roselle petals have been reported as delphinidin-3-sambubioside and cyanidin-3-sambubioside, while the main components of anthraquinones in lac color are laccaic acids. Crocin is classified in the group of carotenoid dyes. Crocin is a digentiobioside ester of dicarboxylic carotenoid (crocetin). The gentiobiose is a diglucoside with a beta 1-6 linkage making the molecule water soluble (Hendry and Houghton, 1992).

Surfactants are generally used in pharmaceutical and cosmetic products. Surfactant molecules at a high concentration form a special structure, so called micelle (Sinko, 2006). The lowest concentration of a surfactant which can form micelle is known as critical micelle concentration (CMC). Organic molecules

solubilized by surfactant molecules reside in non-polar environment of micelle structure and/or interact with surfactant molecules or micelles. Moreover, some reactions are catalyzed in the presence of the micellar system (Fendler and Fendler, 1975). Therefore, changes in physicochemical properties and stabilities of natural colors in the presence of micelles would be very interesting studies.

B. Roselle, lac and gardenia colors

1. Roselle color

Roselle (*Hibiscus sabdariffa* Linn, Malvaceae) is a tropical plant found in many countries such as Thailand (Figure 1). Roselle is an herb used in the treatment of hypercholesterolemia, urinary bladder stone, and hypertension (Delgado-Vargas and Paredes-Lopez, 2002) and it is also a potential source of natural food colorants. The red dyes contained in the red petals of roselle are anthocyanins, which are ideal for producing brilliant red color in gelatin, jams, jellies, and fruit beverages.

The chemical constituents of roselle aqueous extract are organic acids, phenolic compounds, and anthocyanins. The major anthocyanins, which are responsible for reddish-violet color (Sukwattanasinit Burana-0sot and Sotanaphun, 2007) in roselle are delphinidin-3-sambubioside and cyanidin-3-sambubioside (Figure 2) at a relative proportion of delphinidin-3-sambubioside to cyanidin-3-sambubioside is 71.4 and 26.6%, respectively (Briddle and Timberlake, 1996 and Wong et al., 2002).

Figure 1 Roselle (*Hibiscus sabdariffa* Linn, Malvaceae)

The anthocyanins are water-soluble flavonoids, which are responsible for the most spectacular red, blue, and purple colors. In nature, the anthocyanins always exist as glycosides. Aglycones which are degradation products of acid or enzymatic hydrolysis are extremely unstable. Glycosidic substitution increases both stability and water solubility (Tsai, Hsieh and Huang, 2004). Increasing the number of sugar residues tends to increase dye stability (Lauro and Francis, 2000). The most common glycosidic sugars are glucose, galactose, xylose, arabinose, and rhamnose. The substitutions with other disaccharides are rutinose, sophorose, sambubiose, and gentiobiose. Chemical structure modifications on hydroxyl, methoxyl, glycosyl, and especially acyl groups can alter chemical stability of these anthocyanins. In addition, environmental factors such as temperature and light also have significant effects on anthocyanins (Du and Francis, 1973, Lozano and Ibarz, 1979, Gradinaru et al., 2003, Laleh et al., 2006 and NikkHah et al., 2007).

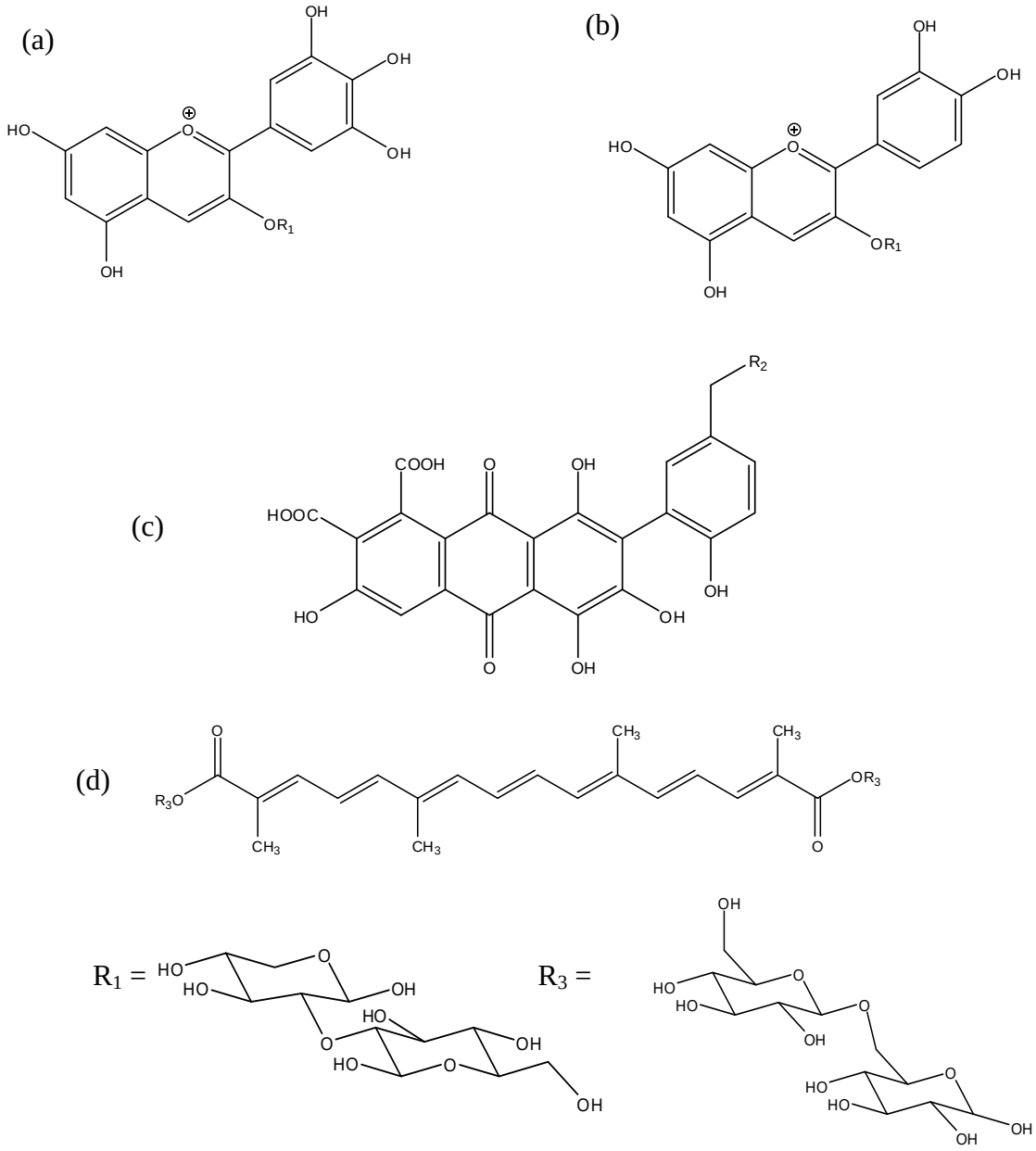

Figure 2 Chemical structures of delphinidin-3-sambubioside (R_1 = sambubiosyl, a), cyanidin-3-sambubioside (R_1 = sambubiosyl, b), laccaic acid (A; R_2 = CH_2NHAc, B; R_2 = CH_2OH, C; R_2 = $CHNH_2COOH$, E; R_2 = CH_2NH_2, c), and crocin (R_3 = gentiobiosyl, d) which are major compounds in roselle, lac, and gardenia, respectively

2. Lac color

Lac dye is a natural food additive extracted from a stick lac (Figure 3), which is a secretion of an insect, *Coccus laccae* (*Laccifer lacca* Kerr). The stick lac is better known as a source of shellac. Red lac dyes consisting of numerous chemical species are extracted with water and generated by the gravid female insect. The major components of lac color are anthraquinones; i.e. laccaic acid A, B, C, and E (Figure 2) (Oka et al., 1998, Watanabe and Terabe, 2000 and Hirata et al., 2001). Most of natural anthraquinones provide red color. Lac dye is widely used as a food colorant. Percentage of laccaic acids in lac is varied according to geography and time of the year depending on where and when the lac resin is harvested (Hendry and Houghton, 1992).

Figure 3 Stick lac from *Coccus laccae* or *Laccifer lacca* Kerr

3. Gardenia color

Gardenia color is a carotenoid giving yellowish color. Gardenia color is obtained by extraction with water or ethanol from the fruit of *Gardenia jasminoides* Ellis (Shan-zhi-I in Chinese, Figure 4). Gardenia is used as herbal medicine and a source natural dye. The gardenia fruit has sedative, antipyretic, diuretic, choleretic, and anti-inflammatory effects (Lauro and Francis, 2000 and Watanabe and Terabe, 2000). The yellow compound in gardenia fruit is crocin, a water soluble dye when compared to other carotenoids (Park et al., 2001) (Figure 2). The fruit also contains various types

of iridoids, of which geniposide is a formed water-soluble polymeric pigment (Mortensen, 2006). As a coloring agent, color from gardenia fruit has been used to give a yellowish color to food products such as noodle, pasta, candies, beverages, and pickled products in Japan (Watanabe and Terabe, 2000).

Crocetin is quite stable under high temperature and light. Crocetin retains it yellow color up to 95% and 85% of its original shade after it is stressed at 80 °C for 30 and 120 minutes, respectively (Choi et al, 2001). In addition, crocetin derivatives are stable after they are exposed to wide range of light wavelengths. The observed temperature and light stabilities are likely to relate to chemical structure of crocetin derivatives but not relate to type of sugar. However, the color tone varies with the sugar linkage. Crocin has two absorbtion maxima at 459 and 435 nm while the absorption maxima of crocetin are at 442 and 420 nm (Lauro and Francis, 2000).

Figure 4 Fruit of *Gardenia jasminoides* Ellis

C. Surfactant

1. Introduction to surfactants

Molecular structure of surfactants is composed of two portions, hydrophobic, and hydrophilic portions (Figure 5). The hydrophobic part is generally readily soluble in oil but sparingly soluble or insoluble in water, while the hydrophilic (or polar) part is sparingly soluble or insoluble in oil but readily soluble in water. The existence of the two totally different parts in the same molecule is responsible for the phenomenon of surface activity, solubilization, and micellization (Attwood and Florence, 1983).

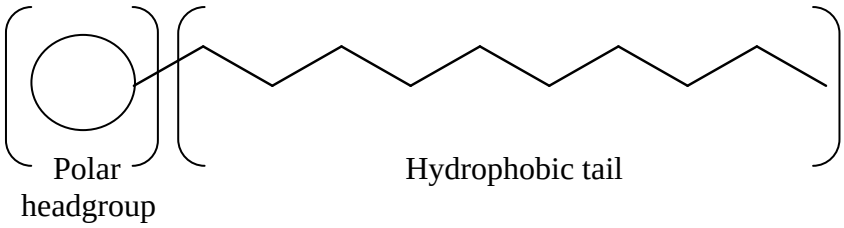

Figure 5 Schematic representation of a surfactant molecule

In aqueous solution, surface active agent rearranges itself as follows. At low concentration of surfactant when its molecules are freely dissolved, surfactant turns its hydrophilic portion towards aqueous solution and turns its hydrophobic segment toward the interphase and aqueous medium where it is energically more favorable than the water-water contacts. In other words, when the contact area between the hydrophobic group of the surfactants and the aqueous surrounding is reduced, free energy the system is reduced. Consequently, surfactant molecules tend to accumulate at interfaces, where the water contact is reduced. Another way to reduce oil-water contact is self-assembly to various structures, so that the surfactant molecules can reduce oil-water contact. Such structures are micelles, microemulsions and a range of liquid crystalline phases (Mukerjee and Mysels, 1971 and Sinko, 2006).

Surfactants are classified into the three groups according to changes on their polar head groups; i.e., anionic, cationic, and non-ionic surfactants.

1.1 Anionic surfactant

The largest group of surfactants is anionic surfactants. Anionic surfactants are products from reactions between high molecular weight organic compounds such as alcohol and an inorganic compounds such as sodium hydroxide or sulfuric acid. Generally, anionic surfactants present as soluble salt forms. Upon dissolution, the molecules dissociate to an ion and an anionic surfactant molecule. The organic part or the hydrophobic part of the molecule has no charge while the water-soluble part of the molecule has a negative charge. Anionic surfactants give good and stable foam. However, solubility of anionic surfactants are pH-dependence and limit in the presence of salt or mineral in water (Attwood and Florence, 1983 and Sinko, 2006).

One example of anionic surfactants is sodium dodecylsulphate (SDS) or sodium laurylsulphate (SLS) which is white or cream color crystals, flakes, or powder with bitter taste and a soapy and faint odor of fatty substance. In pharmaceutical formulations, SDS is used as emulsifying agent (0.5-2.5%), solubilizing agent (at concentrations higher than CMC; >0.0025%), wetting agent (1-2%), or detergent (9-45%). Its chemical formula is $C_{12}H_{25}NaO_4S$ or CH_3-$(CH_2)_{11}$-O-SO_3-Na^+ with a molecular weight of 288.38 (Figure 6). The surfactant is stable at pH 7 but hydrolyzed in solution below pH 4 (Boylan, Cooper and Chowhan, 1986).

1.2 Cationic surfactant

Cationic surfactants are received from reactions between alkyl halides and primary, secondary, tertiary fatty, or quaternary amines, which are ionized at all pH values. Thus, polar head group of the cationic surfactant molecule has a positive charge. Cationic surfactants possess antibacterial activity against a wide range of gram-positive and some negative organisms. They show good wetting property in acid media but they have no detergent action in alkaline solutions. Tissue irritation is a common toxicity of cationic surfactants. Therefore, applications of cationic surfactants in pharmaceutical or cosmetic preparations are limited when compared to that of non-ionic and anionic surfactants. An example of cationic surfactants is cetyltrimethylammonium bromide (CTAB) (Attwood and Florence, 1983 and Karsa, 1998).

Cetyltrimethylammonium bromide (CTAB) or hexadecyltrimethyl-ammonium bromide has chemical formula of $CH_3(CH_2)_{15}N(CH_3)_3Br$ with a molecular weight of 364.45 (Figure 6). Physical appearance of CTAB is a white free flowing powder with a faint characteristic odor. CTAB is water-soluble (1 to 2 of water) and freely soluble in alcohol but it is incompatible with soaps, anionic surfactants, or alkaline hydroxide. CTAB is a quaternary ammonium disinfectant, which has bactericidal activity against both gram-positive and gram-negative organisms. It is usually used in several formulations due to its detergency and emulsifying properties (Boylan, Cooper and Chowhan, 1986 and Reynolds, 1989 and Karsa, 1998).

1.3 Non-ionic surfactant

Non-ionic surfactants get this name due to their uncharged polar headgroups. The polar headgroups usually contain polyethylene oxide, which has a wide range of hydrophile/lipophile balance values (from 0 to 20) with neither a negative nor a positive charge in either part of the molecule. Non-ionic surfactants show excellent compatibility with other types of surfactants due to their uncharged nature. They are also less toxic in terms of skin irritation. Properties of non-ionic surfactants are not pH dependence. On the contrary, they are quite sensitive to salt and temperature. Some non-ionic surfactant solutions get cloudy in warm condition where the temperature exceeds its cloud point (Becher, 1962, Attwood and Florence, 1983 and Koshy, Saiyad and Rakshit, 1996).

An example of non-ionic surfactants is Tween80 (Polysorbate80 or polyoxyethylene 20 sorbitan, $C_{64}H_{124}O_{26}$) with a molecular weight of 1310 (Figure 6). The hydrophilic part of Tween80 is polyethers also known as polyoxyethylene groups. Physical appearance of Tween80 is a viscous, water-soluble yellow liquid. Tween80 is water miscible due to its high HLB value. In pharmaceutical formulation, it has been used as oil-in-water emulsifier (Boylan, Cooper and Chowhan, 1986 and Malmsten, 2002).

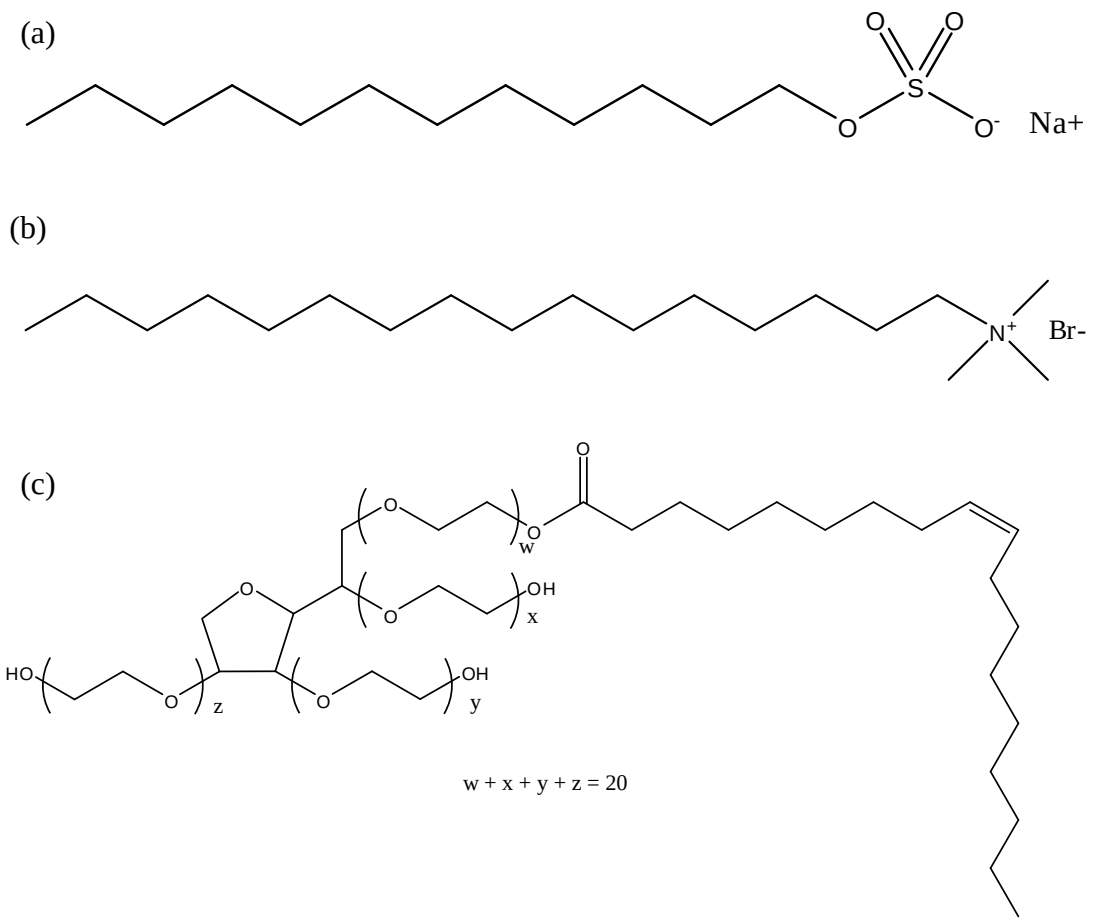

Figure 6 Chemical structure of sodium dodecylsulphate (a), cetyltrimatyl-ammonium bromide (b) and Tweeo80 (c)

2. Micellar system

CMC is a concentration at which surfactant molecules aggregate and form micelles. CMC is an important parameter in predictation micellar characteristics such as stability of substances in micellar system.

Micelle formation phenomena can be explained as follows. At low concentration of surfactant, surfactant molecules are freely dissolved in bulk aqueous and adsorbs at the air-water interface. As surfactant concentration is increased, more surfactant molecules accumulate and occupy all area at the interphase. Above the CMC in aqueous solution, surfactant molecules aggregate, by facing their hydrophilic part outwards to aqueous environment and pointing their hydrophobic tail inwards (Figure 7) . Micelles are dynamic and colloidal structure. Various techniques can be used for determination of CMC value including measurement of surface tension (Fendler and Fendler, 1975 and Sinko, 2006).

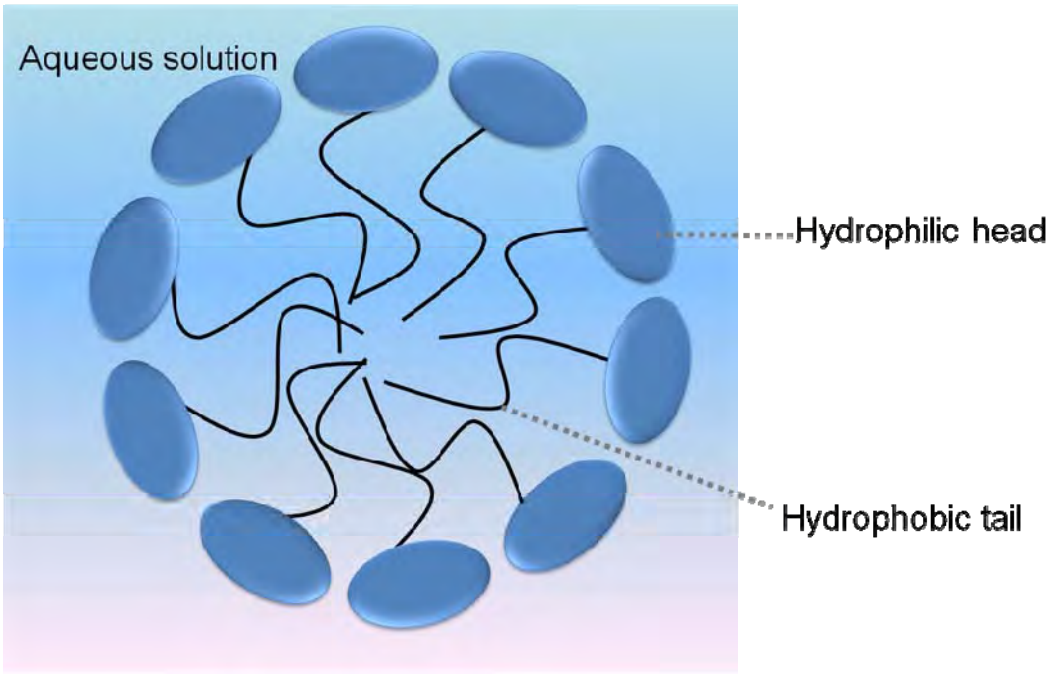

Figure 7 Diagram of micelle in aqueous solution

Shapes of micelles are affected by several factors such as surfactant concentration, temperature, pH, and ionic strength. As the concentration of surfactant increases, the shapes of micells are changed from spherical micelles to cylindrical micelles, middle phase, and neat phase (Figure 8) (Attwood and Florence, 1983).

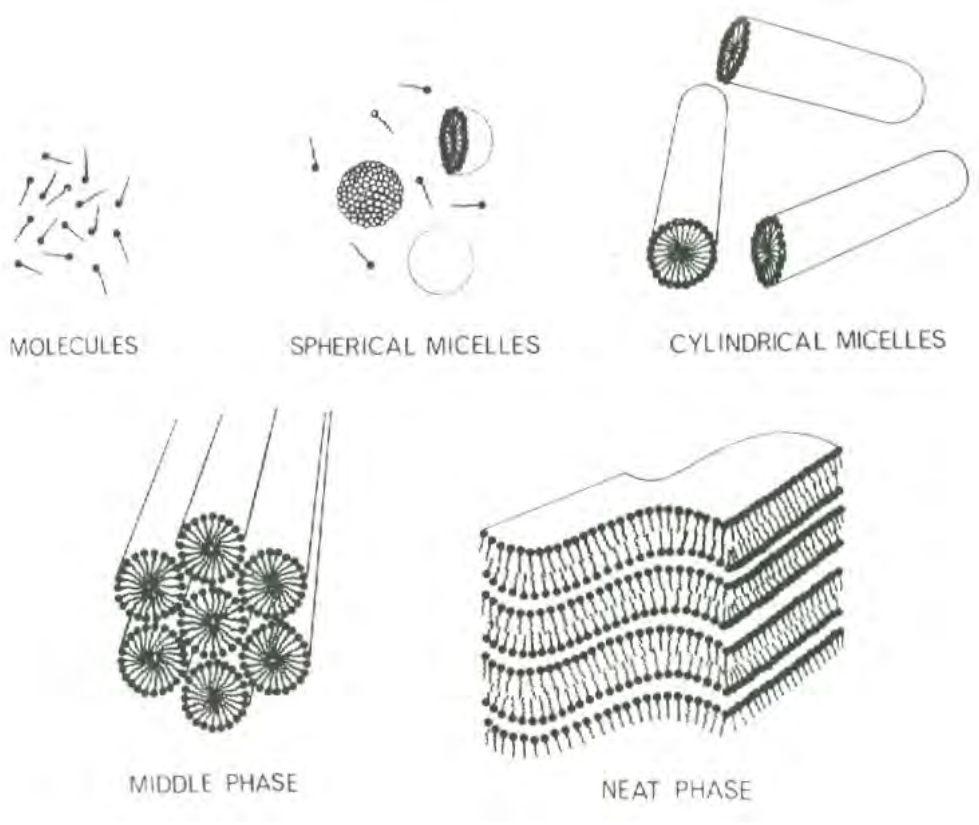

Figure 8 Scheme of micelle shapes as a concentration of surfactant increase in aqueous system (Corkill and Goodman, 1969)

Chemical kinetic reactions in micellar systems were found to be different from the reactions in solution without micelles (Fendler and Fendler, 1975). Micellar systems may facilitate or impede the reaction depending on properties of the reactants and the micelles. In previous study, the base hydrolysis of bis-*p*-nitrophenyl phenylphosphonate in three different micellar systems (SDS, CTAB and polysorbate (20) nonylphenol) was studied (Figure 9). In the presence of cationic surfactant, second-order rate constants for the base hydrolysis of bis-*p*-nitrophenyl phenylphosphonate were slowly increased as CTAB concentration was increased (Figure 9a). At a particular concentration range of CTAB, the rate constants of the reaction were abruptly increased and slowly decrease at very high concentration of CTAB. The concentration range where the reaction rate constants were steeply increased consistent with formation of CTAB micelles. In the other hand, the reaction rate constants were decreased in the presence of SDS and polysorbate (20) nonylphenol (Figure 9b and c).

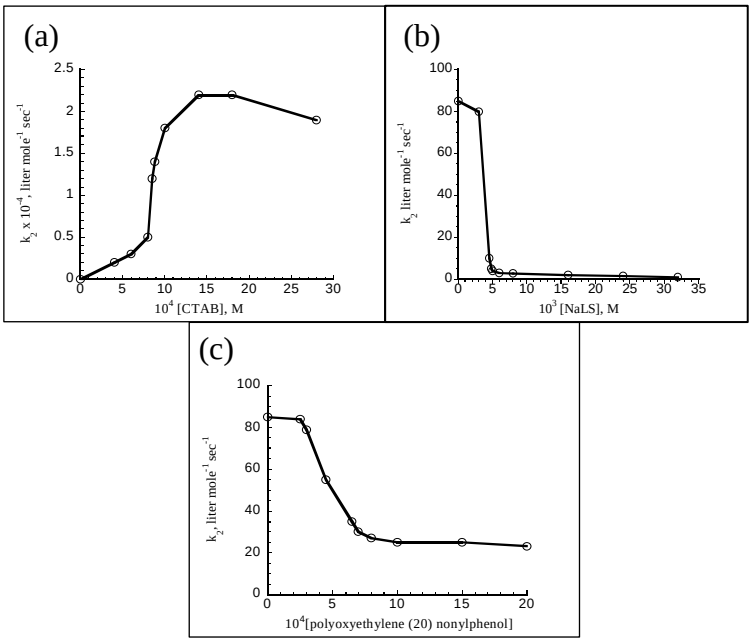

Figure 9 Second-order rate constants for the base catalyzed hydrolysis of bis-p-nitrophenyl phenylphosphonate at 25 °C as functions of the concentration of CTAB (a), SDS (b) and polysorbate (20) nonylphenol (c) in dioxane-water (5:95% v/v) (redraw from Fendler and Fendler, 1975)

In another study, CTAB micellar solution was reported to facilitate degradation of α-naphthyl acetate and dicapthon. However, this effect was retarded by an addition of non-ionic polymer (Werawatganone and Muangsiri, 2006)

3. Factors affecting CMC values

3.1 Hydrophobicity of surfactant molecules

CMC values of both ionic and non-ionic surfactants are decreased as the length of their unbranched hydrocarbon chains are increased (Patist, 1997). Moreover, substitute groups on hydrocarbon chain also affect CMC (Gerry, Jacobs and Anaker, 1997). Substitution of trifluoromethane ($-CF_3$) group on the terminal methyl group ($-CH_3$) of surfactant hydrocarbon chain decreases CMC values because fluorocarbon are more hydrophobic than hydrocarbons. Therefore, fluorocarbon promotes micellization. Chlorine, bromine, iodine, and fluorine show hydrophobic properties resulting in increases of hydrophobicity, decreases of CMC values, and increases of aggregation number.

3.2 Hydrophilicity of surfactant molecules

In general, CMC values of non-ionic surfactants are lower than that of ionic surfactants due to the lack of electrical repulsion force between polar headgroups of surfactant molecules. Anacker and Geer (1971) reported that aggregation of ionic surfactants is increased by the replacement of ethyl group associated with the polar head groups by ethanol group in a series of cationic surfactants. This observation was due to increasing of effective dielectric constant of polar head structure. In the same manner, as hydrophilicity at the polar head groups of surfactant molecules are increased, CMC values of such derivatives are decreased.

3.3 Nature of counterions

Counterions interact with ionic surfactants through ion-ion interaction force. Increase in counterion concentration results decrease in the CMC and increase in aggregation number the counterions neutralize charges on polar head groups of surfactants making the molecules less repulsion force. As a result, the CMC value is decreased and the aggregation is increased (Attwood and Florence, 1983).

3.4 Effect of additives

CMC value of ionic surfactant is decreased after an addition of electrolytes. Charge neutralized by an addition of electrolytes results in thickness reduction of ionic atmosphere surrounding the polar head groups, lowering CMC value, and increasing aggregation (Mukerjee and Mysels, 1971). For polyoxyethylated non-ionic surfactants, an addition of electrolyte lowered CMC values and cloud point (Bacher, 1962).

3.5 Effect of temperature

CMC of both ionic and non-ionic surfactants are altered according to temperature. The observed alteration depends on physicochemical properties of each surfactant. For ionic surfactants at elevated temperatures, water structure around the hydrophobic groups is disrupted resulting in increase of CMC values (Swarbrick and Daruwala, 1969). The major influence of temperature on non-ionic surfactants is the effect on solubility. At a temperature higher than cloud point, solutions of non-ionic surfactants possess a reversible phase separation. On the contrary, at temperature lower than cloud point they possess isotropic solutions.

3.6 Effect of pressure

Pressure elevation caused an increase of CMC followed by the CMC decrease at higher pressures. An induced pressure can increase the dielectric constant of water and other aspects related to water structure (Kaneshinas et al., 1974).

D. Color

1. Visualized Color (Hendry and Houghton, 1992, Berns, 2000, Westland, 2001, Delgado and Paredes, 2002 and MacDougall, 2002)

Color is certain wavelengths of light, which is reflected from an object. Objects can absorb some wavelengths of an incident light, especially at a maximum absorption wavelength (λ_{max}), diffract a portion of the incident light, and refract the rest of the incident light at a reflection angle. Human eyes observe the reflected light and interpret it as visualized color. Table 1 shows a relationship between absorption wavelength and corresponding visualized color of an object.

Table 1 Relation of wavelength of light absorbed and color perception in human eye (Hendry and Houghton, 1992)

Wavelength (nm)	675	600	585	570	540-525	490	460	410
Color absorbed	Red	Orange	Yellow	Yellow-green	Green	Green-blue	Blue	Violet
Color perception	Blue-green	Blue	Violet	Magenta	Orange	Yellow	Yellow-green	Green

Visualization involves eyes and brain. The reflected light stimulates retina in the eyes. The stimuli are transformed to nerve signals and translate to nerve cell known as rods and cones. The rods are sensitive to illumination and responsible for seeing in blur conditions (scopic vision; completely lacking in color), while cones are sensitive to color (photopic vision or color detection cone). There are three different types of rods and cones. Each type of cones absorbed blue, green, and red color at maximum absorption wavelengths of 430, 530, and 660 nm, respectively. When each type of rods couples with a particular type of cones and work together, the couple of rod and cone is called retina ganglion cell, that transfer signal to the brain. Because of this mechanism, the human visualization can characterize the color.

2. Trichromacy theory of vision

Cones in the retina code incident beam as three types of signals or channels before sending the signals to brain. The trichromatic Theory of color was first proposed by Thomas Young. The theory states that there are three primary colors. Each primary color purely generates signal to one of three channels. Other colors are obtained by mixing the three primary colors together at different ratios. This theory is also a basic operational principle in technology such as photography, television, and color printing (Bern, 2000 and Westland, 2001).

3. Color measurement

The common color measuring technique is classified into two techniques; spectroscopy technique and CIELab system.

3.1 Spectroscopy technique

Spectroscopy has been used in measuring color for a long time. Spectrophotometry is a measurement of light that transmits through materials. The principle components of the spectrophotometer are:

1. A stable source of incident light
2. A wavelength selector (filter monochromator) to isolate a desired wavelength from the source
3. Transparent container (cuvette) for housing sample and/or blank
4. A radiation detector (phototube) to convert the received transmitted light to a measurable signal; and a readout device that displays the signal (Berns, 2000).

The spectrophotometer can operate under a visible region (380-750nm) (Figure 10). White light from the light source, such as a tungsten-filament bulb, is spread out into spectrum using a prism or a diffraction grating. A slit is used to select a small portion of the spectrum to illuminate the sample. Wavelength of the light passing through the slit is varied by automatic scanning in order to cover the desired wavelength ranges. The monochromatic radiation illuminates the sample. The transmitted light reaches the detector and is converted to absorbance values present on a display.

According to Beer-Lambert law, the obtained absorbance is directly proportional to sample concentrations. An absorption maximum is a wavelength at which intensity of the transmitted light is the lowest.

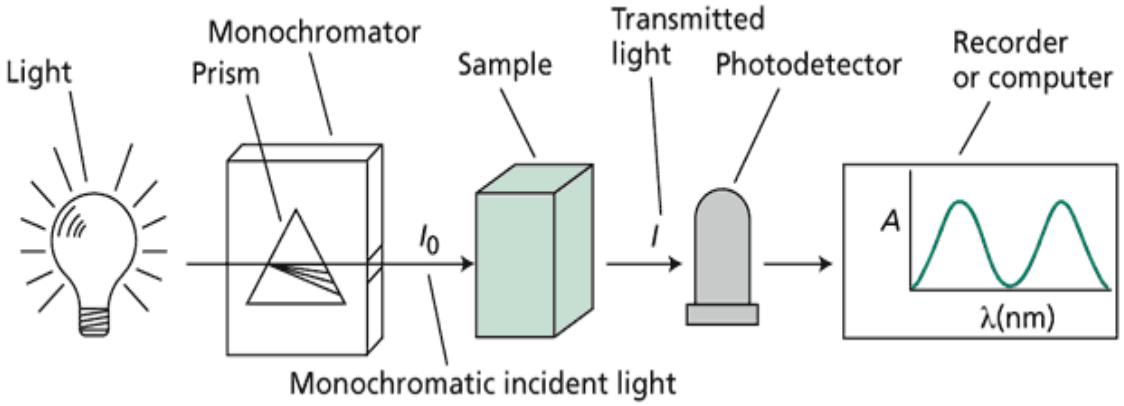

Figure 10 Spectrophotometer schematic diagram (Taiz and Zeiger, 2009)

3.2 CIELab system

Commission Internationale de l'Eclairage (CIE) is the French title of the international commission on light. The CIE developed a system for identification of color stimuli using tristimulus values from three primaries. The CIE created a set of standard colors, which are a mixture of the three primaries, and asked CIE standard observers to describe or identify the standard color stimulus. The results obtained from CIE standard observers are statistically analyzed based on known ratios of the three primaries which are used to create those stimuli. To identify unknown color, the unknown is compared to CIE standard colors. After, the perfect match is identified, color parameters for the unknown, which are *L*, *a*, *b*, and *h*, are known. *L* is lightness; *a* is redness/greenness; *b* is yellowness/blueness values and *h* is appearance of color (Yam and Papadakis, 2004 and Berns, 2000 and Westland, 2001).

There are illuminants that are widely used in color measurement including illuminant A, C, and D. The illuminants A and C are light produced from tungsten filament and mimic natural daylight, respectively. The illuminant C was not a good representation of daylight because it contains insufficient energy at the shorter wavelengths, so it has been replaced by the illuminant D which is the most identical to daylight (Westland, 2001 and Delgado and Paredes, 2002).

Later on CIELab color space was developed in order to allow color identification in term of a three dimensional space shown in Figure 11. The *L*-axis is the lightness, 0 (black) to 100 (white). The two coordinate *a* and *b* depict redness (positive value of *a*) to greenness (negative value of *a*) and yellowness (positive value of *b*) to blueness (negative value of *b*), respectively. For example, a sample with a = b = 0 is an achromatic sample (Gonnet 1998, Westland, 2001).

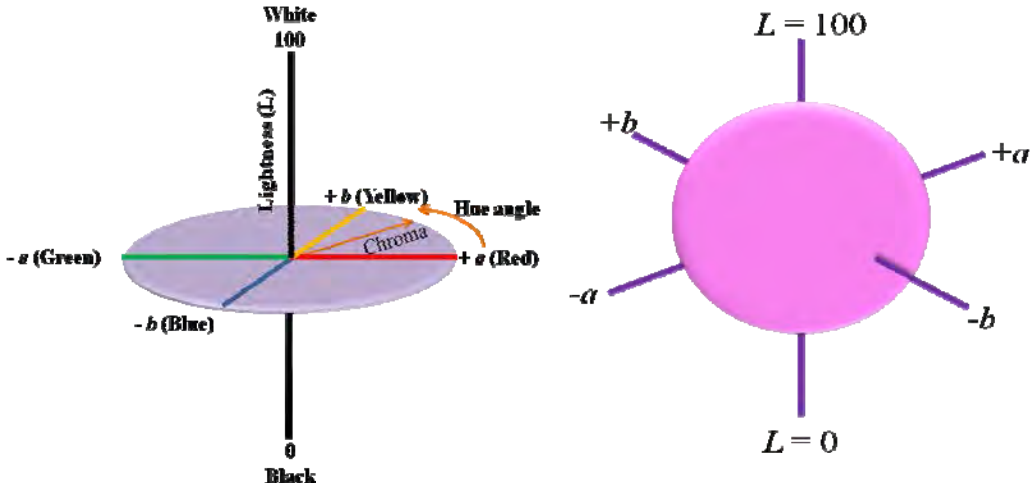

Figure 11 Scheme show CIELab color space (redraw from Westland, 2001)

Color identification is not only done using a point in a Cartesian coordinates (*a* and *b*) but also presents as chroma (*C*), hue (*h*), ΔH and Euclidean distance (ΔE) calculated as from equation 1-4, respectively. ΔH is difference in hue between two points, while ΔE provides information regarding color change in three dimension between two points.

$$C = \sqrt{a^2 + b^2} \qquad \text{Equation (1)}$$

$$h = \tan^{-1}(b / a) \qquad \text{Equation (2)}$$

$$\Delta H = \sqrt{(\Delta a)^2 + (\Delta b)^2 - (\Delta C)^2} \qquad \text{Equation (3)}$$

$$\Delta E = \sqrt{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2} \qquad \text{Equation (4)}$$

CHAPTER III

MATERIALS AND METHODS

Materials

1. Roselle petal (Nongkhai, Thailand)
2. Lac resin (Bangkok, Thailand)
3. Gardenia fruit (Bangkok, Thailand)
4. Sodium dodecylsulphate (CARLO ERBA reagent, Rodano, Italy, Lot No. 3N114094D)
5. Cetyl-trimethylammonium bromide (SIGMA ALDRICH Inc., St. Louis, USA, Lot No. 123K0077)
6. Tween 80® (SIGMA ALDRICH Inc., St. Louis, USA, Lot No. 046K0128)
7. Phosphoric acid (Mallinckrot Baker Inc., Hazelwood, USA, Lot No. C07812)
8. Sodium hydroxide (Merck, Damstadt, Germany, Lot No. B0119798 726)
9. Succinic acid (Ajax Fine Chemical, Seven Hills, Australia, Lot No. AF703191)
10. Oxalic acid (Ajax Fine Chemical, Seven Hills, Australia Lot No. 0710375)
11. Formic acid (CARLO ERBA reagent, Rodano, Italy, Lot No. 2G532272H4)
12. Sodium chloride (Merck, Damstadt, Germany, Lot No. K38447104 807)
13. Disodium hydrogen orthophosphate (Ajax Fine Chemical, Seven Hills, Australia, Lot No. F2F136)
14. Sodium dihydrogen phosphate (Merck, Damstadt, Germany, Lot No. KK220501045 530)
15. Methanol HPLC (Lab-scan analytical sciences, Dublin, Ireland, Lot No. 08040157)
16. Acetonitrile HPLC (Lab-scan analytical sciences, Dublin, Ireland, Lot No. 08020344)

17. Filter papers Whatman® No. 5 (Whatman International Ltd., Maidstone, England)
18. Cellulose acetate membrane with pore size 0.45 μm diameter 25 mm (Swinnex-25® and MF membrane Millipore®, Cork, Ireland, Lot No. R8KN1432)
19. Spectra/ Por® Dialysis membrane MWCO 1,000 (Spectrum Laoratories Inc., Dominguez, USA, Lot No. 3233067)
20. Self-masking Semi-micro flow cell Spectra/ Por® closures (Spectrum Laoratories Inc. , Dominguez, USA, Lot No. 3224285)
21. Vertipure Nylon Syringe filters, 13mm, 0.45μm (Vertical Chromatrography Ltd., Lot No. 51033, Thailand)
22. Quartz glass cuvette (Hellma®, Mullheim, Germany)

Equipment

1. Lyophilizer (Dura-Dry FTS. Systems™, Cambridge, USA)
2. UV-visible spectrophotometer (UV-1601, Shimadzu, Japan)
3. Refrigerated Incubator (FOC 225i, VELP® Scientifica, Milano, Italy)
4. Centrifuge machine (Sigma 302K, Bioblock Scientific, St. Louis, USA)
5. Disposible cuvettes PMMA (Plastibrand®, Belgium)
6. Dynamic contact angle meter and tensiometer (Dataphysics DCAT11, Germany)
7. Water bath (Model RTE17, Therma NESLAB, Waltham, USA)
8. Peristaltic pump (ISMATEC® model IMS829, Glattbrugg, Switzerland)
9. Magnetic stirrer (Model RCT basic, KIKA works Guaunghou, China)
10. pH meter (Orion model 420A, Orion Research Inc., USA)
11. Analytical balance (Mettler Toledo AX105 Delta Range®, Switzerland)
12. Photostability cabinet, Thailand
13. High-Performance Liquid Chromatography (HPLC) equipped with :
    - a solvent delivery module (LC-20AD, Shimadzu Corp., Japan)
    - a variable wavelength UV detector (SPD-M20A Diode Array Detector, Shimadzu Corp., Japan)

- a data integrating software (LC-20, Shimadzu Corp., Japan)
- an automatic sample injector (SIL-20AC HT, Shimadzu Corp., Japan)
- an oven (CTO-20A column oven, Shimadzu Corp., Japan)
- a degasser (DGU-20A_3 degasser, Shimadzu Corp., Japan)
- a communications bus module (CBM-20A, Shimadzu Corp., Japan)

14. Liquid Chromatography-Mass spectrometry (LC-MS) equipped with :
    - Agilent 1100 HPLC system, USA
    - a solvent delivery module (G1312A Binary pump, Agilent, USA)
    - a variable wavelength UV detector (G1315B Diode Array Detector, Agilent, USA)
    - an automatic sample injector (G1313A autosampler, Agilent, USA)
    - an oven (G1316A oven, Agilent, USA)
    - a degasser (G1379A degasser, Agilent, USA)
    - a communications bus module (CBM-20A, Agilent, USA)
    - Mass spectrometer (Bruker Daltonic “Esquire 3000+” Iontrap Mass spectrometer with API-ESI source, USA)

Methods

A. Preparation of lyophilized dyes

1. Extraction and preparation of lyophilized roselle color

Ten grams of roselle petal was soaked in 50 ml of water for 2 hours. The mixture was filtered through Whatman® filter paper number 5. The filtrate was lyophilized until dried powder was obtained.

2. Extraction and preparation of lyophilized lac color

Ten grams of grounded stick lac was soaked in 50 ml of water for 2 hours. The mixture was filtered through Whatman® filter paper number 5. In this study, centrifugation (at 5,000 rpm for 1 hour) and further filtration through cellulose acetate membrane with pore size of 0.45 μm (swinnex-25® and MF membrane Millipore®) were subjected to test if these steps were needed. The filtrate was lyophilized until dried powder was obtained.

3. Extraction and preparation of lyophilized gardenia color

Ten grams of grounded gardenia fruit was soaked in 50 ml of water for 2 hours. The mixture was filtered through Whatman® filter paper number 5. Addition of 95% ethanol (30 ml) and further filtration through Whatman® filter paper number 5 more investigated whether these step were needed. The filtrate was evaporated at 37 ˚C prior to lyophilize until dried powder was obtained.

B. Roselle, lac, and gardenia colors characterization

1. Absorption spectra characteristics of roselle, lac, and gardenia colors in buffer systems under visible light

Hydrochloric acid (0.1N), water, or sodium hydroxide (0.1N) were used as solvent to dissolve lyophilized powder of roselle, lac, and gardenia giving concentrations of 1.26, 0.32, and 0.23 mg/ml, respectively. Visible spectra of the solutions were taken in a visible region (400-700 nm) using a Shimadzu UV-1601 spectrophotometer.

Aqueous solutions of the colors from roselle and lac were prepared in 0.2M succinate buffer pH 4.5 with concentration of 5.2 and 0.25 mg/ml, respectively. Solution of dye from gardenia was prepared in 0.1M phosphate buffer pH 7 at a concentration of 0.23 mg/ml. Aliquots of 1% methyl paraben and 0.1% propyl paraben was added as preservatives to give final concentrations of 0.1% and 0.01% , respectively. Absorbance characteristics of all dye solutions in a visible region (400-700 nm) were studied.

2. pK_a determination of roselle and lac colors

The color solutions of roselle or lac in 0.05M phosphoric acid were placed in a glass jacketed vessel equipped with a magnetic stirrer. The solutions were gradually titrated with 20M sodium hydroxide to cover a pH range of 2 to 9 at 26 ± 1°C. After each addition of sodium hydroxide, the solutions were kept stirring for 30 seconds in order to obtain a homogenous mixture. An Orion Ag/AgCl combination electrode was used to monitor solution pH values. The solution was pumped through a peristaltic pump to a 1 cm path length quartz flow-through cell which was placed in the Shimadzu UV-visible spectrophotometer. The visible spectra were then collected. Absorbances at absorption maxima were determined. At each pH, dissociation constant (K_a) was calculated based on absorbances using an equation present in Albert and Serjeant (1984) (Equation 5).

$$pK_a = pH + \log\left[\frac{A_{HA} - A_{obs}}{A_{obs} - A_{A^-}}\right] \quad \text{Equation (5)}$$

where A_{HA} is absorbance of protonated form

A_{A^-} is absorbance of unprotonated form

A_{obs} is absorbance of color at any pH

The average value for the pK_a and its standard deviation were estimated from the K_a values. All experiments were done in triplicate (n=3).

3. Screening characterization of roselle, lac, and gardenia colors by HPLC

Roselle dye

Roselle dye solution was in 0.2M succinate buffer pH 4.5 to make a final concentration of roselle dye at 5.2 mg/ml. Separation of components in roselle color was performed on a Shimadzu LC20 connected to a C_{18} column (5 μm), 250 x 4.60 mm (Phenomenex®) protected by a guard column containing C_{18} packing (Comesky et al, 2009). The column was maintained at 40 °C. Mobile phase A consisted of 5% formic acid in distilled water, whereas mobile phase B was 100% HPLC grade acetonitrile. Separation was carried out for 20 minutes at an analytical wavelength of 530 nm. The elution profile was a linear gradient elution with 5% B to 20% B from 0 to 10 minutes, 20% B to 80% B from 10 to 13 minutes and then isocratic elution at 80% B from 13 to 17 minutes, finally the linear elution with 80% B to 5% B from 17 to 20 minutes. The flow rate was 0.8 ml/min and injection volume of sample was 5 μl.

Lac dye

Lac dye solution (0.25 mg/ml) was prepared in the 0.2M succinate buffer pH 4.5. Lac dye solution was separated using a Shimadzu LC20 connected to a C_{18} column (5 μm), 250 x 4.60 mm (Phenomenex®) protected by a guard column containing C_{18} packing. Separation system was modified from Oka et al. (1998). Mobile phase was acetonitrile HPLC grade-0.01M oxalic acid (1:4 v/v). The flow rate was 0.8 ml/min and injection volume of sample was 5 μl. Laccaic acids were monitored at 280 nm. Separation was carried out for 25 minutes.

Gardenia dye

Gardenia dye solution (0.23 mg/ml) was prepared in 0.1M phosphate buffer pH 7.0. Separation of crocin was perform on a Shimadzu LC20 equipped with a C_{18} column (5 μm), 250 x 4.60 mm (Phenomenex®) protected by a guard column containing C_{18} packing. The column was eluted with gradient system of distilled water and methanol. Separation system was modified from Lauro and Francis (2000).The elution profile was 50% methanol for 15 minutes and then linear gradient to 100% methanol from 15 to 30 minutes, the finally isocratic elution at 100%

methanol from 30 minutes to 40 minutes. Injection volume of sample was 5 µl and flow rate was 0.75 ml/min. The HPLC chromatogram was monitored at 433 nm.

In all cases, the mobile phase was freshly prepared, filtered through 0.45 µm nylon membrane filter and degassed by sonication for 30 minutes prior to use.

4. Screening characterization of roselle, lac, and gardenia colors by LC-MS

Roselle

Separation of roselle color was conducted on a Symmetry C_{18} (5µm), 250 x 4.6 mm, maintained at 35 °C using a Agilent 1100 HPLC system and photodiode array (PDA) detector operated at 530 nm. Separation system was modified from Comesky et al. (2009). The elution solvents were 5% formic acid in distilled water and 100% HPLC grade acetonitrile. The Separation system was same as in HPLC condition of roselle dye (in 3). The ESI-MS data were acquired in the positive mode using a data-dependent LC-MS method. The ESI voltage, capillary temperature, and sheath gas pressure were 4000 V, 300 °C, and 30 psi, respectively (Comesky et al., 2009).

Lac

Lac dye was prepared in 0.01M oxalic acid and characterized by using the Agilent 1100 HPLC system. A chromatograph equipped with photodiode array (PDA) detectors operated at 280 nm. LC system was developed by Central Instrument Facility, Mahidol University. The chromatographic system included a Symmetry C-18 (5µm), 250 x 4.6 mm with a mixture of acetonitrile : distilled water (1:4 v/v) as a mobile phase at a flow rate of 0.8 ml/min. The mass spectrometer and the data system were performed on an electrospray source operated at a scan rate of 400 u/s in the negative-ion mode. A gas sheath flow of 50 psi nitrogen, electrospray voltage of 4000 V, and capillary temperature of 300 °C were used (Oka et al., 1998).

Gardenia

Gardenia color was characterized using the Agilent 1100 HPLC system. A HPLC chromatogram was monitored at 433 nm using a photodiode array detector (PDA). The separation was performed on a Symmetry C-18 (5µm), 250 x 4.6 mm with the same elution gradient program as the condition of gardenia (in 3) (Lauro and Francis, 2000.). The mobile phase was water and methanol. Flow rate was 0.75 ml/min. The mass spectrometer equipped with an electrospray source operated at a scan rate of 400 u/s under the positive-ion mode and trap drive was 71.6. A gas sheath flow of 40 psi nitrogen, electrospray voltage of 4000 V, and capillary temperature of 300 °C were used (Choi et al., 2001).

C. Preliminary stability of natural colors from roselle, lac, and gardenia color under stress conditions

Stability of color solutions were investigated under six conditions; i.e., in water, at elevated temperature, in acid (0.1N hydrochloric acid, HCl), in base (0.1N sodium hydroxide, NaOH), in 3% hydrogen peroxide (H_2O_2), and under UV light. The lyophilized dye powder was dissolved in the water and kept in dark chambers at 4, 30, and 72˚C or in a UV light chamber (Photostability cabinet, Thailand) at 30˚C. 0.1N HCl, 0.1N NaOH, or 3% H_2O_2 were used to dissolved roselle, lac, and gardenia to give concentrations of 1.26, 0.32, and 0.23 mg/ml, respectively. Samples were kept in glass vials tightly sealed with rubber stoppers and aluminium seal. At appropriate time, UV absorbances of the samples were measured. Percentage of color remaining was calculated based on absorbance at maximum wavelengths (λ_{max}) and Equation (6).

$$\% \text{ color remaining} = \frac{A_{\lambda_{max},t}}{A_{\lambda_{max},to}} \text{ x } 100 \qquad \text{Equation (6)}$$

D. Critical micelle concentration (CMC) determination of sodium dodecylsulphate (SDS), cetyltrimethylammonium bromide (CTAB), and polysorbate80 (Tween80)

In order to obtained CMC values, surface tension measurements were performed on a Dataphysics DCAT11 tensiometer equipped with a Wilhelmy plate at 26 ± 1 °C. A series of CTAB, SDS, or Tween80 at various concentrations were prepared in 0.1M phosphate buffer pH 7.0 or 0.2M succinate buffer pH 4.5 with an addition of 0.1% methyl paraben and 0.01% propyl and adjusted ionic strength to 0.25M using sodium chloride. Surface tension values of the solutions were measured. Surface tension of distilled water was evaluated to ensure that the instrument was in a good operational condition. To calculate CMC for each system, the surface tension values were plotted against the surfactant concentrations on a semilogarithmic scale. All experiments were done in triplicate (n=3).

E. Stability of roselle, lac, and gardenia colors in micellar systems

Roselle or lac solutions at concentrations of 5.2 and 0.25 mg/ml, respectively were prepared in 0.2M succinate buffer pH 4.5 while 0.23 mg/ml gardenia fruit color solution was prepared in 0.1M phosphate buffer pH 7 in the represent or in the absence of CTAB, SDS, or Tween80 micellar systems. Concentrations of micellar systems were varied to be 1, 5, 10, and 15 folds of their CMC values. Each system was adjusted ionic strength 0.25 M using sodium chloride and preserved using an addition of 0.1% methyl paraben and 0.01% propyl paraben. The solutions were filled in well-closed glass vials, protected from light and kept in a stability chamber at 30 ˚C for 2 months or 3 half lives of the colors. At appropriate time, samples were analyzed for pH values and for color stability using spectrophotometry technique and CIELab system. The color solutions in the absence of surfactant under the test conditions were used as controls. All experiments were done in triplicate (n=3).

F. Evaluation of color changes and color stability of roselle, lac, and gardenia dye solutions

1. Determination of color change and color stability using spectroscopy technique

At appropriate time, samples which were prepared in step E were taken and evaluated for color change using UV-visible spectrophotometer under a visible range (400-700 nm). Absorbances at absorption maxima at initial time ($A_{\lambda max,\ t0}$) and at time t ($A_{\lambda max,\ t}$) were determined. The percentage of color remaining was calculated using Equation (6)

$$\%\ \text{color remaining} = \frac{A_{\lambda_{max},t}}{A_{\lambda_{max},to}} \text{ x } 100 \qquad \text{Equation (6)}$$

Color changes were illustrated by plots between percentages of color remaining versus time. Two addition parameters; i.e. color intensity (CI) and color density (CD), were also employed in evaluation of color stabilities. CI is absorbance at absorption maxima at any time (Equation 7). CD is a summation of absorbance at 420, 520, and 620 nm (Equation 8) (Fan et al, 2008)

$$\text{color intensity} = A_{\lambda_{max}} \qquad \text{Equation (7)}$$

$$\text{color densiy} \quad = A_{420} + A_{520} + A_{620} \qquad \text{Equation (8)}$$

where $A_{\lambda\ max}$ is absorbance at maximum wavelength at time t

A_{420} is absorbance at 420 nm

A_{520} is absorbance at 520 nm

A_{620} is absorbance at 620 nm

2. Determination of color change and color stability using CIELab system

At appropriate time, samples, which were prepared in step E, were taken and evaluated for color change using CIELab system. The color solutions were transferred into disposable cuvettes which were later scanned by a HP scanjet G4010. The scanned pictures were saved as TIFF files. The digital files were analyzed for colors using Adobe Photoshop software CS version in terms of lightness (L),

redness/greenness (*a*), and yellowness/blueness (*b*) values (Yam and Papadakis, 2004). These parameters were employed in calculation of *C*, *h*, Δ*H*, and Δ*E* using the following equations

$$C = (a^2 + b^2)^{0.5} \quad \text{Equation (1)}$$

$$h = \tan^{-1}(b/a) \quad \text{Equation (2)}$$

$$\Delta H = ((\Delta a)^2 + (\Delta b)^2 - (\Delta c)^2)^{0.5} \quad \text{Equation (3)}$$

$$\Delta E = (\Delta L^2 + \Delta a^2 + \Delta b^2)^{0.5} \quad \text{Equation (4)}$$

where
- *C* is chroma, which is intensity or shade of color
- *h* is hue, which is appearance of color
- Δ*H* is different of hue at any time
- Δ*E* is information regarding color change in three dimension between two points

In all measurement, lightness of a white paper was used as a base line to ensure that light was not a confounding parameter. The color measurements were performed on eight positions of each cuvette picture as described in Figure 12.

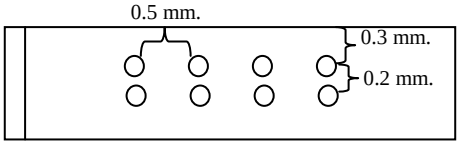

Figure 12 Diagram showing the positions of color measurement in CIELab system

CHAPTER IV

RESULTS AND DISCUSSION

A.Preparation of lyophilized dyes

In this study, the colors were extracted using water extraction technique and lyophilized to obtain dried powder. The physical appearance of lyophilized dyes from roselle, lac, and gardenia are pink-red bulky powder, dark red bulky powder, and yellow fibered-like cake, respectively. Absorbance and visible-spectra characteristics of dye solutions (before lyophilization) and reconstituted dye solutions (after lyophilization) at the same concentration were compared. Visible-spectra of the dye solutions before and after lyophilization process were superimposed (Appendix A.3) and insignificant change in visible absorbance was observed. Therefore, the lyophilization process did not cause degradation of the dyes.

Percent yield of roselle, lac, and gardenia from extraction process are shown in Table 2. Roselle extract gave the highest yield. However, it was highly likely that the dried powder was a mixture of delphinidin, organic acid, and sugar which were presence in roselle petal (Tsai et al., 2002, Wong et al., 2002 and Hsieh et al., 2008). Organic acids and sugar may directly affect stability of dye (Hubbermann et al., 2006) but these compounds were inseparable using the simple and the most popular extraction technique, the water extraction technique. Regarding lac and gardenia, high standard deviations of percent yields of lac and gardenia lyophilized dye were observed probably due to inconsistency of the extraction procedure. In preliminary study, lac dye solution in acidic condition showed some precipitation, while the gardenia lyophilized powder possessed a fiber-like characteristic implying the presence of polymer in the lyophilized extract. Therefore, extraction method of lac and gardenia would be further developed in order to obtain reproducible extraction procedure and dyes with higher purities.

Table 2 The percent yields of roselle, lac, and gardenia by water extraction

Color extract	Percent yield (Mean ± SD)
Roselle	31.96 ± 0.32
Lac	1.99 ± 1.47
Gardenia	9.59 ± 2.52

Further purification of lac and gardenia dyes was done by filtration through cellulose acetate membrane with pore size of 0.45 μm and addition of 95% ethanol, respectively. Ethanol was employed to precipitate the polymer presence in gardenia dye solution. The mixture was then filtered through a Whatman® filter paper number 5 in order to separate the precipitated polymer. The filtrate was dried using a water bath at 37 °C followed by further lyophilization. After purification, the obtained percent yields of lac and gardenia dyes were 2.79 ± 0.23 and 13.66 ± 0.88%, respectively. These results showed high percent yield when compared to that of the preliminary studies. This observation was probably due to variation between batches of raw materials and better extraction procedure. This speculation was consistent with the fact that variation in sources and harvesting time of the year was proven to be a source of % ingredient variation (Hendry and Houghton, 1992 and Lauro and Francis, 2000). The physical appearance of lyophilized dyes from roselle, lac and gardenia are pink-red bulky powder, dark red bulky powder, and yellow flake, respectively (Figure 13).

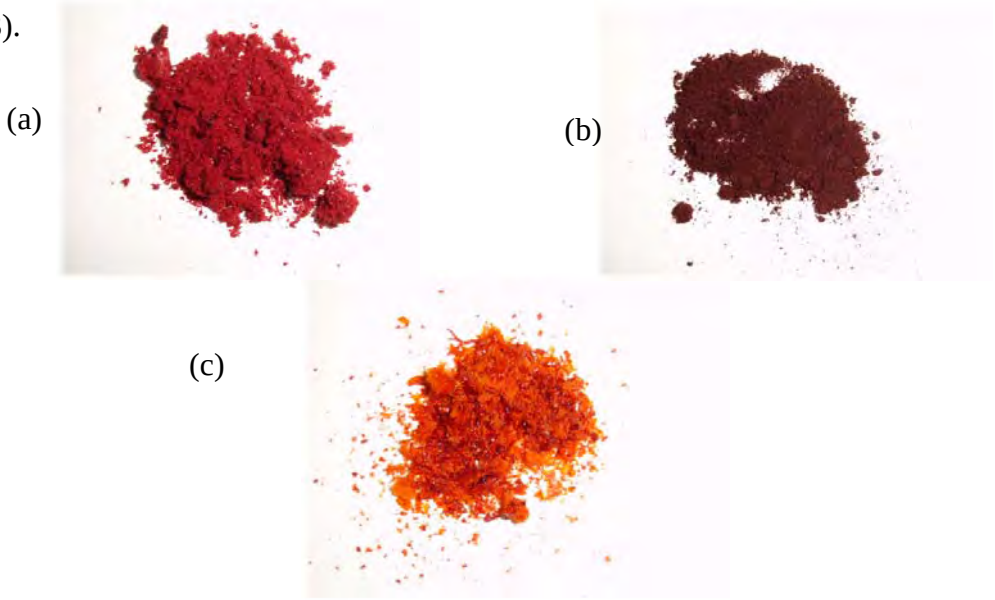

Figure 13 The lyophilized dye of roselle (a), lac (b), and gardenia (c)

B. Roselle, lac, and gardenia color characterization

Stability of roselle, lac, and gardenia dyes were evaluated under stress conditions. Visible characteristic of dyes in 0.1N HCl, water and 0.1N NaOH are shown in Figure 14.

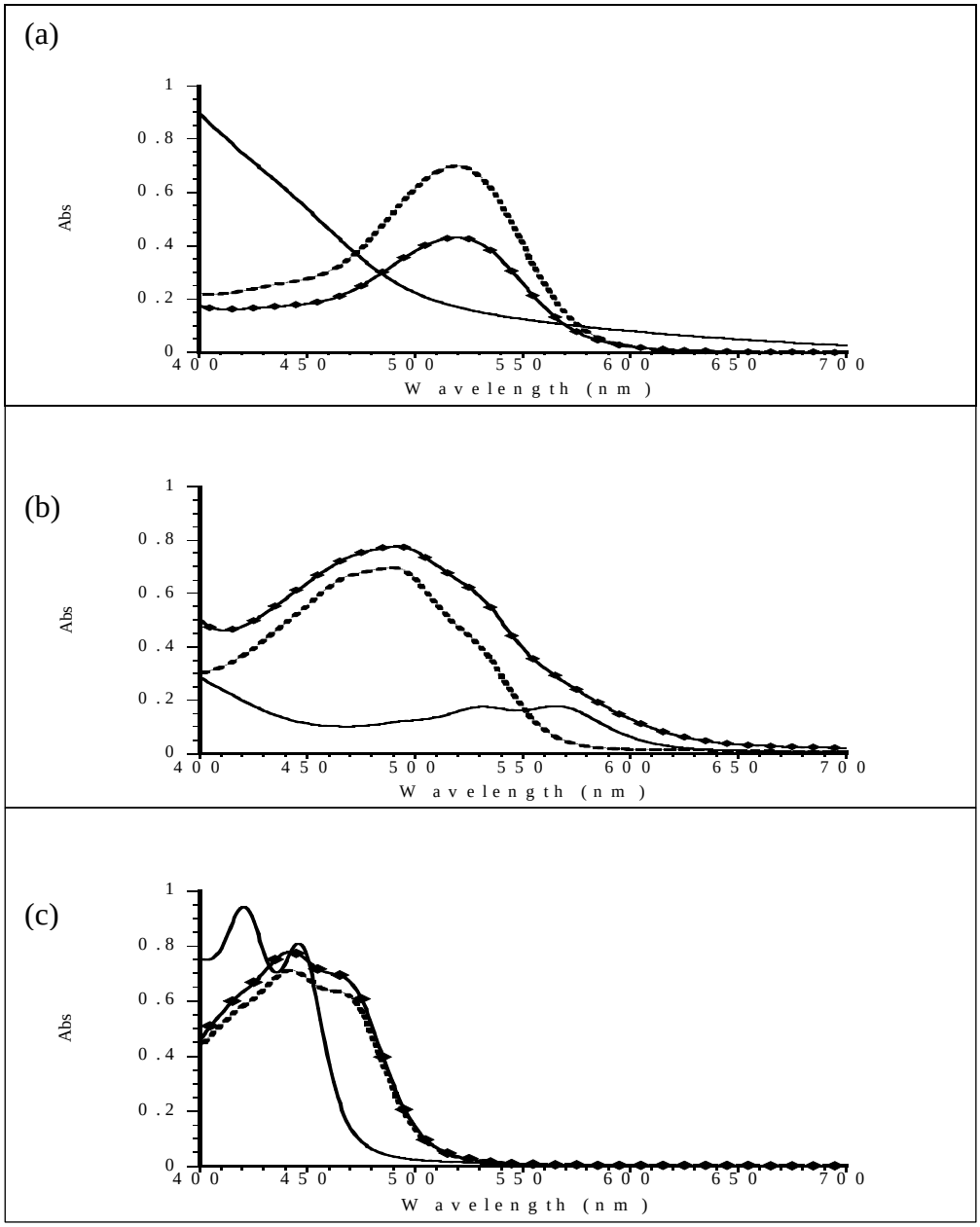

Figure 14 UV-visible spectra of roselle (a), lac (b), and gardenia (c) colors in 0.1N HCl (-----), 0.1N NaOH (—), and water (—◆—)

The observed color of roselle dye solution was varied from red-pink, pink to yellowish in different solvents ranging from acid (1N HCl), neutral (water) to alkaline (1N NaOH), respectively. The λ_{max} of roselle color in acid condition and water was 520 nm (Figure 14a). In alkaline condition, the absorbance at 520 nm disappeared and higher absorbance was observed at 420 nm which was consistent with a result reported by Fan et al. (2008). In other words, roselle solution underwent hypsochromic shift in alkaline condition. Delphinidin-3-sambubioside has been reported as a major coloring compound in roselle (Sukwattanasinit Burana-Osot and Sotanaphun, 2007), which has different chemical forms depending on pH. In acid condition, most of delphinidin-3-sambubioside is in a form of flavylium cation giving red color. As pH value increases, the flavylium cation form is deprotonated to blue quinonoidal base and further degraded to colorless carbinol pseudobase which is finally transformed to yellowish chalcone (Figure 15). In other words, in alkaline conditions, the anthocyanins degrade to yellow chalcone (Ovando et al., 2008, Markakis, 1982 and Lauro and Francis, 2000) as shown in the below degradation scheme.

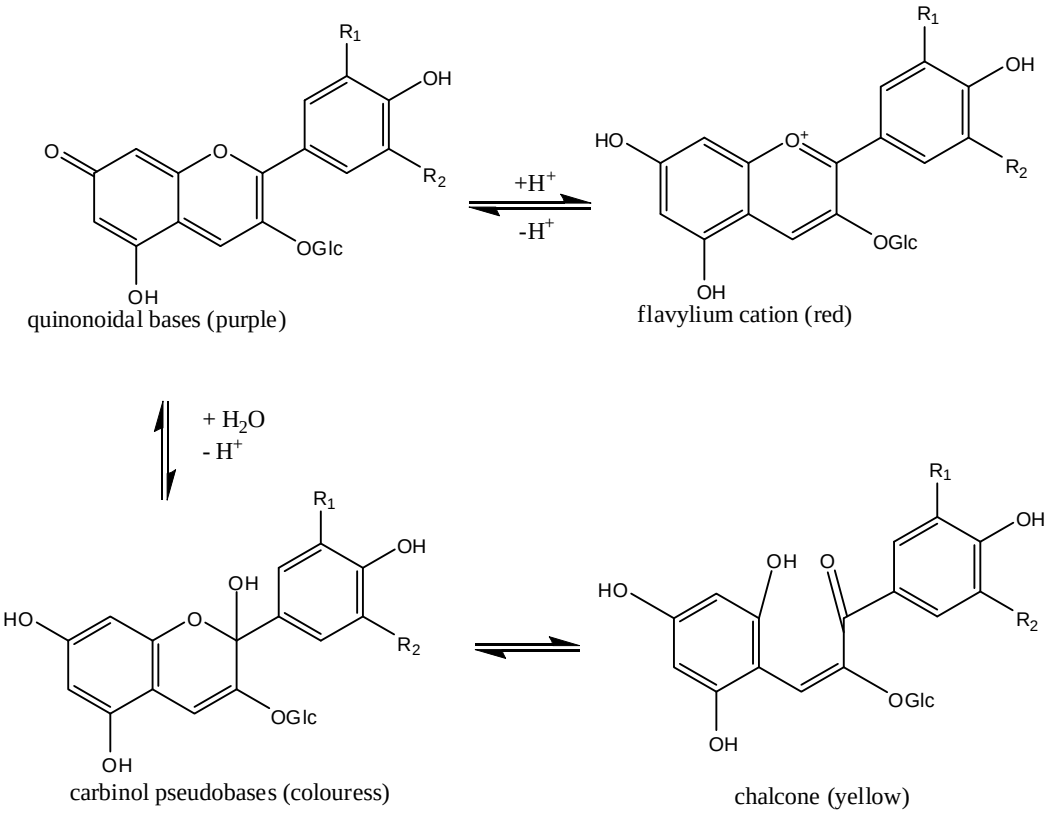

Figure 15 Degradation scheme of anthocyanins in various pH

HPLC analysis was employed to characterize chemical compounds presence in roselle dye solution using a modified HPLC method developed by Comeskey et al. (2009). The HPLC chromatogram of roselle dye revealed two major peaks at retention times of 8.9 and 9.5 minutes (Figure 16a). The observed chromatographic pattern was consistent with the chromatographic pattern previously reported (Comeskey et al., 2009 and Wong et al., 2002). In the studies by Wong et al. (2000) and Sukwattanasinit et al. (2007), HPLC chromatograms of roselle dye have two major peaks correspondence to delphinidin-3-sambubioside and cyanidin-3-sambubioside, proven by TLC and HPLC-DAD. Delphinidin-3-sambubioside and cyanidin-3-sambubioside chemical structures are shown in Figure (2a and 2b) with theoretically molecular weight of 597.3 and 581.2, respectively (Wang, Race and Shrikhande, 2003, Tian et al., 2005 and Hsieh et al., 2008). However, in this current study, data on retention time and peak pattern were insufficient to identify whether the two major compounds presented in roselle dye were delphinidin-3-sambubioside and cyanidin-3-sambubioside. Thus, LC-MS was employed in order to determine molecular weight of compounds eluting at 8.9 and 9.5 minutes. Mass spectra of peak at a retention time of 8.9 minutes showed mass to charge ratios of 597.2 and 303.0 (Figure 16b), which corresponded to theoretical molecular weights of delphinidin-3-sambubioside (molecular weight of 597.3) and delphinidin (molecular weight of 303), respectively (Figure 2a and 2b). While mass spectra of the peak at 9.5 minutes showed mass to charge ratios of 581.1 and 287.0 (Figure 16c) corresponding to theoretical molecular weight of cyanidin-3-sambubioside (molecular weight of 581.2) and cyanidin (molecular weight of 287). The presence of mass corresponding to delphinidin and cyanidin was due to fragmentation of delphinidin-3-sambubioside and cyanidin-3-sambubioside during ionization step. Based on HPLC peak pattern and molecular mass of each LC peak, the major dye components presence in roselle aqueous extract were delphinidin-3-sambubioside and cyanidin-3-sambubioside which were consistent with the previous reports (Wang, Race and Shrikhande, 2003, Tian et al., 2005 and Hsieh et al., 2008).

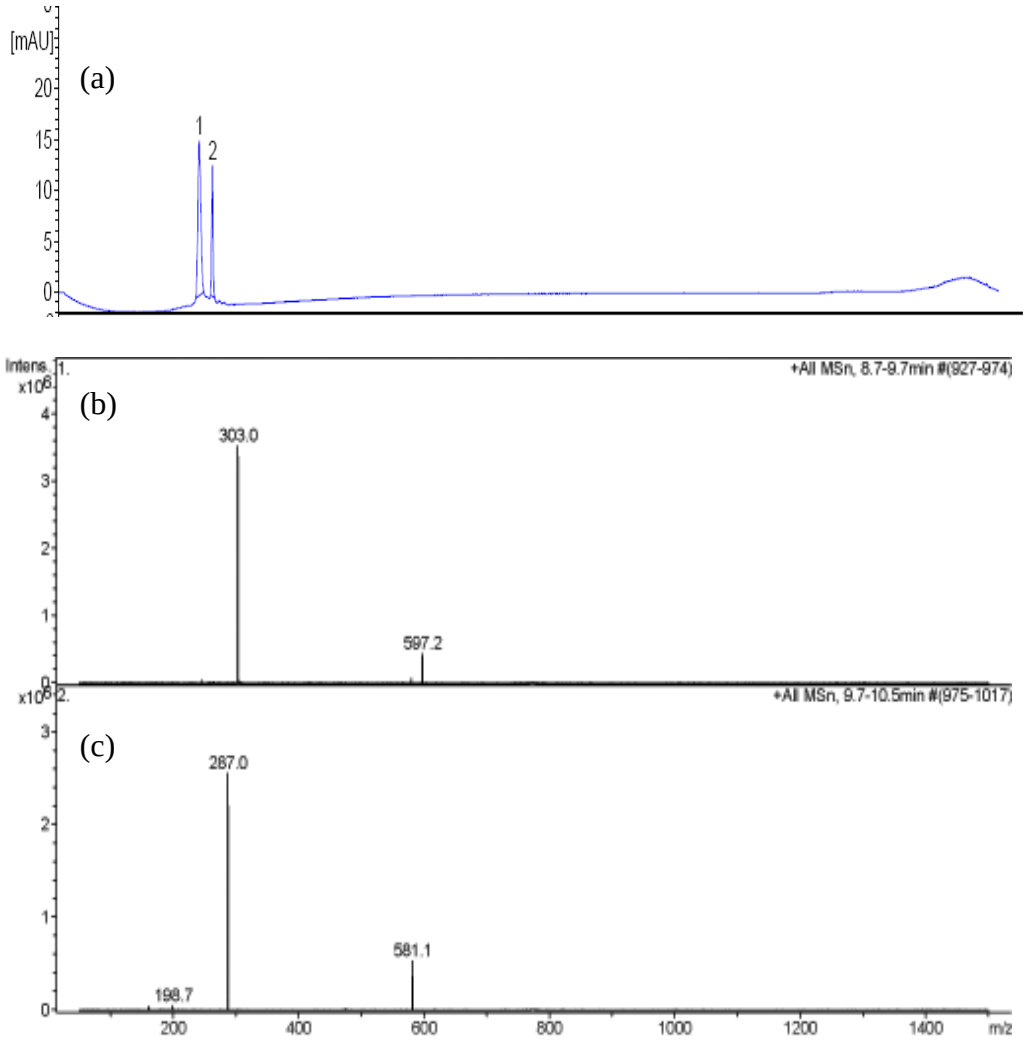

Figure 16 LC-MS of roselle dye, i.e. HPLC chromatogram of roselle dye (a), mass spectra of the peak at retention time of 8.9 minutes (b), and mass spectra of the peak at retention time of 9.5 minutes (c)

Apparent pK_a of roselle and lac dyes are reported in Table 3. As mentionted earlier, visualized colors of roselle and lac are pH-dependence. Moreover, the observed pK_a of roselle dye has never been reported elsewhere. In this study, the apparent pK_a was determined using UV-spectroscopic titration technique and equation (5). Absorbances at 520 nm were employed to estimate apparent pK_a of roselle dye. Thirteen UV spectra were obtained over pH range 2 to 9 at 26 ± 1 °C as shown in Figure 17a. Color change was observed over the pH range. Changes in visible spectra were due to the pH-sensitive anthocyanins in roselle dye (Markakis, 1982, Hendry and Houghton, 1992, Lauro and francis, 2000, Delgado-Vargas and Paredes-Lopez, 2002 and Wrostad, Durst and Lee, 2005). There is one ionizable functional group on chemical structure of delphinidin (Figure 15). The apparent pK_a of roselle dye was calculated to be 3.00 ± 0.08. The apparent pK_a of roselle was consistent with pK_a values of other anthocyanins from grape skin, elderberry, and black currant which were reported to be 3.68, 3.32, and 3.37, respectively (Wrolstad, 2000).

Table 3 pK_a value of roselle and lac dye

Dye	pK_a (Mean ± SD)
Roselle	3.00 ± 0.08
Lac	5.96 ± 0.15

In conclusion, water extract of roselle composed of two major coloring compounds. They were expected to be delphinidin-3-sambubioside and cyanidin-3-sambubioside with molecular masses of 597.2 and 581.1, respectively. In addition, the observed pK_a of roselle extract was in a vicinity of other anthocyanin's pK_a.

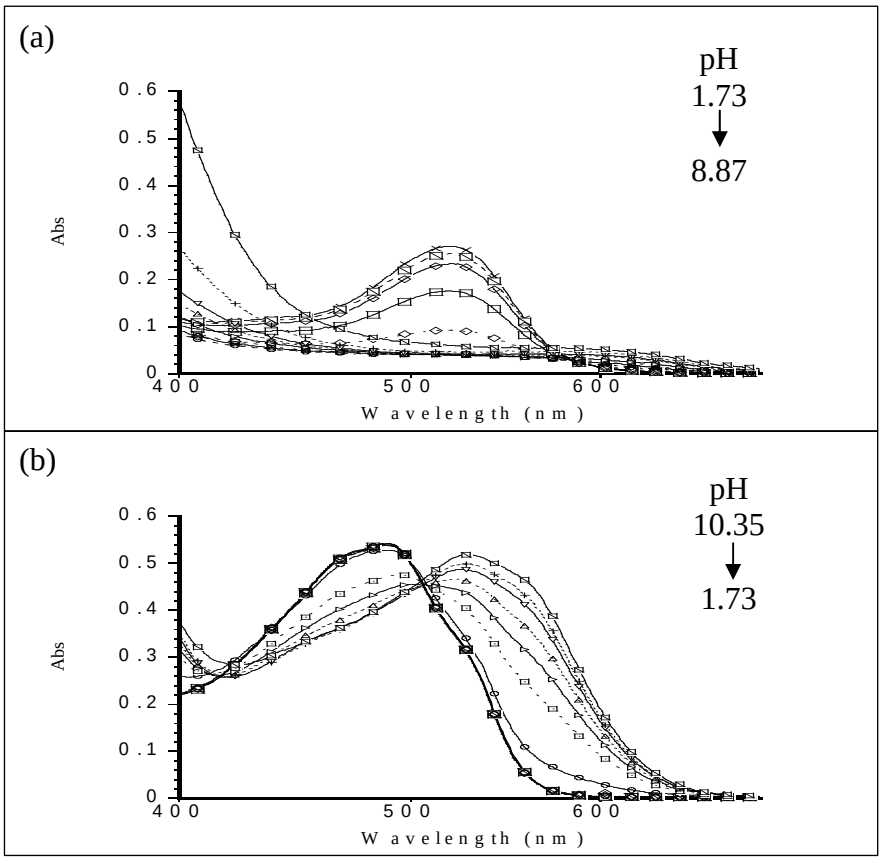

Figure 17 Visible spectra of roselle (a) and lac (b) dyes at various pH (roselle: (✳; pH 1.73), (--▧--; pH 1.97) (◈; pH 2.26), (--◇--; pH 2.76), (⊟; pH 3.51), (--○--; pH 5.35), (⊖; pH 5.89), (--□--; pH 6.16), (⊡; pH 6.62), (--△--; pH 6.98), (▽; pH 7.17), (--+--; pH 7.68) and (▨; pH 8.87) and lac: (✳; pH 1.73), (--▧--; pH 2.00) (◈; pH 2.52), (--◇--; pH 2.91), (⊟; pH 4.16), (--○--; pH 5.58), (⊖; pH 5.81), (--□--; pH 6.08), (⊡; pH 6.39), (--△--; pH 7.02), (▽; pH 7.54), (--+--; pH 8.69), and (▨; pH 10.35))

Color of lac dye solution varied from orange, red to magenta in acid (1N HCl), water, and alkaline (1N NaOH), respectively. The λ_{max} of lac solution in acid condition and water was 490 nm while the λ_{max} shifted to 530 nm in alkaline condition as shown in UV spectra (Figure 14b).

In this study, HPLC chromatogram of lac dye showed three major peaks at 8.9, 14.7, and 16 minutes (Figure 18a) while HPLC chromatogram of lac solution reported by Oka et al. (1998) showed four major peaks corresponding to laccaic acid A, B, C, and E using similar HPLC conditions. Analysis of the peak at 16 minutes revealed that this peak was coelution of compounds with retention times of 16.4, and 16.7 minutes (Figure 18b). Molecular mass of compounds with HPLC retention times of 8.9, 14.7, 16.4, and 16.7 minutes were determined to be 537.9, 519.9, 535.9, and 494.9, respectively using mass spectra obtained from LC-MS technique (Figure 18 c-f). Molecular weights at retention times of 8.9, 16.4, and 16.7 minutes were identified as laccaic acid C, A, and B with theoretical molecular masses of 539, 537, and 496, respectively (Oka et al., 1998, Oka et al., 1998 and Park, 2001). The peak at retention time of 14.7 minutes, which has mass to charge ratio of 519.9, was an unidentified compound.

Apparent pK_a of lac dye was determined using UV-spectroscopic titration technique. Thirteen of visible spectra were obtained over pH range 2 to 10 at 26 ± 1 °C. The obtained visible spectra showed an isobestic point at 500 nm (Figure 17b) and the observed color of lac dye changed from orange to magenta. The apparent pK_a values were estimated based on absorbance at 560 nm at various pH using Equation (5). Major components of lac dye are anthraquinone dicarboxylic acids (laccaic acid A, B, C, and E), which contained two ionizable groups in chemical structures as shown in Figure 2c (Oka et al, 1998). Therefore, the lac dye color changed when it was in different pH condition. The colorants in lac showed pH-dependent characteristic with an apparent pK_a of 5.96 ± 0.15 corresponding to pK_a of one of the carboxylic groups presents in laccaic acid structure. In conclusion, the major colorants in lyophilized lac extract possessed molecular mass of 539, 537, and 496, which were laccaic acid C, A, and B, repectively.

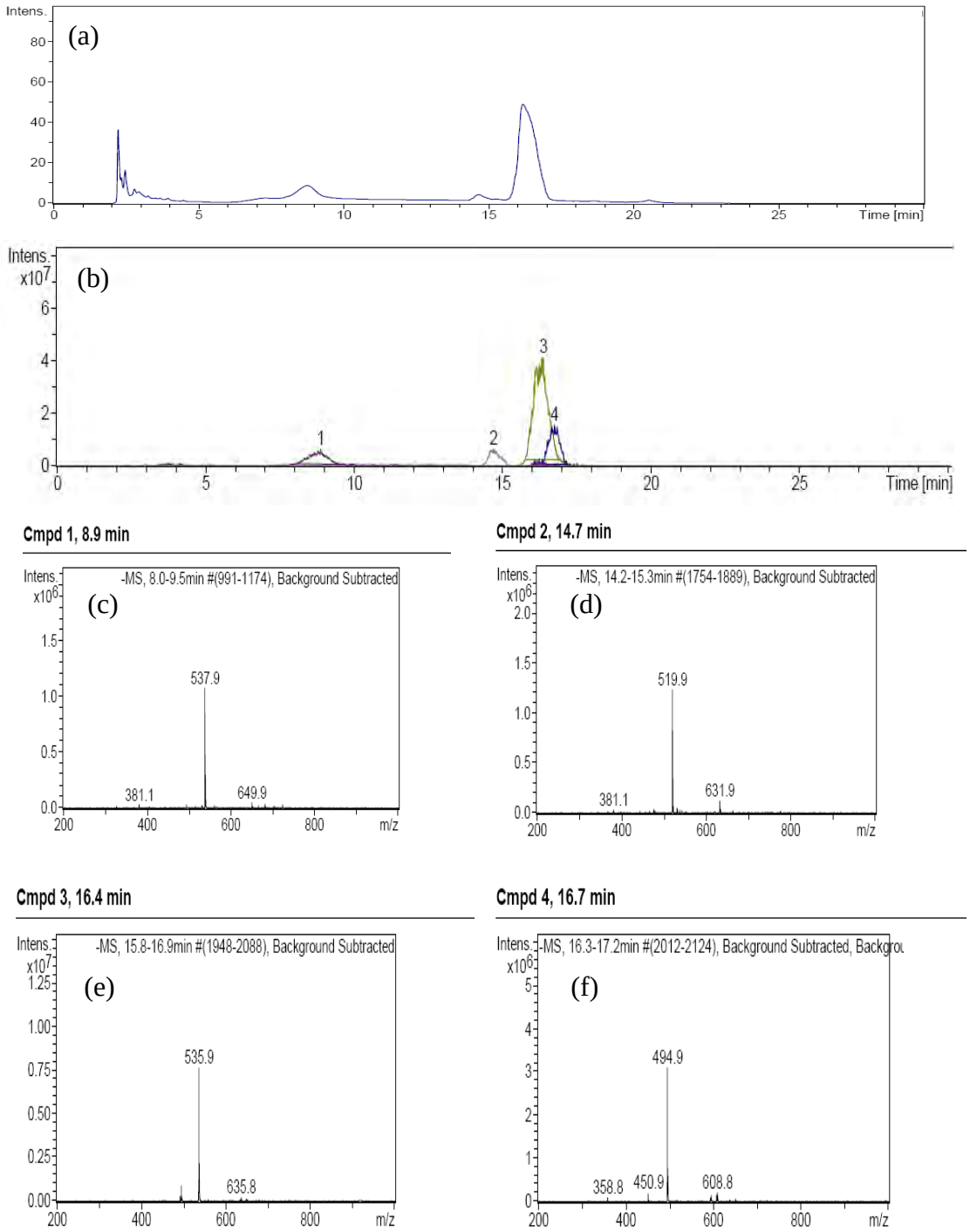

Figure 18 LC-MS of lac dye, i.e. HPLC chromatogram of lac dye (a and b), mass spectra of the peak at retention time of 8.9 minutes (c), mass spectra of the peak at retention time of 14.7 minutes (d), mass spectra of the peak at retention time of 16.4 minutes (e), and mass spectra of the peak at retention time of 16.7minutes (f)

Visible characteristics of gardenia dye solution changed as medium pH changed over pH range of 2 to 10 but color of dye solutions were yellow in all conditions. λ_{max} of gardenia dye in 0.1 N HCl and water was 443 nm while it shifted to 420 nm in alkaline condition with change in visible spectrum pattern (Figure 14c). Crocin, which is expected to be a major component of gardenia dye, is an ester compound (Figure 4d) (Lauro and Francis, 2000, Choi et al, 2001 and Park et al., 2001). The shift in visible characteristic was probably due to loss of digentiobiosyl group via ester hydrolysis (Chen et al., 2008). Furthermore, there is no ionizable group in crocin chemical structure; therefore, the observed color change is not related to substrate ionization. Thus, the pKa of gardenia dye cannot be determined.

HPLC chromatogram of gardenia dye has four peaks, which resembled with HPLC chromatogram pattern of gardenia dye reported in previous study (Lauro and Francis, 2000). Molecular mass of compounds with HPLC retention times at 7.3, 12.3, 29.5, and 32.7 minutes were determined to be 999.4, 837.3, 675.2, and 999.4, respectively using mass spectra obtained by LC-MS technique (Figure 19). The mass to charge ratio of 999.4 is sodium salt of crocin. The obtained molecular weights of peaks at 7.3, 12.3, and 29.5 minutes are specified to be molecular weights of crocin, crocetin monogentiobiosyl monoglucosyl ester, and crocetin monogentiobiosyl ester, respectively, while a peak at retention time of 32.7 minutes corresponded to an unidentified compound (Choi et al., 2001 and Park et al., 2001). In summary, gardenia dye is composed of crocin, crocetin monogentiobiosyl monoglucosyl ester, crocetin monogentiobiosyl ester as coloring compounds.

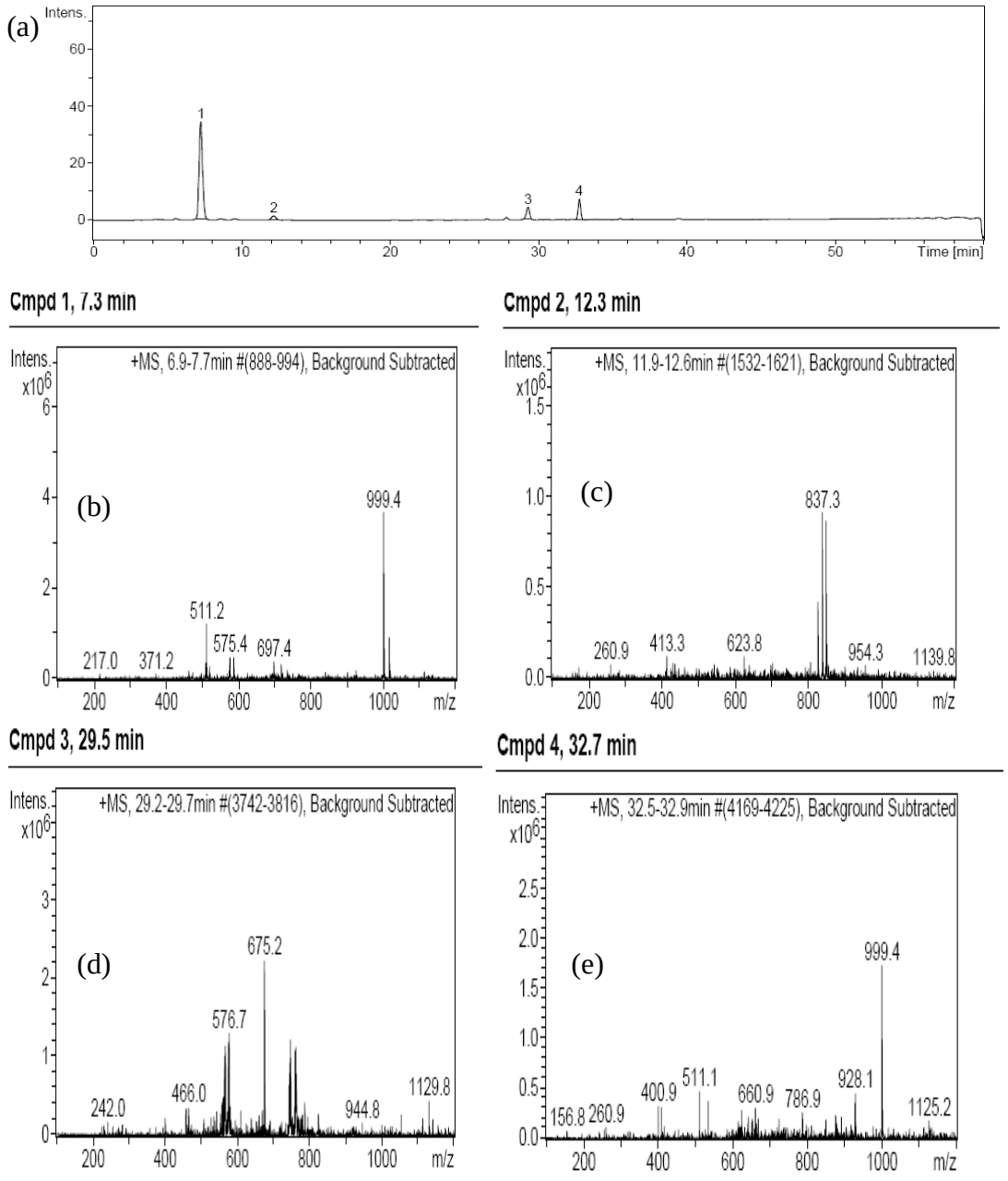

Figure 19 LC-MS of gardenia dye, i.e. HPLC chromatogram of gardenia dye (a), mass spectra of the peak at retention time of 7.3 minutes (b), mass spectra of the peak at retention time of 12.3 minutes (c), mass spectra of the peak at retention time of 29.5 minutes (d), and mass spectra of the peak at retention time of 32.7 minutes (e)

C. Preliminary stability of natural colors from roselle, lac, and gardenia color under stress conditions

Stability of roselle color under stress conditions; acid, alkaline, hydrolysis, UV-light exposure and high temperature, are shown in terms of percentage color remaining as a function of time (Figure 20). Roselle color was rather stable in water and acidic conditions. In acid condition, a major fraction of delphinidin is presence as flavylium cation. In alkaline condition, delphinidin is unlikely to undergo deprotonation and further degrade to carbinol pseudobase (colorless) and chalcone (yellowish) as shown in Figure 15. Thus, delphinidin was more stable under acid condition (Cevallos-Casals and Cisneros-Zevallos, 2004). The degradation rate was also accelerated at high temperature (72 ˚C) and in the presence of UV light (Figure 20a and 20b) which was consistent with the results reported by Furtado et al. (1993), Ahmed, Shivhare and Raghavan (2004), Wrostad, Durst and Lee (2005) and Cisse et al. (2009). Furthermore, in the presence of oxidizing agent (3% H_2O_2), roselle color was changed from red to colorless within 5 minutes. The colorless product was speculated to be hydroquinone derivatives resulting from oxidation at delphinidin phenol group. This result was consistent with anthocyanin oxidation in the previous study (Francis, 1989 and Majangos, Varona and Villota, 2006).

Lac dye was stable in acidic condition and in water (Figure 20c and 20d) but the color gradually faded over 150 hr in the presence of 3% H_2O_2. Both UV-light and 3% H_2O_2 may facilitate oxidation of laccaic acids at phenol groups giving rise to colorless hydroquinone derivatives (Majangos, Varona and Villota, 2006). Moreover, lac color degraded in alkaline condition through unknown pathway. However, among these conditions, lac color was very stable at high temperature (72 ˚C) (Figure 20c).

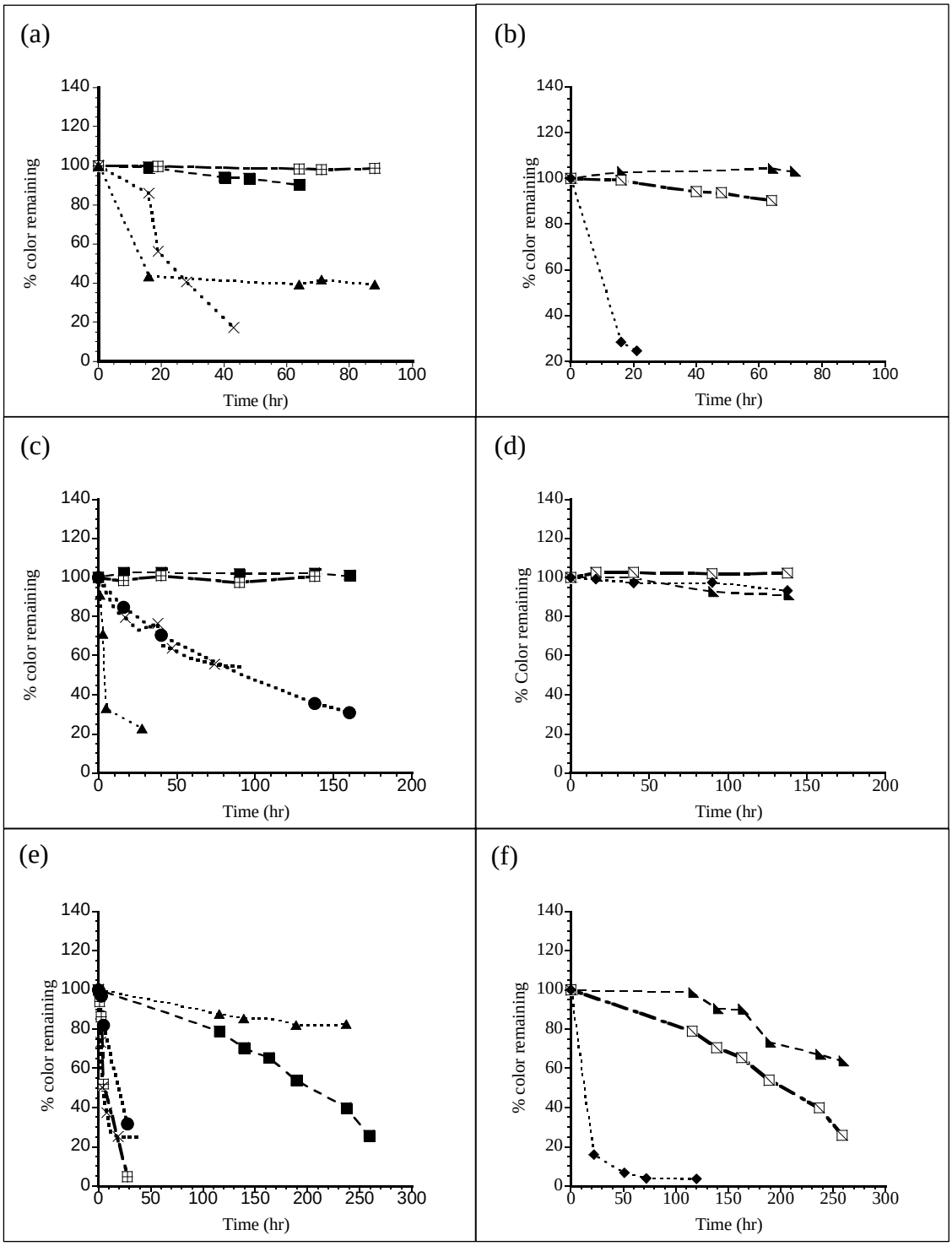

Figure 20 Percentage of color remaining of roselle dye (a), lac dye (c), and gardenia dye (e) in five conditions: water (■), acid (⊞), alkaline (▲), UV light (✕), oxidation (●), and temperature effect on roselle dye (b), lac dye (d) and gardenia dye (f) at 4 ˚C (◣), 30 ˚C (▨), and 72 ˚C (◆)

Gardenia color was also unstable (Figure 20e and 20f). According to degradation of crocin from saffron and gardenia fruit, crocin degradation was accelerated in the presence of light, acid, oxidation and increasing temperature (Bors et al., 1982, Romero and Bakker, 2000, Tsimidou et al., 2006, Reyes and Cisneros-Zevallos, 2007 and Chen et al., 2008). Therefore, among these conditions, gardenia color was most stable in alkaline solution.

Anthocyanins in roselle and crocin in gardenia have disaccharides connected to the core structures, delphinidin and crocetin, respectively, through ester bonds. Therefore, both compounds can undergo acid-base catalyzed ester hydrolysis resulting in loss of color intensity (Tsai, Hseih and Huang, 2004 and Nikkhah et al, 2007). Anthocyanin and crocin changed from their initial colors to brown at high temperature and in the presence H_2O_2 because the disaccharides may undergo Millard reaction and oxidation (Romeo and Bakker, 2000 and Tsai et al, 2005).

D. Critical micelle concentration (CMC) determination of sodium dodecylsulphate (SDS), cetyltrimethylammonium bromide (CTAB), and polysorbate80 (Tween80)

This study intends to observe natural color stability in three different micellar systems, sodium dodecylsulphate (SDS), cetyltrimethylammonium bromide (CTAB) and polysorbate80 (Tween80).

The obtained surface tensions of CTAB, SDS and Tween80 in phosphate buffer pH 7 and succinate buffer pH 4.5 were plotted against surfactant concentrations on a semilogarithmic scale as shown in Figure 21 and 22.

In this study, the concentrations of SDS, CTAB and Tween80 were varied from 5×10^{-7} to 10^{-2} M for CMC determination. As the concentrations of CTAB, SDS, and Tween80 were increased, surface tensions decreased and reached a plateau at high surfactant concentrations. The inflection point represented a situation when both interface and bulk phase became saturated with monomers of surfactants. The presence of any additional surfactant molecules leaded to aggregation of surfactant molecules and form micelles in the bulk phase. In other words, the inflection point on

the surface tension versus log concentration curve represents CMC (Figure 21 and 22).

CMC determination was done by separating data into two regions where surface tension was inversely proportional to surfactant concentrations and where surface tension was independent of surfactant concentrations. Then each group of data was fit in a linear equation using linear regression technique. A surfactant concentration at intersection of these two linear equations was estimated to be CMC. The apparent CMC values of the three surfactants in two buffer systems were calculated and reported in Table 4.

Table 4 CMC of SDS, CTAB, and Tween80 in phosphate buffer pH 7 and succinate pH 4.5

Type of Surfactant	Phosphate buffer pH 7 (M, Mean ± SD)	Succinate buffer pH 4.5 (M, Mean ± SD)
SDS	$4.97 \times 10^{-4} \pm 1.55 \times 10^{-5}$	$5.12 \times 10^{-4} \pm 2.82 \times 10^{-5}$
CTAB	$2.05 \times 10^{-5} \pm 2.87 \times 10^{-6}$	$2.05 \times 10^{-5} \pm 1.85 \times 10^{-6}$
Tween80	$1.85 \times 10^{-5} \pm 1.25 \times 10^{-6}$	$1.84 \times 10^{-5} \pm 8.23 \times 10^{-6}$

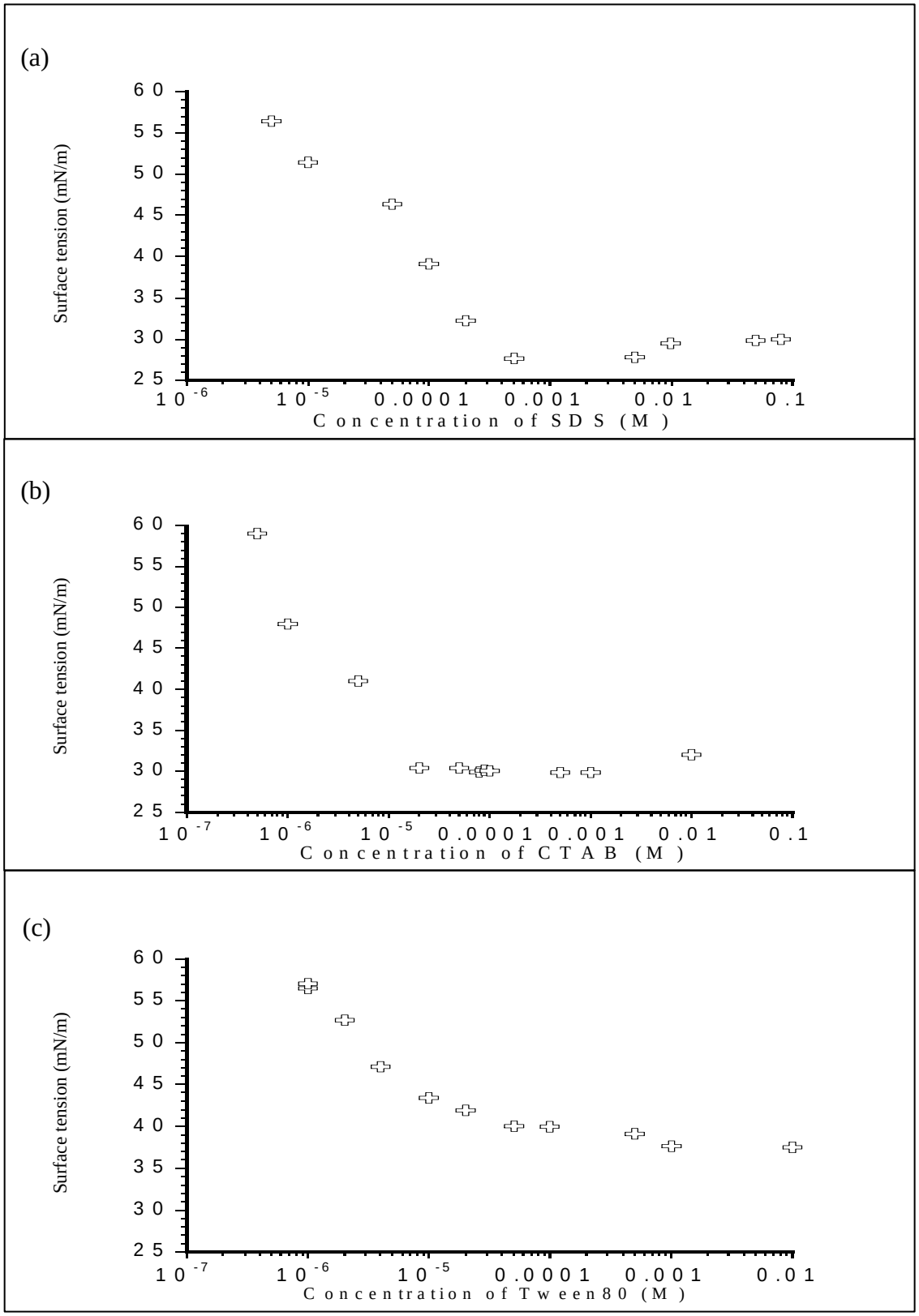

Figure 21 showing surface tension of SDS (a), CTAB (b), and Tween80 (c) plotted against log concentration in phosphate buffer pH 7

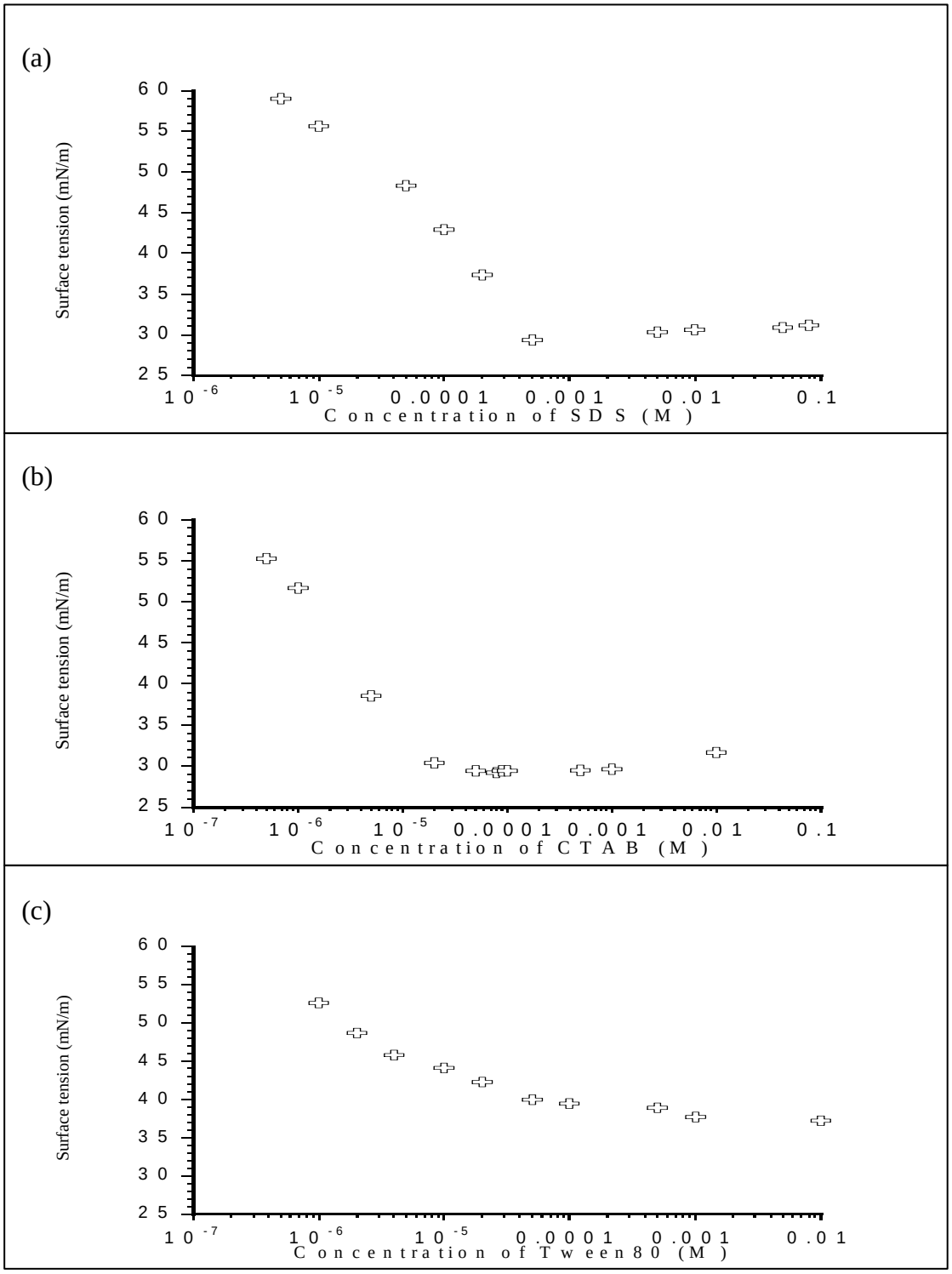

Figure 22 showing surface tension of SDS (a), CTAB (b), and Tween80 (c) plotted against log concentration in succinate buffer pH 4.5

The CMC of SDS, CTAB, and Tween80 in buffer solutions were compared to that of in water. The CMC of SDS, CTAB, and Tween80 in water were reported to be 8×10^{-3} M (at 37 °C) (Boylan, Cooper and Chowhan, 1986 and Kodama M., Kubota Y. and Miura M., 1972), 1×10^{-3} M (at 25 °C) (Modaressi et al., 1972), and 1.2×10^{-5} M (at 23 °C) (Zheng and Obbard, 2002). The CMC of SDS and CTAB, which are anionic and cationic surfactants, in buffer systems were lower than that of the surfactants in aqueous solution because the presence of electrolytes in the systems reduced thickness of the ionic layer surrounding polar headgroups. As a consequence, the repulsion force between polar head group was reduced. Therefore, the CMC of SDS and CTAB were decreased. While the CMC of Tween80, a non-ionic surfactant in two buffer systems were similar to that of Tween80 in water.

The CMC of SDS, CTAB and Tween80 did not change as the solvent was changed from phosphate buffer pH 7 to succinate buffer pH 4.5. Normally CMC of surfactants is altered depending on ionic strength rather than pH of the solutions. In this study, ionic strength of both buffer systems was kept constant at 0.25M. Therefore, alteration of CMC was not observed. The obtained CMC of SDS, CTAB and Tween80 in phosphate buffer pH 7 and succinate buffer pH 4.5 were used to prepare their micellar systems in next study.

E. Stability of roselle, lac, and gardenia colors in micellar systems

As previously mentioned in section C, the roselle and lac colors were found to be very stable in acidic condition while the most stable condition for gardenia dye was in alkaline condition. pH of most preparations are in a range of 6-8, which pH safe and does not irritate to skin. Therefore, to study stability of these natural colors in the presence of micelles, succinate buffer pH 4.5 was selected to be used as a solvent for roselle and lac dyes, while phosphate buffer pH 7 was chosen to be a solvent for gardenia dye in each micellar system. According to CMC of the surfactants, the concentrations of surfactants used in this study are shown in Table 5. Ionic strength of all solutions were kept constant at 0.25M using sodium chloride. The mixtures were then incubated at 30 °C throughout the studies. At appropriate time, samples were taken to determine color remaining of the samples using both UV-visible spectrophotometry and CIELab system.

Table 5 The concentrations of surfactants used in stabilities study

CMC	SDS, M	CTAB, M	Tween80, M
1 CMC	5.00×10^{-4}	2.05×10^{-5}	1.85×10^{-5}
5 CMC	2.50×10^{-3}	1.03×10^{-4}	9.25×10^{-5}
10 CMC	5.00×10^{-3}	2.05×10^{-4}	1.85×10^{-4}
15 CMC	7.50×10^{-3}	3.08×10^{-4}	2.76×10^{-4}

In the presence of CTAB systems, the solutions turned cloudy after an addition of roselle, lac, or gardenia dyes. CTAB is cationic surfactant, which have cloud point around 30 °C (Alam, Naqvi and Din, 2007). Addition of some electrolyte decreased the cloud point (Yu and Xu, 1989) and CMC of CTAB. Therefore, more micellar structures were formed which eventually could make the colloidal solution of micelles turned turbid. The ionized forms of flavylium cation in roselle could lower the cloud point of CTAB. However, laccaic acids in succinate buffer pH 4.5 and crocin in phosphate buffer pH 7 were presence as unionized species. In addition, the observed cloudiness was probably due to interaction between CTAB with other unidentified components presence in the dyes. Thus, the CTAB micellar system was withdrawn from this experiment.

In the absence of micelles, roselle color in succinate buffer pH 4.5 was pink with visible absorption maximum at 528 nm (Figure 23, Table 6). It was noticed that λ_{max} of roselle dye in succinate buffer pH 4.5 underwent significant red shift when compared to that of the dye in water (λ_{max} = 520 nm). The observed red shift of roselle in buffer systems was also reported by Lauro et al. (2000). The observed red shift was not due to difference in pH values of roselle in water (pH ≈ 5) and roselle in succinate buffer pH 4.5 since the red shift was not found during pK_a determination of roselle (Figure 17a). Therefore, the observed red shift was probably due to ionic interaction between buffer species and delphinidin.

Table 6 The absorption maxima (λ_{max}) of roselle, lac, and gardenia dyes in aqueous, buffer, and micellar systems

Dye	λ_{max} (nm)			
	H_2O	Buffer	SDS	Tween80
Roselle	520	528	528	528
Lac	490	490	490	490
Gardenia	443	440	443	442

(a) (b) (c)

Figure 23 Depicted color of dyes, roselle (a) and lac (b) in succinate buffer pH 4.5 and gardenia (c) in phosphate buffer pH 7

UV-visible absorbances of roselle dye in SDS micellar system were increased as the concentration of SDS micelles was increased (Figure 24a). In succinate buffer pH 4.5, delphinidin is in a flavylium cation form. Therefore, the flavylium cations interacted with negative charge of SDS micelles through ionic interaction force (Malyukin, Efimova and Kemnitz, 2001 and Bielska, Sobczynska and Prochaska, 2009). Ionic interaction facilitated incorporation of hydrophobic part of delphinidin in the palisade layer of SDS micelles. Both ionic interaction and non-polar environment increased absorbance of delphinidin. As concentration of SDS was increased, the volume of non-polar environment was increased, the micelles could accommodate more delphinidin, and consequently absorbance of delphinidin was increased.

UV-visible characteristics of roselle dye in the presence or in the absence of Tween80 were superimposable (Figure 24b). In addition, absorption maxima and absorbances of roselle were not a function of Tween80 micellar concentration. Interaction between flavylium cation of dephinidin and non-ionic Tween80 was unlikely. In addition, the ionized form of delphinidin is very water-soluble. Therefore, without ionic interaction between delphinidin and Tween80, delphinidin would not have a tendency to reside in micellar environment of Tween80 micelles. As a result, changes in UV-visible characteristics of delphinidin in the presence of Tween80 micelles were not observed.

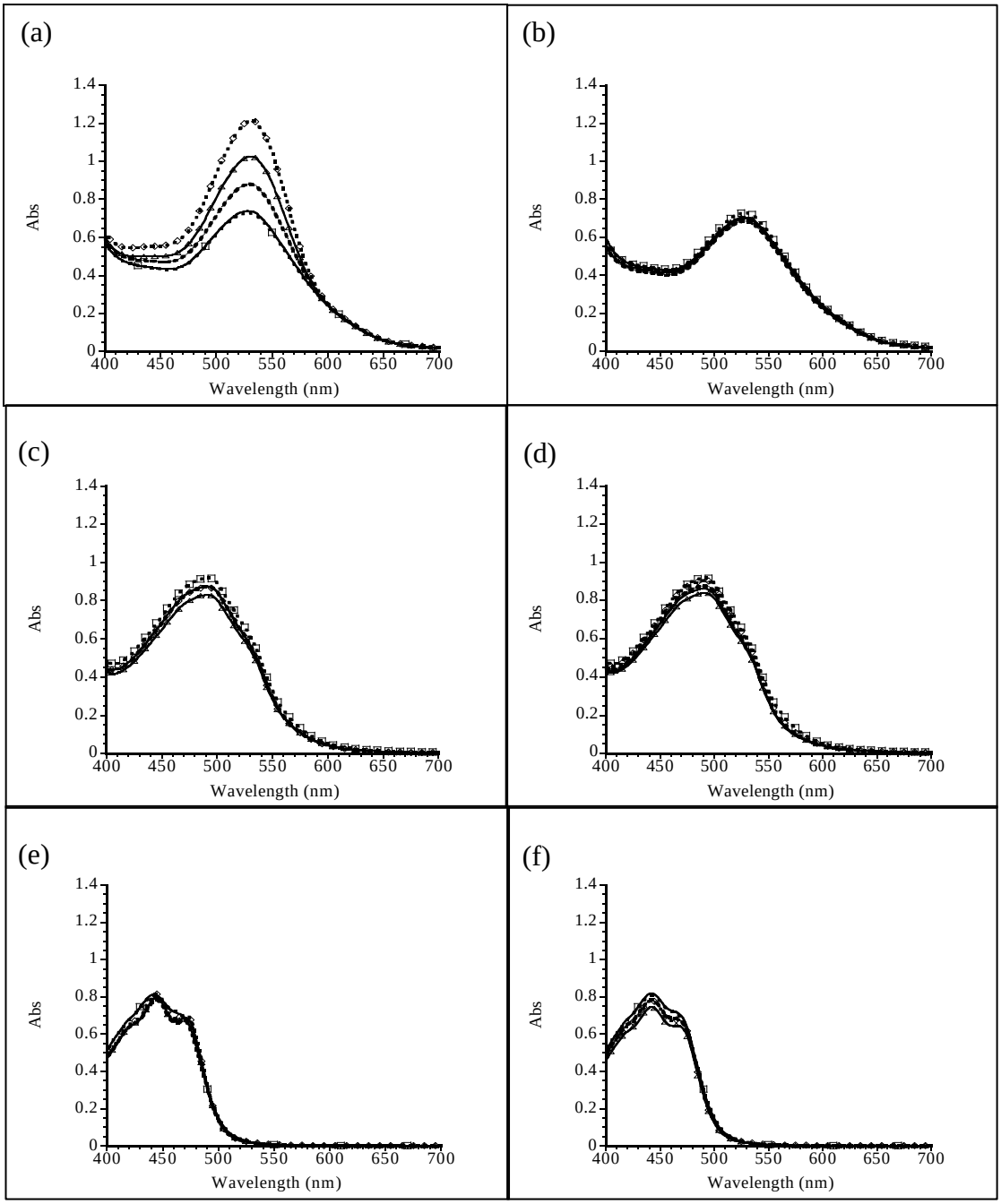

Figure 24 UV-visible spectra of dyes in micellar solutions; roselle in SDS (a), roselle in Tween80 (b), lac in SDS (c), lac in Tween80 (d), gardenia in SDS (e), and gardenia in Tween80 (f) at concentration of 0 CMC (---□---), 1 CMC (—), 5 CMC (-----), 10 CMC (△), and 15 CMC (--◇--)

Stability of roselle dye in succinate buffer pH 4.5 in the absence and in the presence of micelles at concentrations of 1-15 folds of their CMC values was monitored at 30 °C. Roselle color in the presence of SDS or Tween80 faded overtime which was consistent with decreasing of visible-absorbances at 528 nm and increasing in visible-absorbance at 420 nm or color changed from pink to yellowish within 12 days (Figure 25, 26, and 27). The color change of roselle in the absence and in the presence of SDS micelle was mainly due to delphinidin degradation as proving by HPLC analysis (Figure 28).

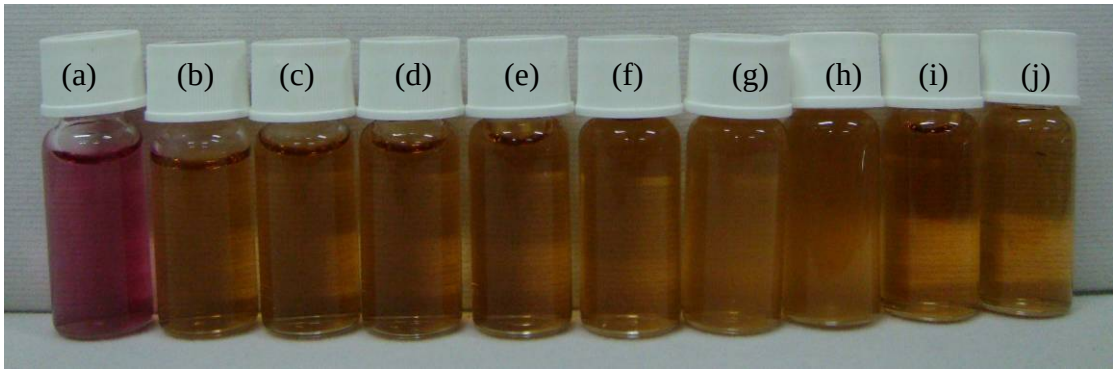

Figure 25 Color of roselle dye succinate buffer pH 4.5 at day 0 (a), succinate buffer pH 4.5 at day 12 (b), 1 CMC of SDS at day 12 (c), 5 CMC of SDS at day 12 (d), 10 CMC of SDS at day 12 (e), 15 CMC of SDS at day 12 (f), 1 CMC of Tween80 at day 12 (g), 5 CMC of Tween80 at day 12 (h), 10 CMC of Tween80 at day 12 (i), and 15 CMC of Tween80 at day 12 (j)

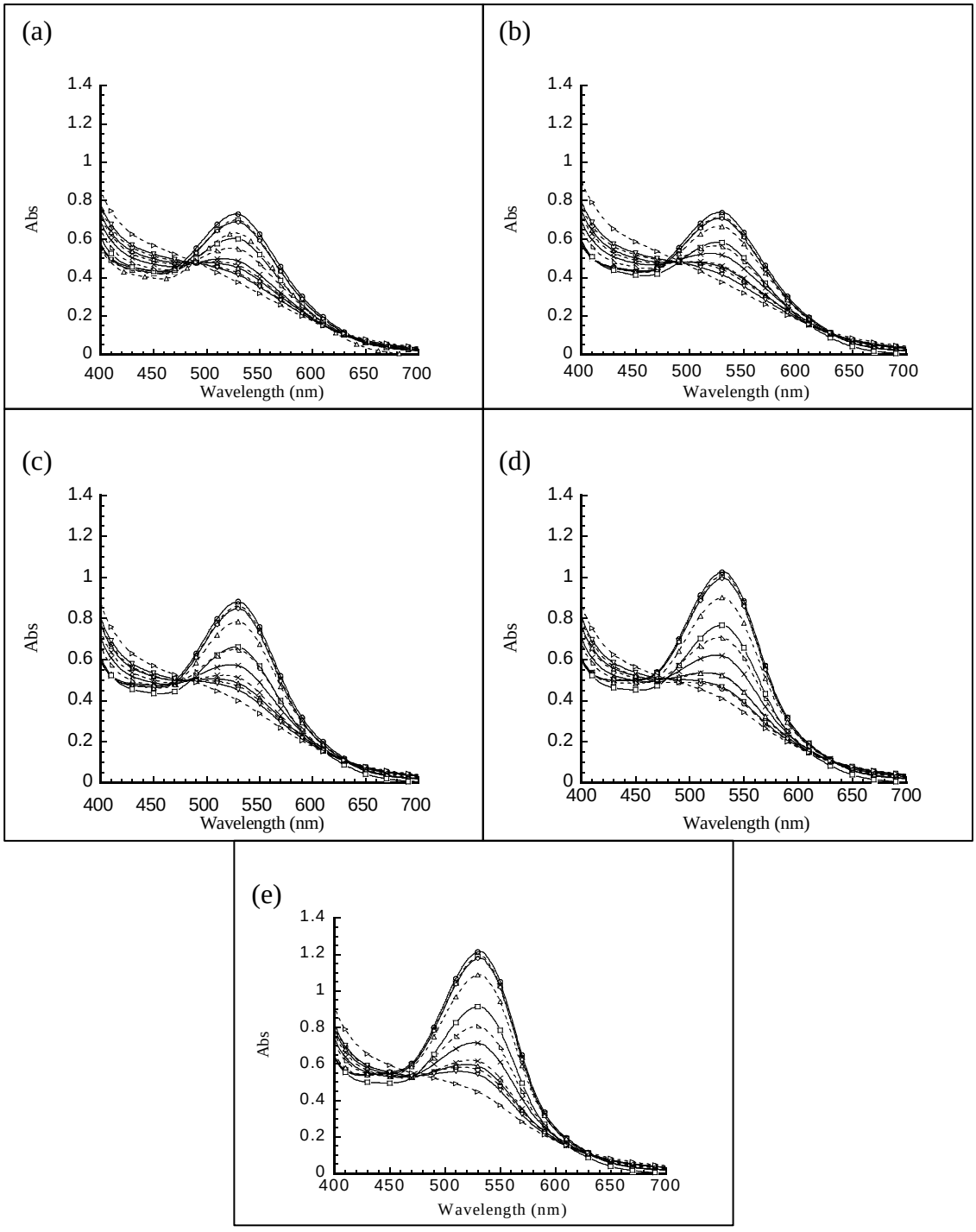

Figure 26 UV-visible spectra of roselle color in succinate buffer pH 4.5 in the absence or in the presence SDS of micellar systems: no SDS (a), 1 CMC (b), 5 CMC (c), 10 CMC (d) and 15 CMC (e) at day 0 (⊖), day 0.17 (--□--), day 0.42 (◈), day 2 (--△--), day 3 (⊟), day 4 (--◺--), day 5 (✶), day 6 (--×--), day 7 (⧋), day 10 (--○--), and day 12 (▽)

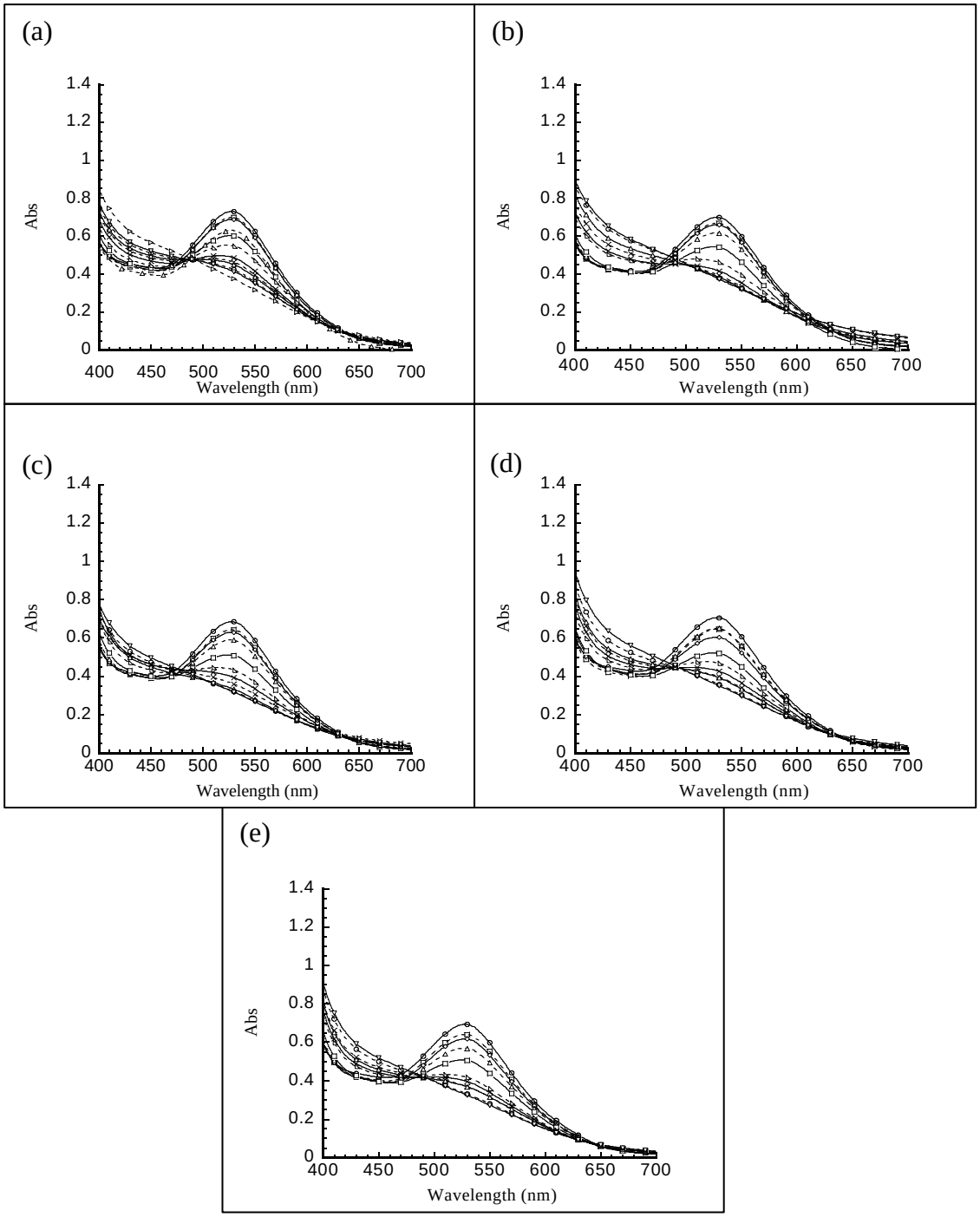

Figure 27 UV-visible spectra of roselle color in succinate buffer pH 4.5 in the absence or in the presence of Tween80 micellar systems: no Tween80 (a), 1 CMC (b), 5 CMC (c), 10 CMC (d) and 15 CMC (e) at day 0 (⊖), day 0.17 (--□--), day 0.42 (◈), day 2 (--△--), day 3 (⊟), day 4 (--◺--), day 5 (✳), day 6 (--×--), day 7 (⧋), day 10 (--○--), and day 12 (▽)

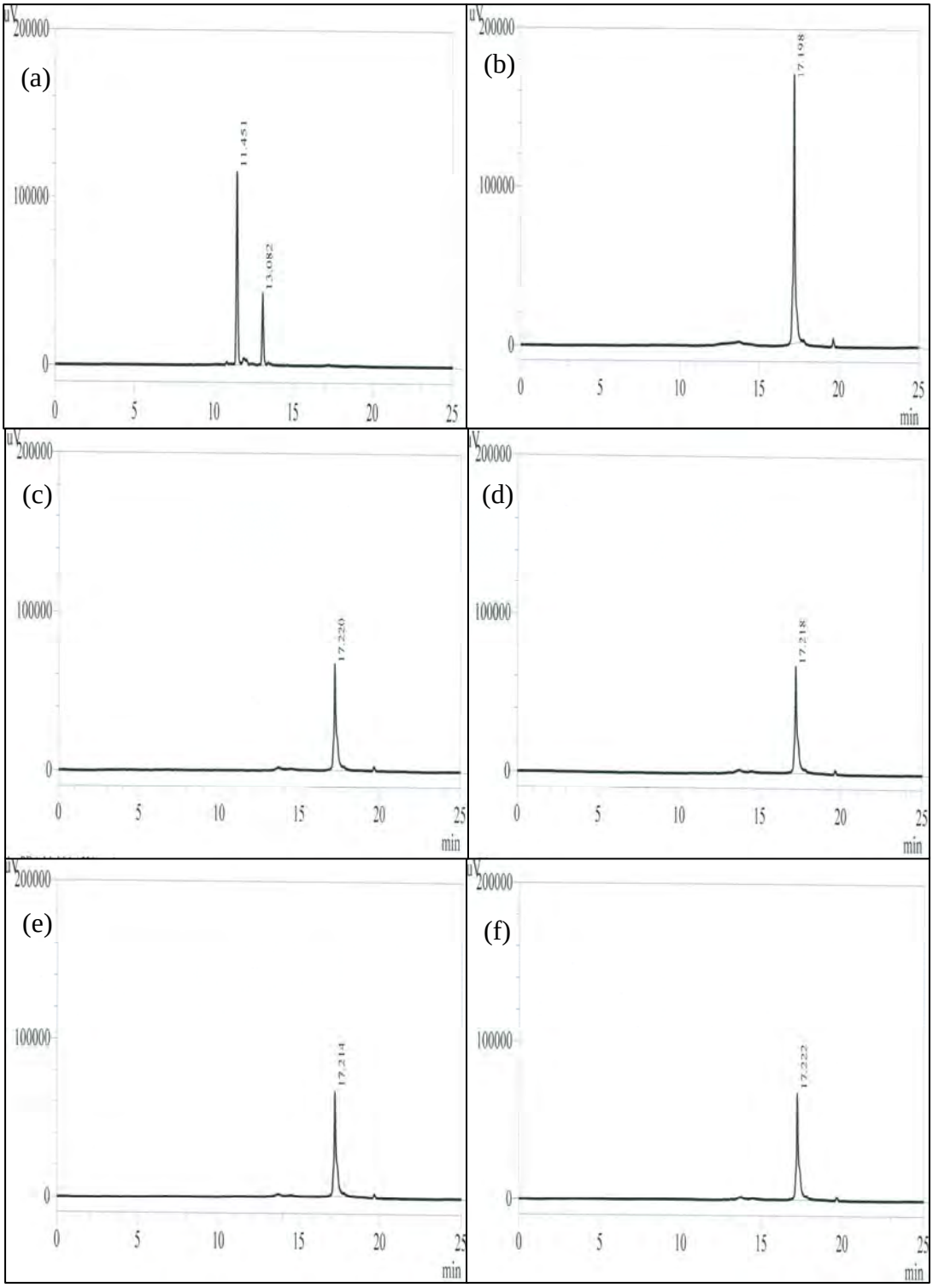

Figure 28 HPLC chromatograms of roselle dye in succinate buffer pH 4.5 (at day 0 (a) and day 12 (b)) and SDS micellar systems at concentrations of 1 CMC (c), 5 CMC (d), 10 CMC (e), and 15 CMC (f) at day 12

At the initial time, the HPLC chromatogram of roselle dye in the absence of micelle showed two major peaks at retention time of 11 and 13 minutes corresponding to delphinidin-3-sambubioside and cyanidin-3-sambubioside, respectively. At the end of experiment, the peaks at 11 and 13 minutes disappeared but a new peak at retention time of 17 minutes of an degradation product was present. In the presence of SDS micelles at all concentrations, HPLC chromatograms of roselle dye at day 12 of the experiment also showed the peak at 17 minutes. These results indicated that anthocyanins was completely degraded within 12 days and yield one degradation product the HPLC retention time of 17 minutes. According to the degradation scheme of anthocyanins as shown in Figure 15, final degradation product of anthocyanins is chalcone, giving yellow color. Therefore, at the end of day 12 of study, pink color of roselle was changed to yellow color of chalcone.

Roselle color in the presence of Tween80 at concentrations of 1 and 5 folds of CMC did not only fade to yellow but the mixtures also turned cloudy within 12 days. Roselle solution in the presence of Tween80 micelles at concentrations of 10 and 15 folds of CMC did not turn to cloudy within 12 days of the experiment but they turned cloudy sometimes later on. The observed cloudiness of these mixtures was speculated to be due to decrease of Tween80 cloud point. Cloud point is a unique property of non-ionic surfactant with poly (ethylene oxide) chains. At temperature above cloud point, the surfactant phase separates and precipitates. Factors promoting cloud point depression are the presence of both organic and inorganic additives, which promote water structure or bind to water molecules around surfactant molecules (Koshy, Saiyad and Rakshit, 1996, Na et al, 1999 and Mahajan et al., 2008). Many common salts including sodium chloride are very effective in cloud point depression. In this study, the color solutions contained electrolytes such as succinic acid and sodium chloride including other component in the dye. Therefore, the observed cloudiness of roselle color could be a result from the cloud point depression.

Baseline of UV-visible spectra of cloudy solution is higher than that of clear solution. Pattern of UV-visible spectra of the translucent roselle solutions were similar to those clear roselle solutions, but the absorbance values at all wavelength were increased. Cloudy solution scatters UV-visible incident beam at all directions making a lower fraction of transmitted light reach detector. So, the instrument overestimates sample absorbances. To eliminate light scattering effect due to cloudy solution, the baseline was readjusted to zero by subtraction absorbances at any wavelengths with the absorbance at 700 nm. This baseline adjustment technique was reported to be an effective technique in data treatment of colloidal system (Prodanov et al., 2005 and Sheridan et al., 2007). In this study, spectra of cloudy samples were readjusted baseline using the baseline subtraction technique before they were displayed and compared with other spectra obtaining from clear solutions (Figure 27b and c).

Stabilities of roselle in the presence and in the absence of micellar systems were investigated using parameters such as color intensity (CI), color density (CD) and percentage of color remaining (% color remaining). CI is absorbance at λ_{max}; CD is a summation of absorbances at 420, 520, and 620 nm, which represent yellow, red, and blue; % color remaining is reported as percent absorbance at 520 nm at time t when compared to that at initial time. CI, CD, and % color remaining of roselle color in micellar systems as a function of time are shown in Figure 29.

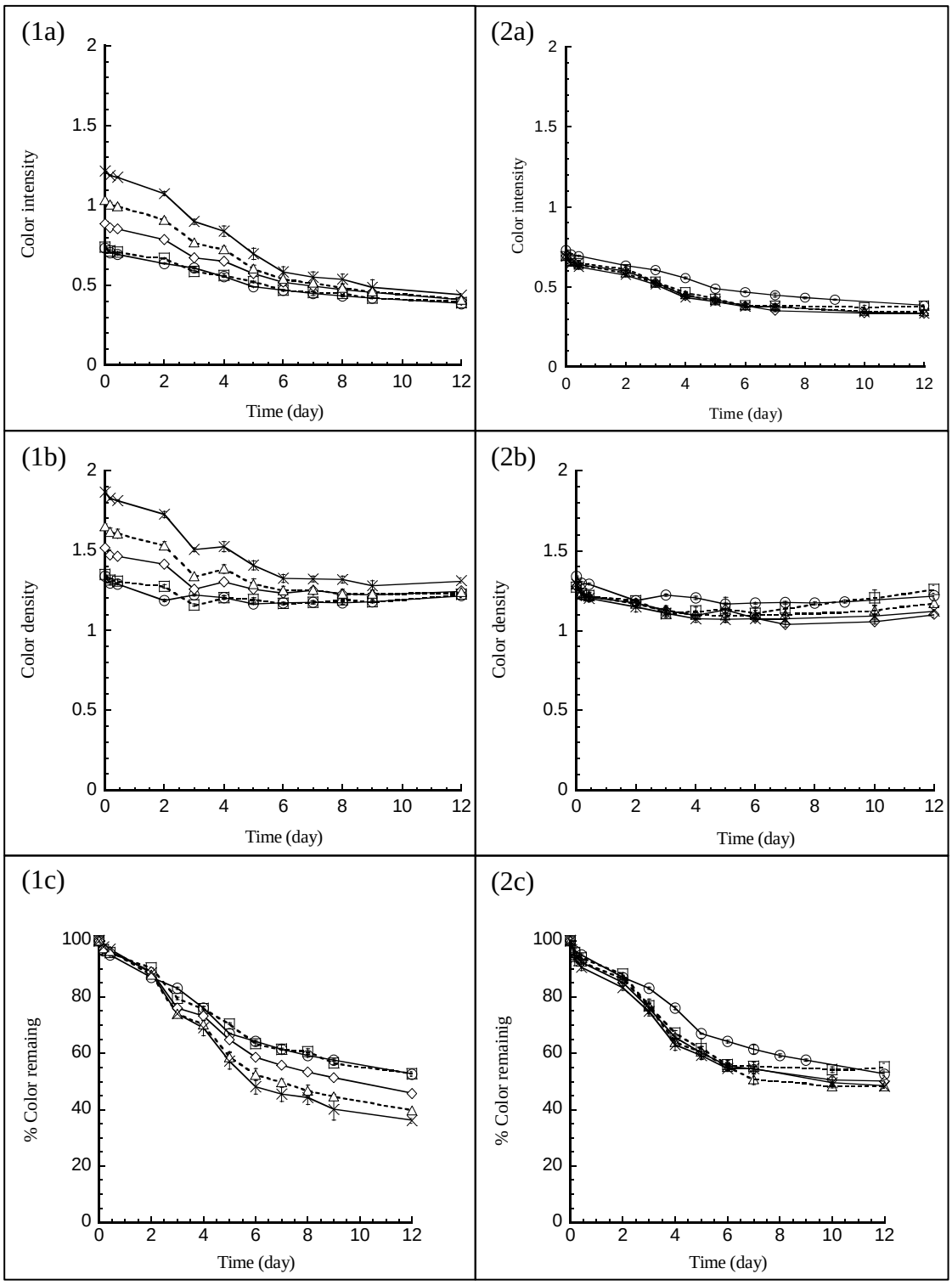

Figure 29 Color intensity (a), color density (b), and percentage of color remaining (c) of roselle dye in SDS (1) or Tween80 (2) micellar systems, i.e. succinate buffer pH 4.5 (⊖), 1CMC (--□--), 5 CMC (◈), 10 CMC (--△--), and 15 CMC (✳)

CI and CD of roselle dye in the presence of SDS micelles at concentration higher than 1 CMC were greater than that in the absences of micelles; in other words, a greater absorptivity was observed in the presence of SDS micelles at high concentrations. CI of roselle color in various concentrations of SDS micelles decreased overtime and gave comparable CI values at the end of experiment (Figure 29a). In the presence of SDS micelles, the observed decrease in CI was proportional to concentration of micelle. At a concentration of 15 folds of CMC, CI was decreased in the fastest extent, while at a concentration of 1 CMC, decrease in CI was comparable to that of the system without micelles. Furthermore, at the end of reaction, all systems showed almost the same CI values. The decrease in CI or absorbance at 528 nm was likely due to degradation of delphinidin. These observations implied that SDS micelle could not deter degradation of delphinidin in the presence of micelle at equilibrium, a portion of delphinidin was partitioned into micelles while the rest stayed in the bulk phase. When the delphinidin in the bulk phase degraded to chalcone, delphinidin inside the micelles was partitioned out to the bulk phase in order to keep the equilibrium. This process progressed until delphinidin completely degraded. The fastest decreased rate of CI in the presence of SDS at a concentration of 15 folds of CMC could be due to two reasons. Firstly, delphinidin degradation led to lower concentration of delphinidin presence in the palisade layer; thus, decrease in visible absorbance was observed. Secondly, chalcone (degraded product) is a neutral and water-soluble molecule, so chalcone had no interaction with SDS micelles leading to comparable CI values independent of SDS micelle concentration.

In the absence and in the presence of SDS micelles at a concentration of 1 CMC, CD of roselle was not significantly changed over the time (Independent-t test, $p > 0.05$) (Figure 29, 1b). CD is a summation of absorbance at 420, 520, and 620 nm. In the presence of SDS micelles at concentrations of 5, 10, and 15 folds of CMC, CD values at the end of experiment were significantly decreased from the values at initial time (Paired-t test, $p < 0.05$). The decrease CD of roselle dye solution indicated that intensity of roselle color decreased.

The % color remaining of roselle dye was not different in the absence and in the presence of SDS micelles over 2 days. After 2 days, the % color remaining of roselle dye in various SDS concentrations decreased and % color remaining of roselle

in SDS micelles at 15 folds of CMC decreased in the greatest extent. This result also indicated that the interaction between delphinidin and SDS micelles did not deter degradation rate of delphinidin even in the high concentration of SDS micelle. Decrease in CD and % color remaining at high concentration of SDS micelles were the fastest due to decrease of UV absorptivity of delphinidin (Hendry and Houghton, 1991 and Lima et al., 2002).

Change in hue (*h*) and chroma (*C*) as a function of time of roselles in the absence and in the presence of SDS micelles were illustrated in Figure 30, 1a. Chroma values of all solutions gradually decreased over 12 days. Chroma represents color intensity or distance away from the origin on a Cartesian coordinate (Figure 11). Color faded or gets intense when *C* value decreases or increases, respectively. In this study, chroma of roselle in the absence and in the presence of SDS micelles at a concentration of 1 CMC were superimposable. This observation on *C* was consistent with the CD obtained from UV-visible technique as mention earlier. At initial time, greater *C* values were increase as concentrations of SDS were increased. However, the *C* values at various SDS concentrations became comparable at the end of experiment. Changes in hue as a function of time were depicted as sigmoidal profiles (Figure 30, 1a (solid lines)). The *h* values were slowly increased during day 0 to day 3. Then, the hue were steeply increased during day 4 to day 6. The hue were gradually increased during day 7 to day 12. All profiles were almost superimposable and obviously were not different from each other at all times except for hue of roselle in the presence of SDS at a concentration of 15 CMC during day 0 to day 3 of the reaction. In the CIELab system, hue is the parameter describing color in terms of angle on a Cartesian coordinate. Therefore, the hue profiles in this experiment showed that roselle color changed from red to yellowish over 12 days. The hue profile of roselle in SDS micelles at concentration of 15 CMC also showed that at initial time the intensity of roselle color did not only increase but the color also visually changed. However, this color change could not be detected by UV-visible spectroscopy as a shift in absorption maxima.

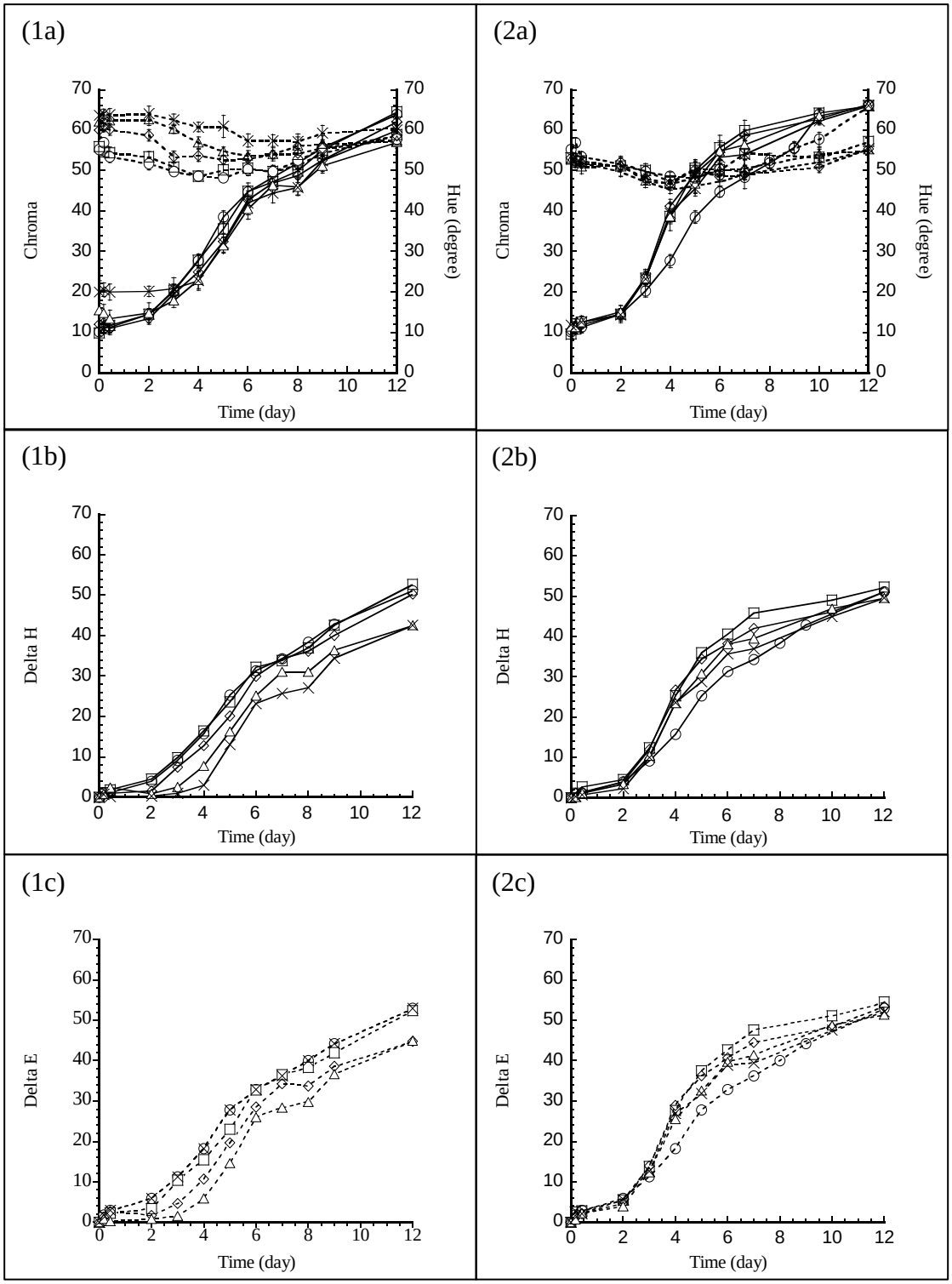

Figure 30 Chroma (a, ---), hue (a,—), Δ*H* (b), and Δ*E* (c) of roselle dye in SDS (1) or Tween80 (2) micellar systems, i.e. succinate buffer pH 4.5 (⊖), 1CMC (□), 5 CMC (◇), 10 CMC (△), and 15 CMC (×)

ΔH and ΔE profiles of roselle in the absence and in the presence of SDS micelles were shown as sigmoidal profiles (Figure 30, 1b and 1c). ΔH and ΔE represent changes in color positions on the CIELab sphere. Figure 30, 1b also illustrated that, in the absence and in the presence of low concentration of SDS micelles (1CMC), changes in ΔH could be detected as early as day 2 of the experiment. As concentration of SDS micelle increased, it could prolong changes in ΔH up to 4 days. Therefore, the SDS micelles at high concentration could be slow down degradation reaction of roselle color at the initial time.

CI and % color remaining of roselle dye in the absence and in the presence of Tween80 micelles gradually decreased overtime (Figure 29, 2a and 2c). These results implied that the redness of roselle dye decreased as a function of time. However, color density did not obviously change suggesting that color of roselle dye was changed but could not specified using color density (Figure 29, 2b). Flavylium cation in roselle dye was expected to show weak interaction with non-ionic micelles (Tween80); therefore, stability of roselle dye at various concentrations of Tween80 was comparable to that of in the absence of micelles. This speculation was supported by the fact that visible-absorption enhancement or red shifts in absorption maxima were not observed. CI, CD, and % color remaining profiles of roselle dye in the presence all various concentrations of Tween80 were similar to each other but they were significantly different from those values in the absence of micelles (ANOVA, $p < 0.05$) (Figure 29, 2a, 2b, and 2c). CI and % color remaining of roselle in the absence of Tween80 showed that color was more stable than those in the presence of Tween80 micelles during first 10 days. These finding also suggested that there was a weak interaction between delphinidin and Tween80 micelles in a way that delphinidin degradation was facilitated by the presence of Tween80 micelles and the facilitation was independent of micelle concentration.

Employing CIELab system in determination of roselle color change in the absence and in the presence of Tween80 micelles was illustrated by hue and chroma profiles in Figure 30, 2a. Chroma of all solutions were similar to CD in UV-spectroscopy technique representing intensity of all color in the sample (Figure 29b and 30b). These *C* and CD values did not change much over 12 days and no effect of Tween80 concentration was observed. However, alteration of *h* was obviously

noticeable. Changes in *h* as a function of time were depicted as sigmoidal profiles (Figure 30, 2a (solid lines)). These profiles were almost superimposable and did not show obviously different from each other at all times except for hue of roselle in the absence of Tween80. The hue profiles in this experiment suggested that roselle color was change from red to yellowish over 12 days in the same manner as what was observed in SDS micelles. Hue profile of roselle in the absence of Tween80 micelles obviously showed that color of roselle dye was changed from red to yellow at a different rate from that in the presence of Tween80 micelles. However, by the end of the experiments, color of roselle in all solutions was changed in the same extent and reached the same hue values. ΔH and ΔE profiles of roselle in the absence and in the presence of Tween80 micelles were shown as sigmoidal profiles (Figure 30, 2b and 2c), illustrated that, in the absence and in the presence of Tween80 micelles, changes in ΔH and ΔE was detectable as early as within 2 days of the experiment. Moreover, the ΔH and ΔE of roselle in the absence Tween80 micelles was significantly lower than that in the presence Tween80 micelles at all concentrations (ANOVA, $p < 0.05$) and became indifferent at the end of study.

Stability of lac dye in succinate buffer pH 4.5 in the absence and in the presence of micelle at concentrations of 1-15 folds of SDS or Tween80 micelles was studied. Lac color in the absence and in the presence of SDS or Tween80 changed from orange to orange-red (Figure 31). According to HPLC chromatograms of lac in the absence and in the presence of SDS micelles at initial time and at the end of experiment were shown in Figure 32. At initial time, HPLC chromatogram showed two major peaks at retention times of 7.5 and 18.7 minutes corresponding to laccaic acid C and a mixture of laccaic acid A and B which were co-eluted, respectively. The peak height of laccaic acid A and B in all SDS solutions at day 60 were decreased when compared with initial time, while peak height of lacaic acid C was gradually decreased as concentration of SDS micelles were increased. Laccaic acid A, B, and C degradation led to color change. However, information regarding degradation of laccaic acids was very few. Therefore, discussion on possible degradation products could not be done at this moment. Further studies need to be done in order to clarify these points.

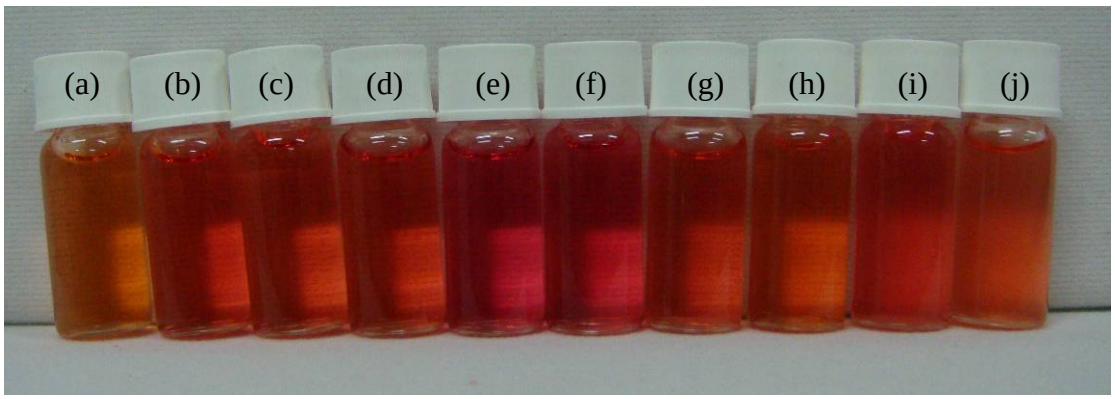

Figure 31 Color of lac dye in succinate buffer pH 4.5 at day 0 (a), succinate buffer pH 4.5 at day 60 (b), 1 CMC of SDS micelle at day 60 (c), 5 CMC of SDS micelle at day 60 (d), 10 CMC of SDS micelle at day 60 (e), 15 CMC of SDS micelle at day 60 (f), 1 CMC of Tween80 micelle at day 60 (g), 5 CMC of Tween80 micelle at day 60 (h), 10 CMC of Tween80 micelle at day 60 (i), and 15 CMC of Tween80 micelle at day 60 (j)

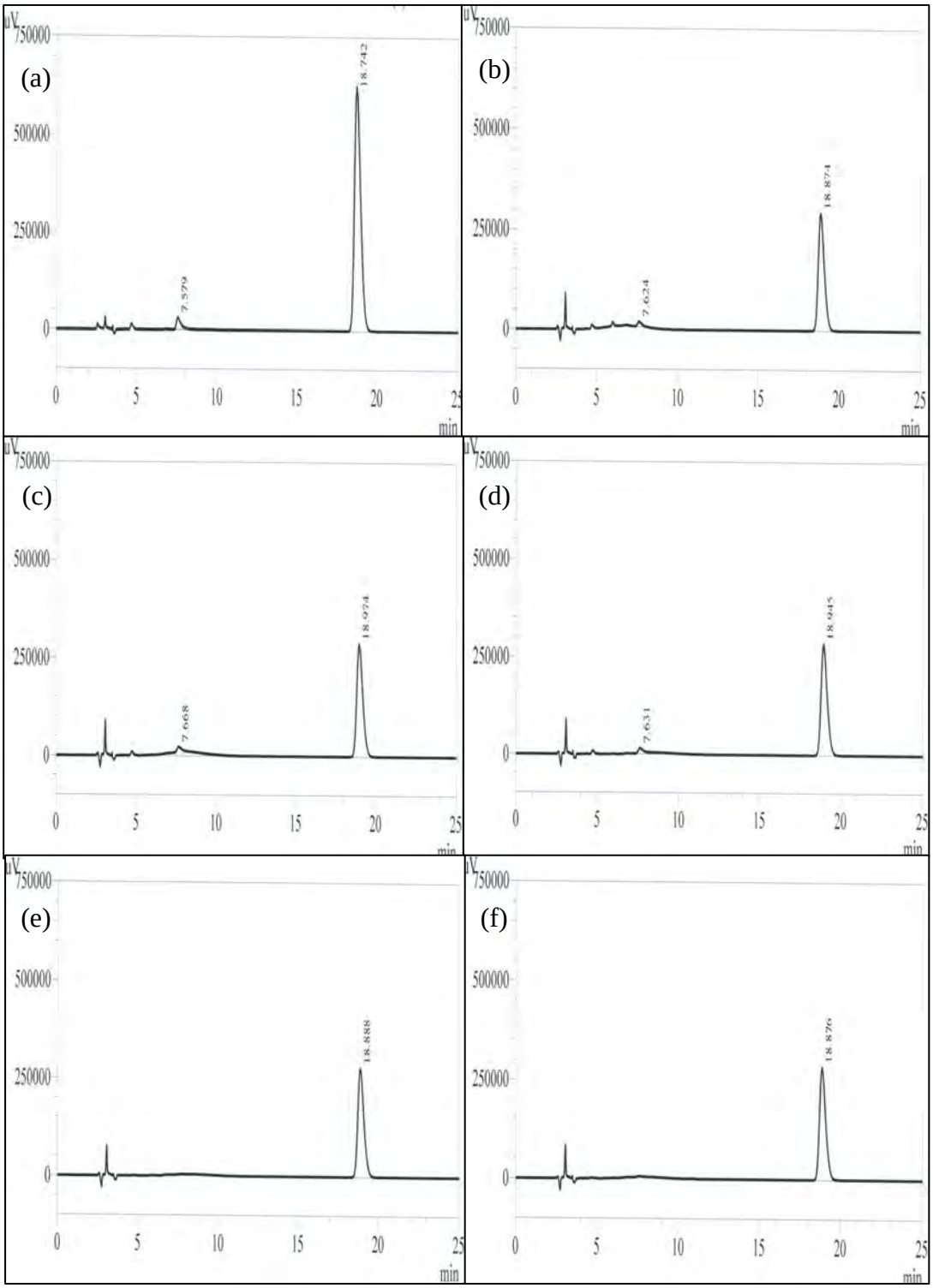

Figure 32 HPLC chromatograms of lac dye in succinate buffer pH 4.5 (at day 0 (a) and day 60 (b)) or SDS micellar systems at concentrations of 1 CMC (c), 5 CMC (d), 10 CMC (e), and 15 CMC (f) at day 60

Visible spectra of lac in the presence of SDS micelles at all concentrations were identical during the first 2 days while visible spectra of lac dye in the absence of micelle showed some changes within 2 days (Figure 33). At initial time, lac dye possessed visible absorption maxima at 490 nm corresponding to orange color. After kept lac dye solutions at 30 °C and pH 4.5, absorbances at 490 nm decreased very slowly. In addition, a new absorption maxima and a shoulder at 520 and 560 nm, respectively, were became obviously detectable. Therefore, lac dye in the presence of SDS micelles was lasting about 2 days before the change. Overall this observed visible characteristics of lac color in the presence of SDS at all concentrations were similary to visible characteristic of lac dye in the absence of micelles. Color change of lac dye was delayed by the presence of SDS micelles for few days. At pH 4.5, water soluble lac dye, which has pK_a of 5.96, are in an unionized form and showed weak interaction with SDS micelles.

In case of lac, CI was determined from absorbances at 490 nm and % color remaining is presented as percentage of absorbance at 490 nm at time t to that at initial time. CI, CD, and % color remaining of lac color in SDS as a function of time are shown in Figure 34. CI and color remaining of lac dye in the presence of SDS micelles were not significantly different when compared to lac dye in the absence of SDS micelles throughout 2 months (ANOVA, $p > 0.05$). CI and % color remaining were calculated from absorbances at 490 nm, which was close to isobestic point (Figure 17b). Thus, CI and % color remaining of lac color did not change while UV spectra of lac dye in the absence and in the presence SDS micelles changed obviously. The alteration of lac color could be observed from the increase in CD value during the first 3 day of the experiment (Figure 34, 2b). CD values are a summation of absorbances at three wavelengths of 420, 520, and 620 nm as mentioned above. When the absorbance of three primaries: red, yellow, and blue, were taken into consideration, the increase of CD was due to increase of redness at 520 nm. However, UV-spectroscopy parameter could not clearly arbitrate color change of lac; therefore, CIELab was used to assure the change of lac color.

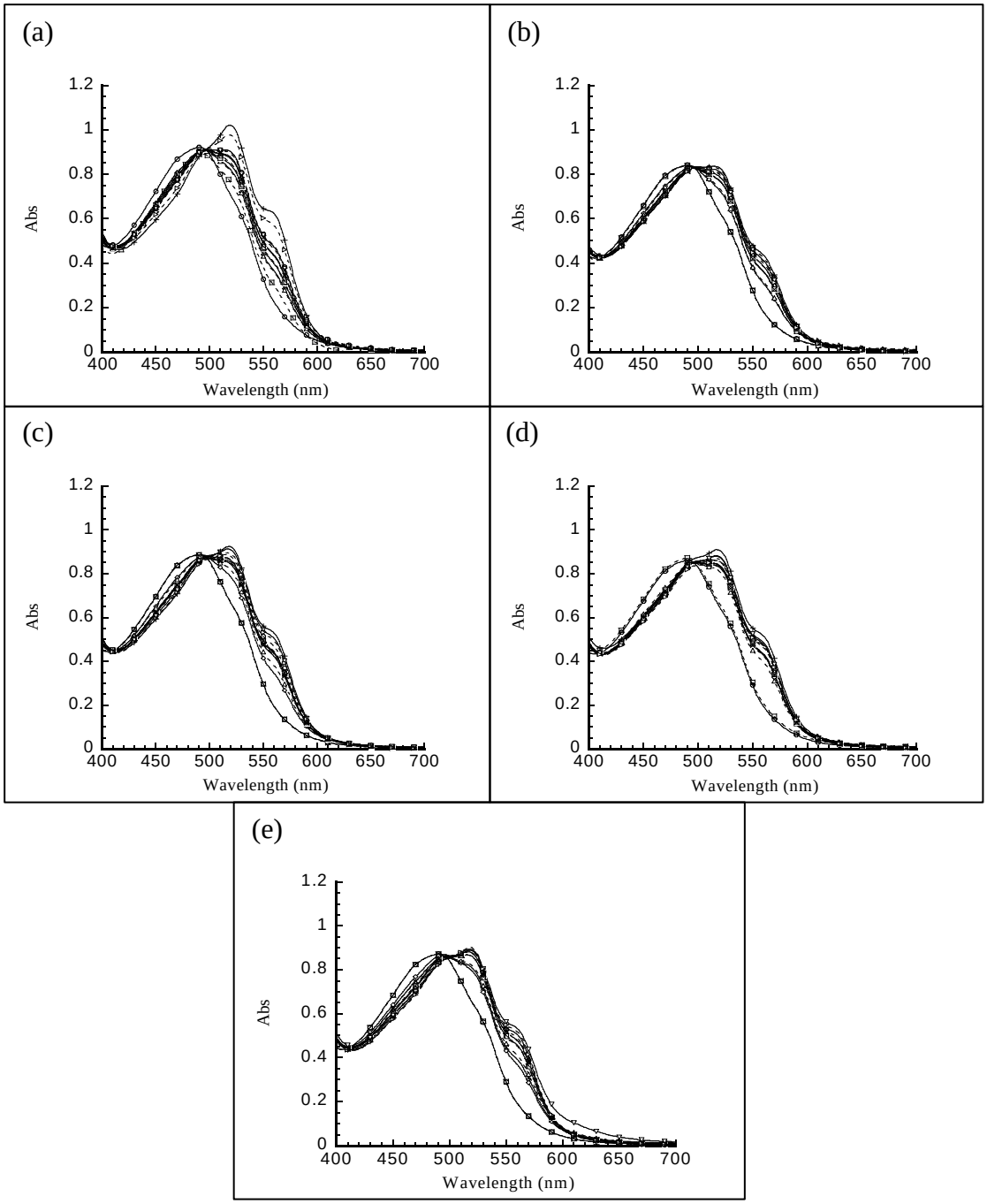

Figure 33 UV-visible spectra of lac color in succinate buffer pH 4.5 in the absence and in the presence of SDS micellar system: no SDS (a), 1 CMC (b), 5 CMC (c), 10 CMC (d) and 15 CMC (e) at day 0 (⊖), day 2 (--□--), day 5 (◈), day 8 (--△--), day 14 (⊟), day 18 (--◺--), day 21 (✳), day 24 (--×--), day 31 (⧋), day 38 (--○--), day 45 (▽), day 52 (--▷--), and day 60 (+)

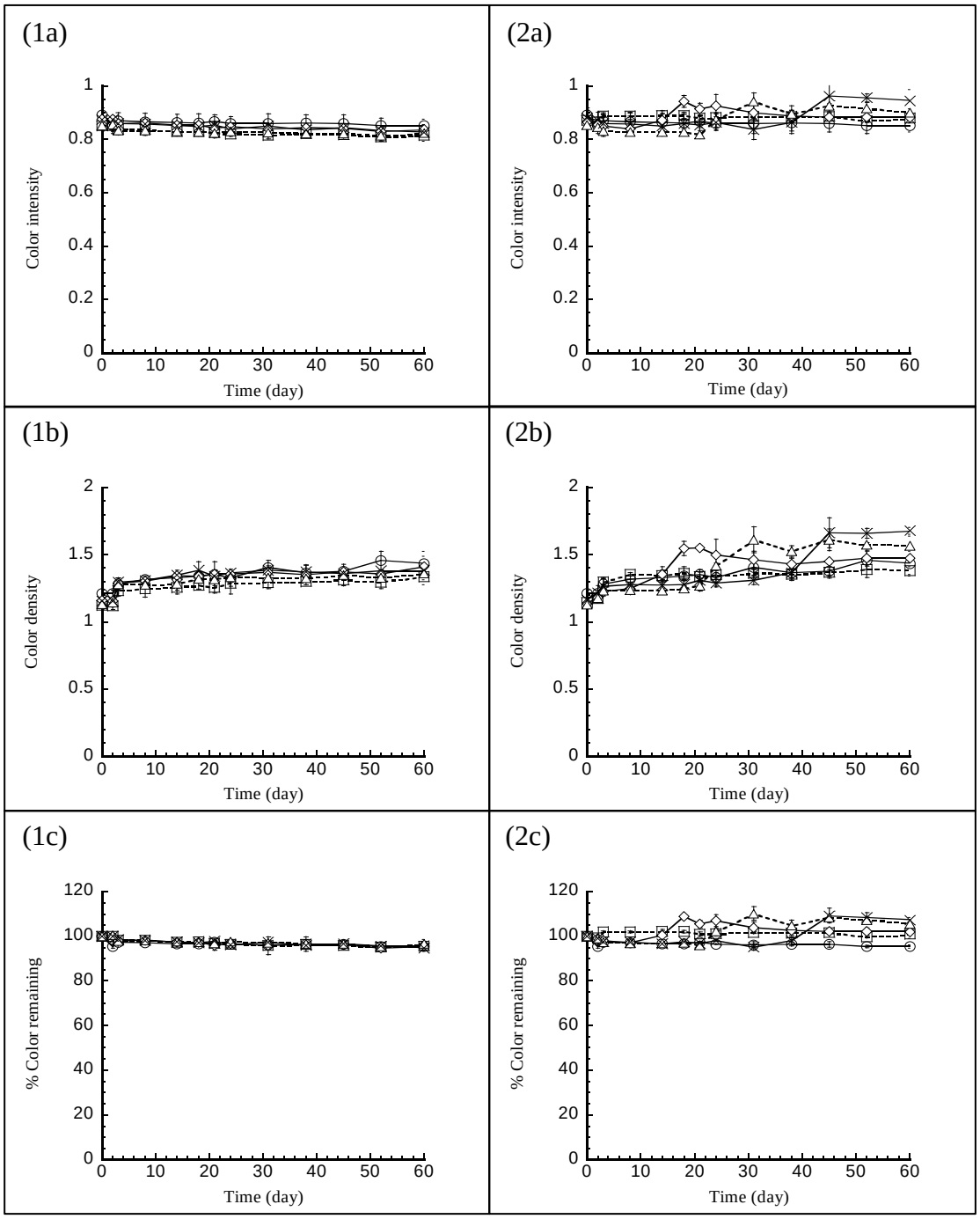

Figure 34 Color intensity (a), color density (b), and percentage of color remaining (c) of lac dye in SDS (1) or Tween80 (2) micellar systems, i.e. succinate buffer pH 4.5 (⊖), 1CMC (--□--), 5 CMC (◈), 10 CMC (--△--), and 15 CMC (✶)

Change of chroma (*C*) and hue (*h*) as a function of time of lac in the absence and in the presence of SDS micelles were depicted in Figure 35a, 1a. Over time, chroma profiles and hue profiles of lac in the absence and in the presence of SDS micelles were almost superimposable. Chroma values at initial time and at any time point were not significantly different (Paired-t test, $p > 0.05$) while a small decrease in hue value was observed during first few days and then remained at about 45 degree corresponding to orange. Therefore, it could be concluded that intensity of lac color in all conditions remained almost constant and color of lac were also red throughout the experiments. These results supported the data from UV-spectroscopy technique.

ΔH and ΔE profiles of lac in the absence of SDS micelle and in the presence of SDS micelle at a concentration of 1 CMC were almost identical (Figure 35b and c). ΔH and ΔE profiles of lac in the presence of SDS micelles at concentration of 5, 10, and 15 CMC were obviously depicted lac color change since ΔH and ΔE values increased from 0 to about 10 after 2 days. After 2 days, ΔH and ΔE profiles showed minor changes. These results suggested that lac dyes in the absence and in the presence of SDS micelles at a concentration of 1 CMC, underwent less color change than those in the presence of SDS micelles at concentration of 5, 10, and 15 CMC. This difference in color change of various solutions could not be detected using UV-spectroscopy technique. Although CD profiles (Figure 34, 1b) showed that most of color change took place within 2 days of the experiment, they could not distinguished the difference of color change at 0 and 1 CMC of SDS concentrations from the rest.

Lac color in the presence of Tween80 micelles at concentration of 5, 10, and 15 CMC became cloudy. The observed cloudiness of these mixtures were speculated to be due to decrease of the Tween80 cloud point as mention earlier. Therefore, cloudy solution was adjusted to zero by substration absorbances with the absorbance at 700 nm. The visible spectra after baseline adjustment were displayed in Figure 36. During storage time, visible spectra of lac in the absence of Tween80 showed red shift to absorption maxima of 520 nm while UV spectra of lac in Tween80 micelles showed spectra broadening and hyperchromic shift. The obviously hyperchromic shift were observed in the absence and in the presence of Tween80 at concentration of 5, 10, and 15 CMC. The observed band broadening and hyperchromic shift at pH 4.5 suggested that unionized form of lac dye interacted with non-ionic micelles of

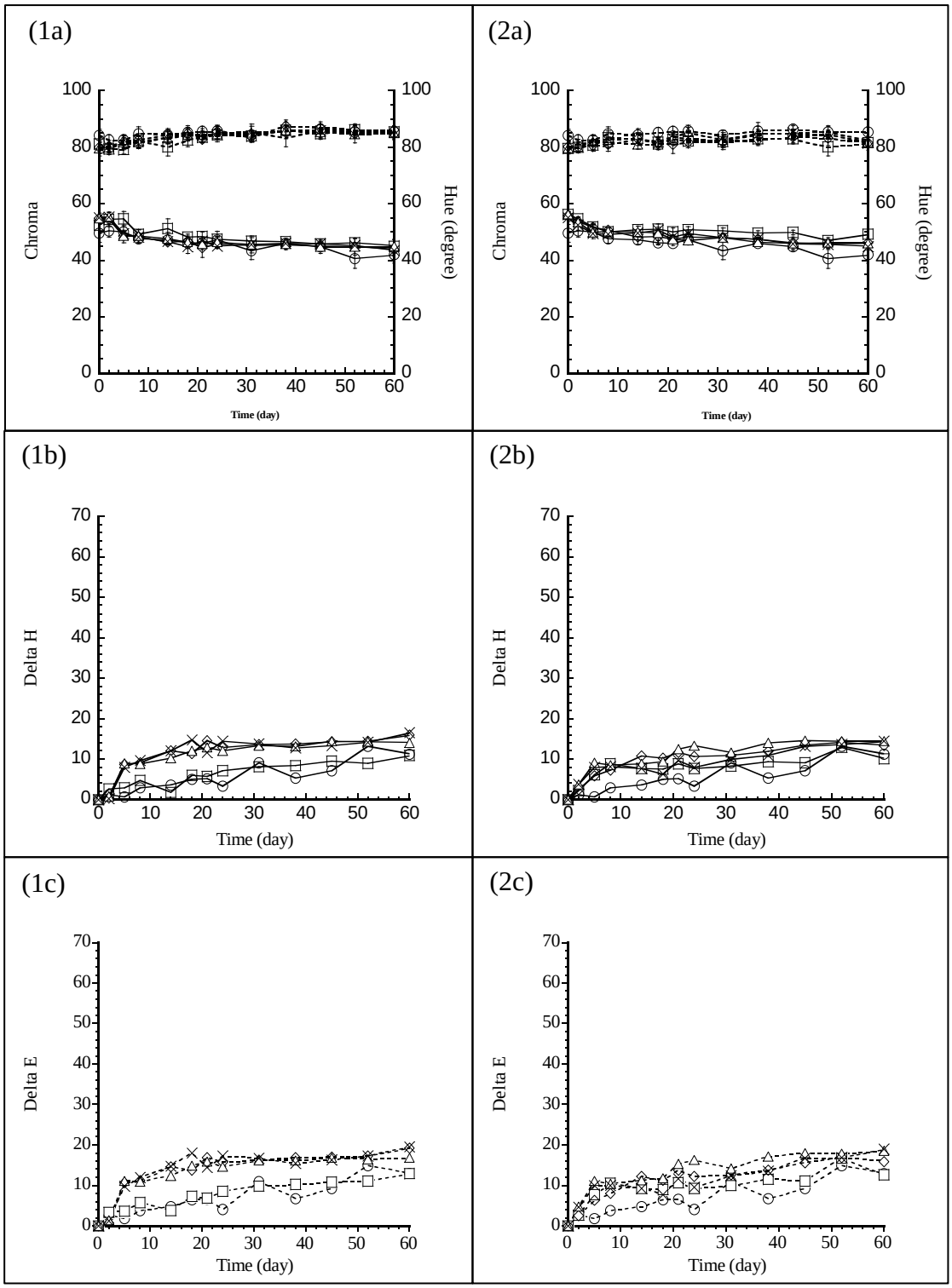

Figure 35 Chroma (a,---), hue (a,—), Δ*H* (b), and Δ*E* (c) of lac dye in SDS (1) or Tween80 (2) micellar systems, i.e. succinate buffer pH 4.5 (⊖), 1CMC (□), 5 CMC (◇), 10 CMC (△), and 15 CMC (×)

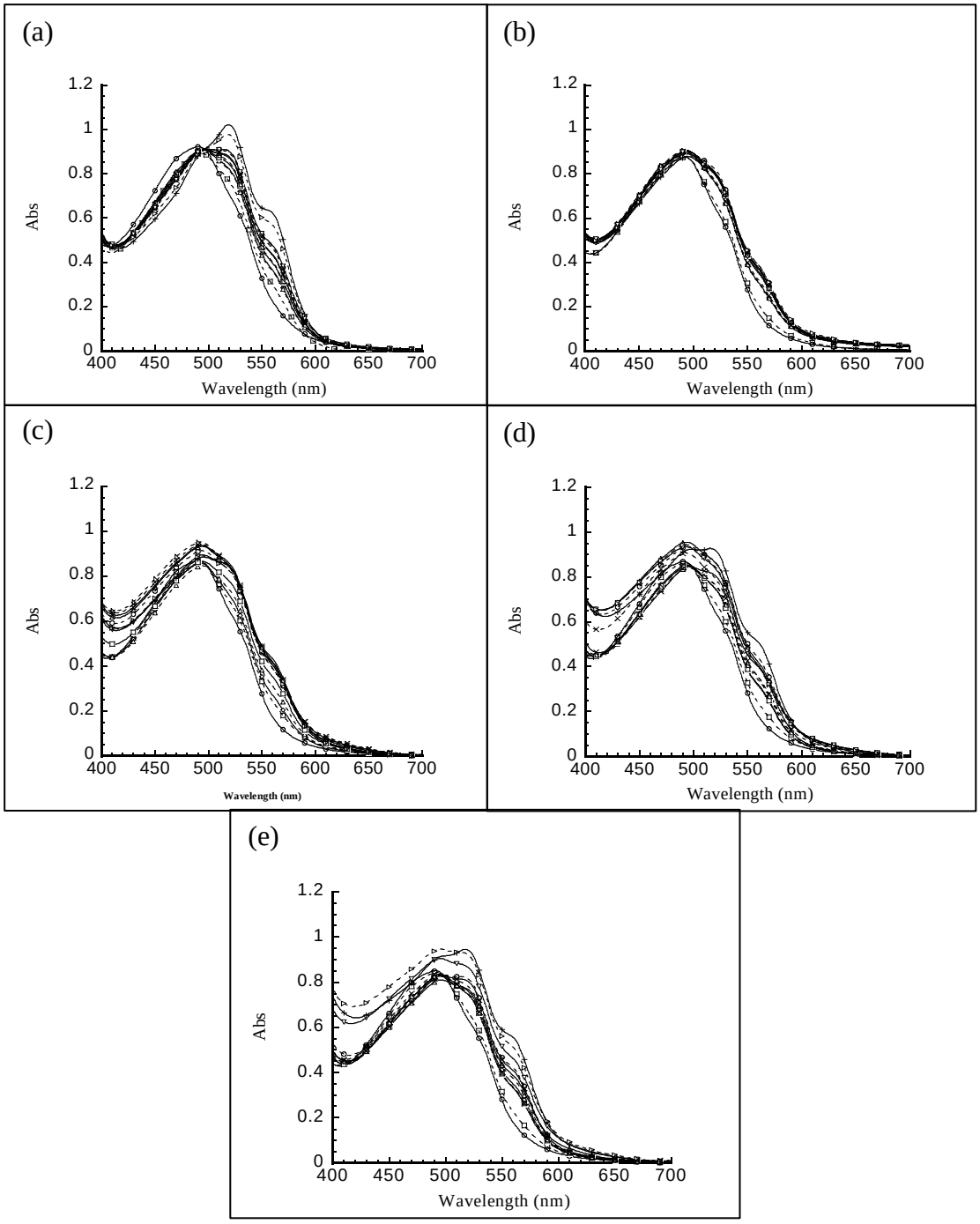

Figure 36 UV-visible spectra of lac color in succinate buffer pH 4.5 in the absence or in the presence of Tween80 micellar system: no Tween80 (a), 1 CMC (b), 5 CMC (c), 10 CMC (d) and 15 CMC (e) at day 0 (⊖), day 2 (--□--), day 5 (◈),day 8 (--△--), day 14 (⊟), day 18 (--◺--), day 21 (✳), day 24 (--×--), day 31 (⧋), day 38 (--○--), day 45 (▽), day 52 (--▷--), and day 60 (┼)

Tween80 at some extent. In addition, the solution cloudiness showed that there were physical instabilities between lac dye and Tween80 at high concentration.

CI and % color remaining profiles of lac in the presence of Tween80 were similar to that in the absence of Tween80 (Figure 34, 2a and 2c). CD of lac in the absence and the presence of Tween80 after day 60 were increased when compared to CD values at the initial time especially during the first few days of experiment. However, lac color change could not be clearly elaborated from CI, CD, and % color remaining data.

Employing CIELab method, data are shown in Figure 35, 2a-c. No change of chroma was observed during the study in the systems with and without Tween80. Hue profiles showed that lac color in all solutions were in red shade but the red color of lac in the absence of Tween80 was more in different in red tone (lower hue values) from that in the presence of Tween80. Similar to the effect of SDS, obvious changes of ΔH and ΔE in the presence of Tween80 were observed during the first few days and gradually increased after that.

Gardenia dye was dissolved in phosphate buffer pH 7, SDS or Tween80 micellar systems. All solutions were yellow and could not be distinguished from each other by eyes. After 60 days in the dark at 30 °C, gardenia color in the absence of micelles, in the presence SDS at concentration of 1CMC, and in the presence of Tween80 at concentrations of 1, 5, 10, and 15 CMC faded overtime while gardenia in the presence of SDS at concentrations of 5, 10, and 15 CMC remained yellowish (Figure 37). HPLC chromatogram of gardenia color at initial time showed three major peaks at retention times of 13.7, 27.8, and 29.6 minutes corresponding to crocin, crocetin monogentiobiosyl monoglucosyl ester, and crocetin monogentiobiosyl ester, respectively (Figure 38a). Over 60 days crocetin derivative peaks were disappeared from all solutions (Figure 38).

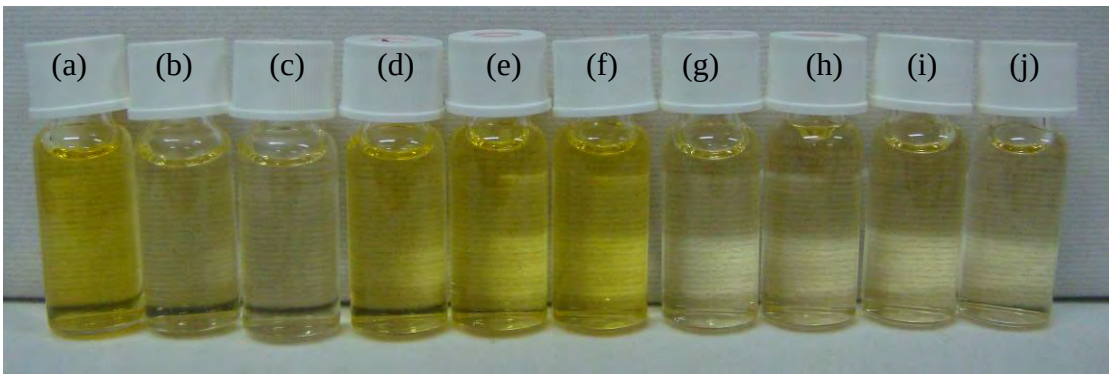

Figure 37 Color of gardenia dye in phosphate buffer pH 7 at day 0 (a), phosphate buffer pH 7 at 60 day (b), 1 CMC of SDS micelle at day 60 (c), 5 CMC of SDS micelle at day 60 (d), 10 CMC of SDS micelle at day 60 (e), 15 CMC of SDS micelle at day 60 (f), 1 CMC of Tween80 micelle at day 60 (g), 5 CMC of Tween80 micelle at day 60 (h), 10 CMC of Tween80 micelle at day 60 (i), and 15 CMC of Tween80 micelle at day 60 (j)

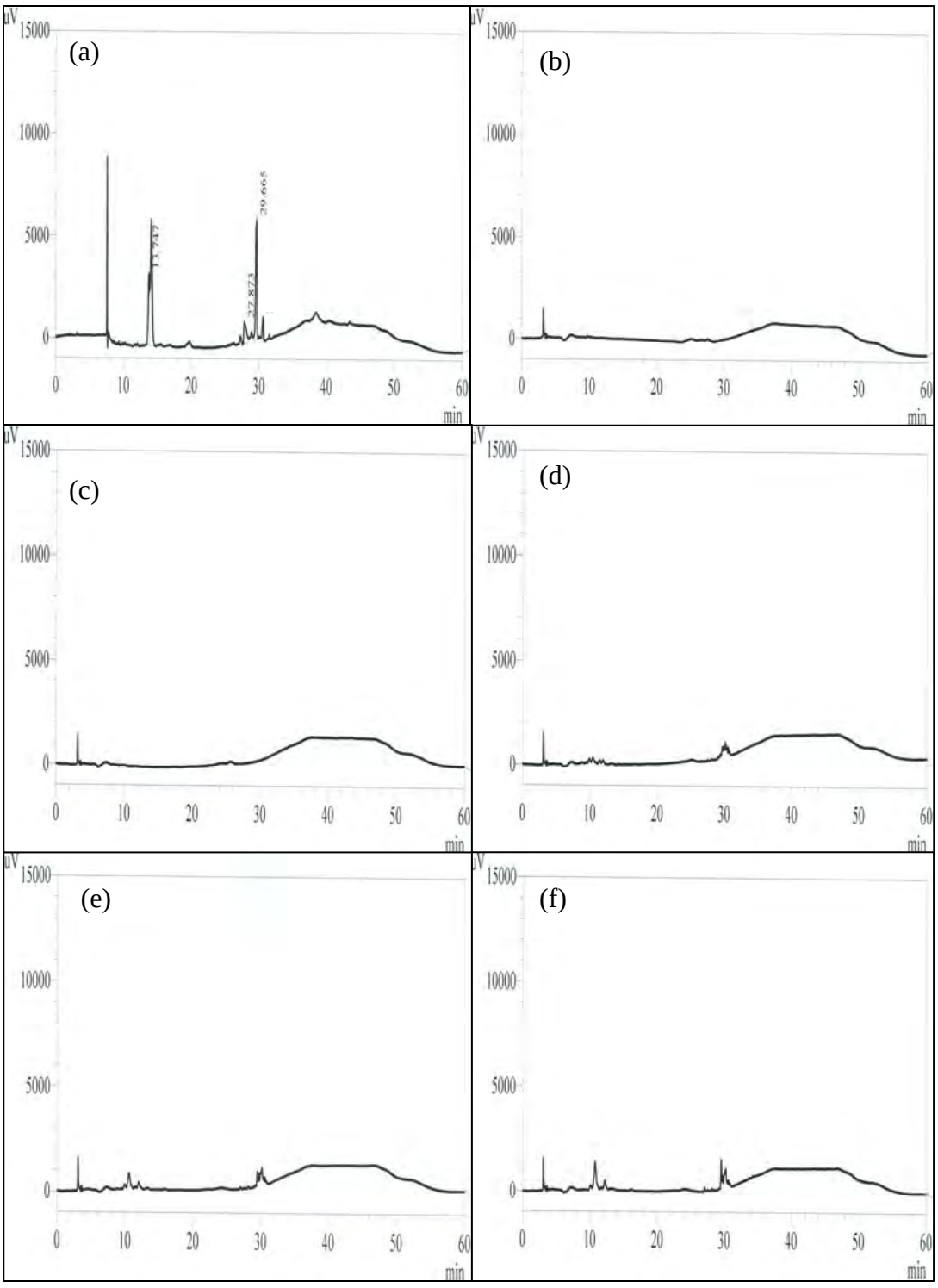

Figure 38 HPLC chromatograms of gardenia dye in phosphate buffer pH 7 (at day 0 (a) and day 60 (b)) or SDS micellar systems at concentrations of 1 CMC (c), 5 CMC (d), 10 CMC (e), and 15 CMC (f) at day 60

At initial time, visible spectra of gardenia color in the absence and in the presence of SDS micelles were similar to UV-visible absorption maximum at 443 nm and a shoulder at 470 nm. Over 60 days, visible spectra of gardenia in SDS solution at concentrations of 0 and 1 CMC showed the same characteristics as those at initial time with decrease in absorbance (Figure 39a, b) while in the presence of SDS concentrations of 5, 10, and 15 CMC, the shoulder of visible spectra at 470 nm was prominent and the absorbances more slowly decreased than the absorbance in the absence and in the presence of SDS at concentration of 1CMC (Figure 39). In other words, SDS micelles at high concentration could deter fading of gardenia color. Crocin (Figure 4), a major component in gardenia, is a non-ionic dye. Thus, the crocin was not expected to have ionic interact with SDS micelles. However, hydrophobic structure of crocetin may interact with SDS micelles through hydrophobic-hydrophobic interaction. As a result, visible characteristic alteration was observed in the presence of high SDS concentrations. The crocin molecule possesses two disaccharides (digentiobioside) connected to crocetin through ester bonds as shown in Figure 4d, so some crocin molecules which do not reside in the micellar structure undergoes hydrolysis eventually giving crocetin which has lower molar absorptivity than that of crocin (Lauro and Francis, 2000 Park et al., 2001 and Ketmaro, Muangsiri and Werwatganone, 2008). Therefore, in the presence of high SDS micelle concentrations, micellar structure could accommodate crocin and retard crocin hydrolysis by lowering accessibility of water molecules.

CI, CD, and % color remaining of gardenia color in the absence or in the presence of SDS at concentrations of 1, 5, 10, and 15 CMC were depicted in Figure 40, 1a-c. CI, CD, and % color remaining curves of gardenia color in the absence and in the presence of SDS micelle at concentration of 1 CMC were superimposable while color fading of gardenia in the presence of SDS at concentration of 5, 10, and 15 CMC was inversely related to concentration of SDS micelle. At concentration of 15 CMC, CI, CD, and % color remaining decreased in the slowest extent. This result indicated that the SDS micelles at high concentration could retard gardenia color fading.

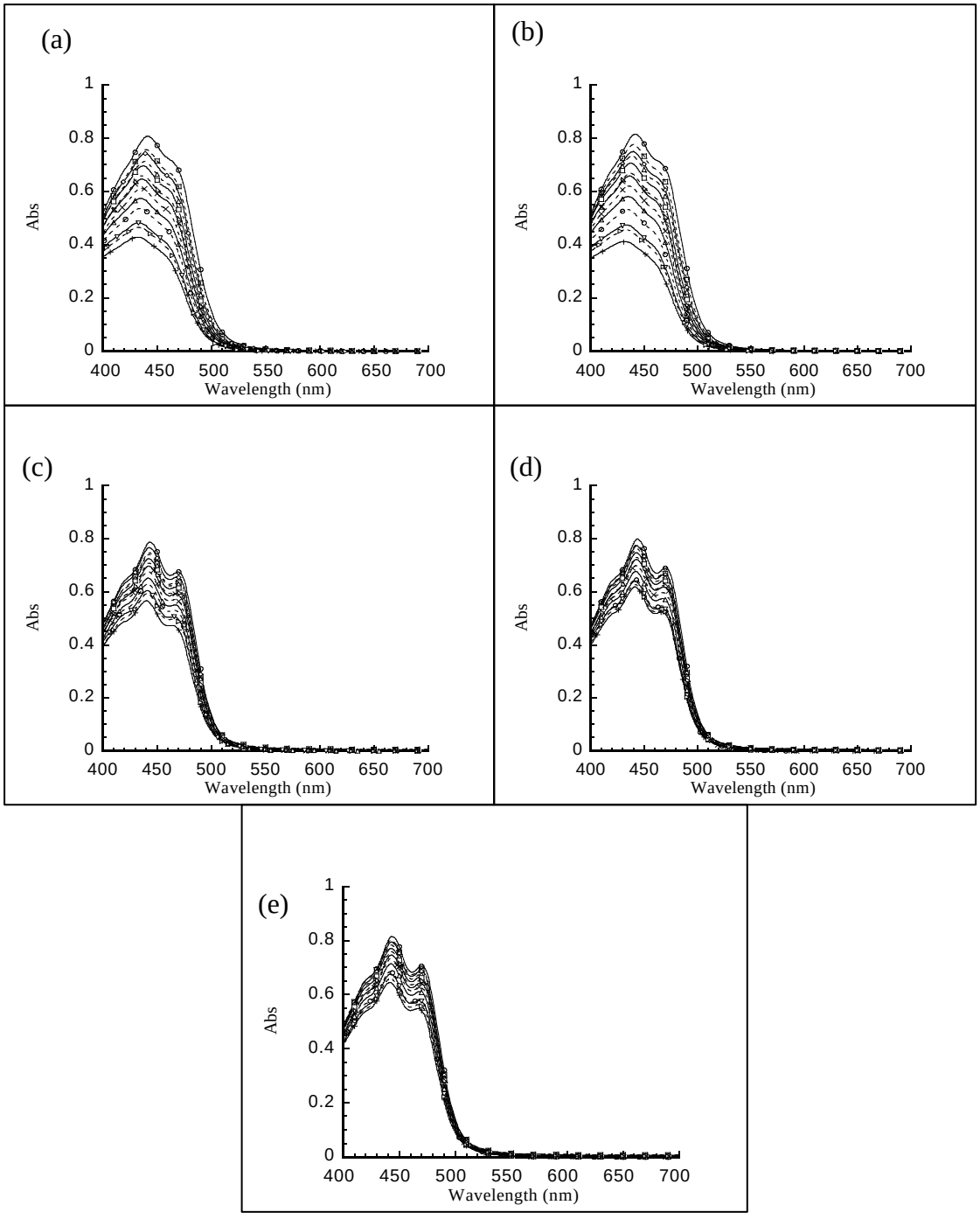

Figure 39 UV-visible spectra of gardenia color in phosphate buffer pH 7 in the absence or in the presence of SDS micellar system: no SDS (a), 1 CMC (b), 5 CMC (c), 10 CMC (d) and 15 CMC (e) at day 0 (⊖), day 4 (--□--), day 6 (◈), day 9 (--△--), day12 (⊟), day 16 (--◺--), day 19 (✳), day 23 (--×--), day 29 (⧋), day 39 (--○--), day 46 (▽), day 53 (--▷--), and day 60 (+)

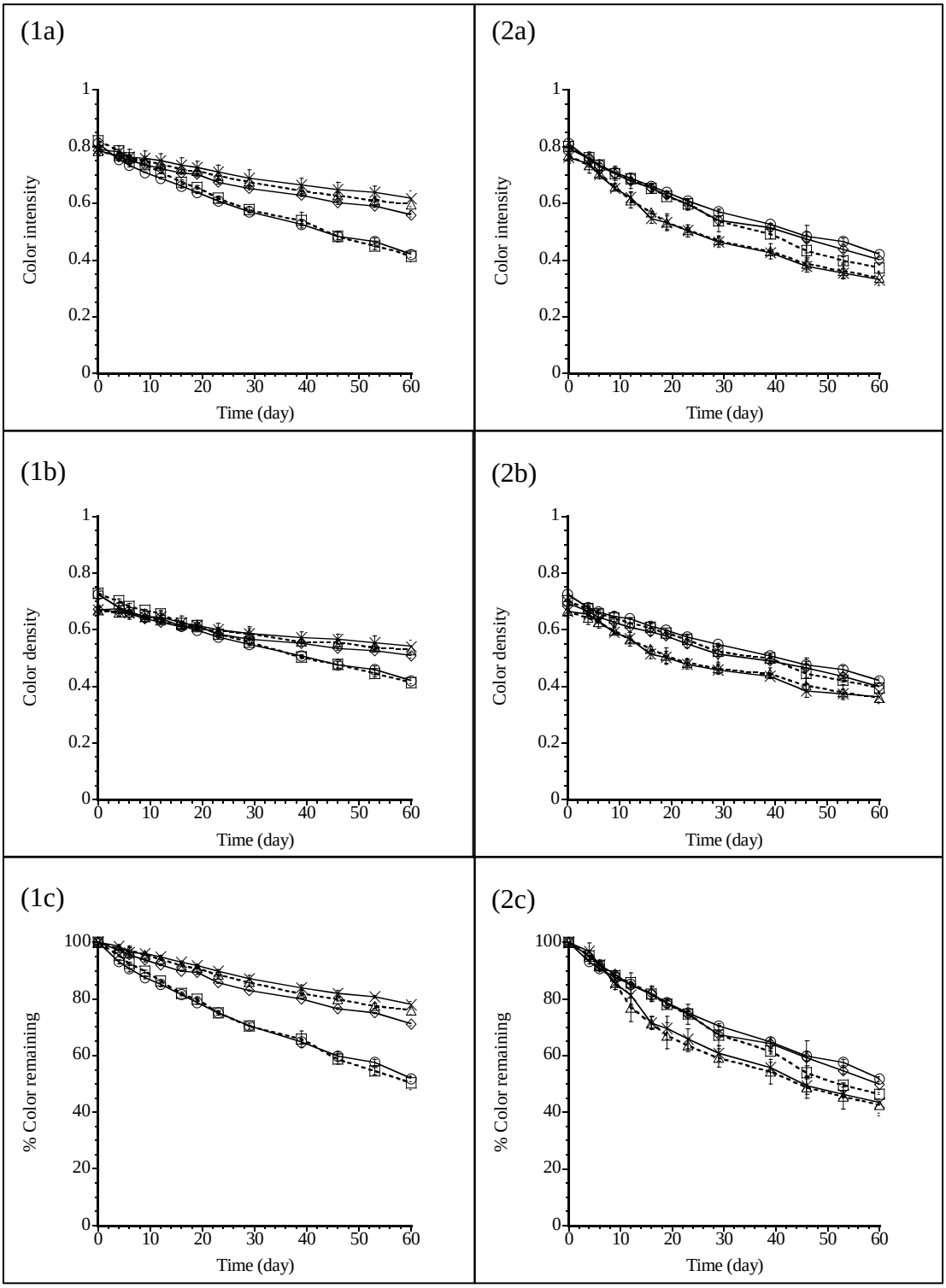

Figure 40 Color intensity (a), color density (b), and percentage of color remaining (c) of gardenia dye in SDS (1) or Tween80 (2) micellar systems, i.e. phosphate buffer pH 7 (⊖), 1CMC (--□--), 5 CMC (◈), 10 CMC (--△--), and 15 CMC (✳)

CIELab values of gardenia color in the absence and in the presence of SDS micelles at concentration of 1, 5, 10, and 15 were displayed in Figure 41, 1a-c. Chroma curve of gardenia color in the absence and in the presence of SDS micelles at all concentrations were almost constant until day 23 of the experiment after that chroma profiles of gardenia in buffer and in the presence of SDS micelles at a concentration of 1 CMC decreased while the rest of chroma profiles remained constant. Hue profiles of gardenia color in the absence and in the presence of SDS micelles were slowly increased over 60 days. The hue values were in a range of 90 to 100 consistent with the visualized color, yellow. In the absence and in the presence of SDS micelles at a concentration of 1 CMC, gardenia color was observed to fade (decrease in chroma) over time. On the contrary, gardenia color in the presence of high SDS concentrations did not change over 60 days. ΔH curves of gardenia color in the absence and in the presence of SDS at a concentration of 1 CMC were superimposable and depicted color change in a greater extent than those in the presence of SDS at high concentrations. ΔE profiles of gardenia in the absence and in the presence of SDS micelles at concentration of 1 CMC were similar to each other which showed obvious change in ΔE values after day 23. This observation was consistent with the result obtained from chroma profiles. ΔE curves of gardenia in the presence of SDS at concentrations of 5, 10, and 15 were superimposable and were not significantly changed throughout the experiment ($p > 0.05$).

Visible characteristics of gardenia color in the presence of Tween80 micelles were similar to that of gardenia color in the absence of Tween80 (Figure 42). The UV spectra showed a UV absorption maximum at 442 nm with a shoulder at 465 nm and the absorbances at these two wavelengths decreased over time. Both crocin and Tween80 are non-ionic compounds. Hydrophobic structure of crocetin suggested that hydrophobic interaction between crocin and Tween80 micelles should take place. However, based on the observed UV characteristics of gardenia color, it seemed that there was weak interaction between gardenia dye and Tween80 micelles. The CI, CD, and % color remaining profiles of gardenia color in the absence and in the presence of Tween80 at concentrations of 1 and 5 CMC were superimposable and illustrated that gardenia color in such conditions was more stable than gardenia color in the presence of Tween80 at concentrations of 10 and 15 CMC (Figure 40, 2a-c). Therefore, the

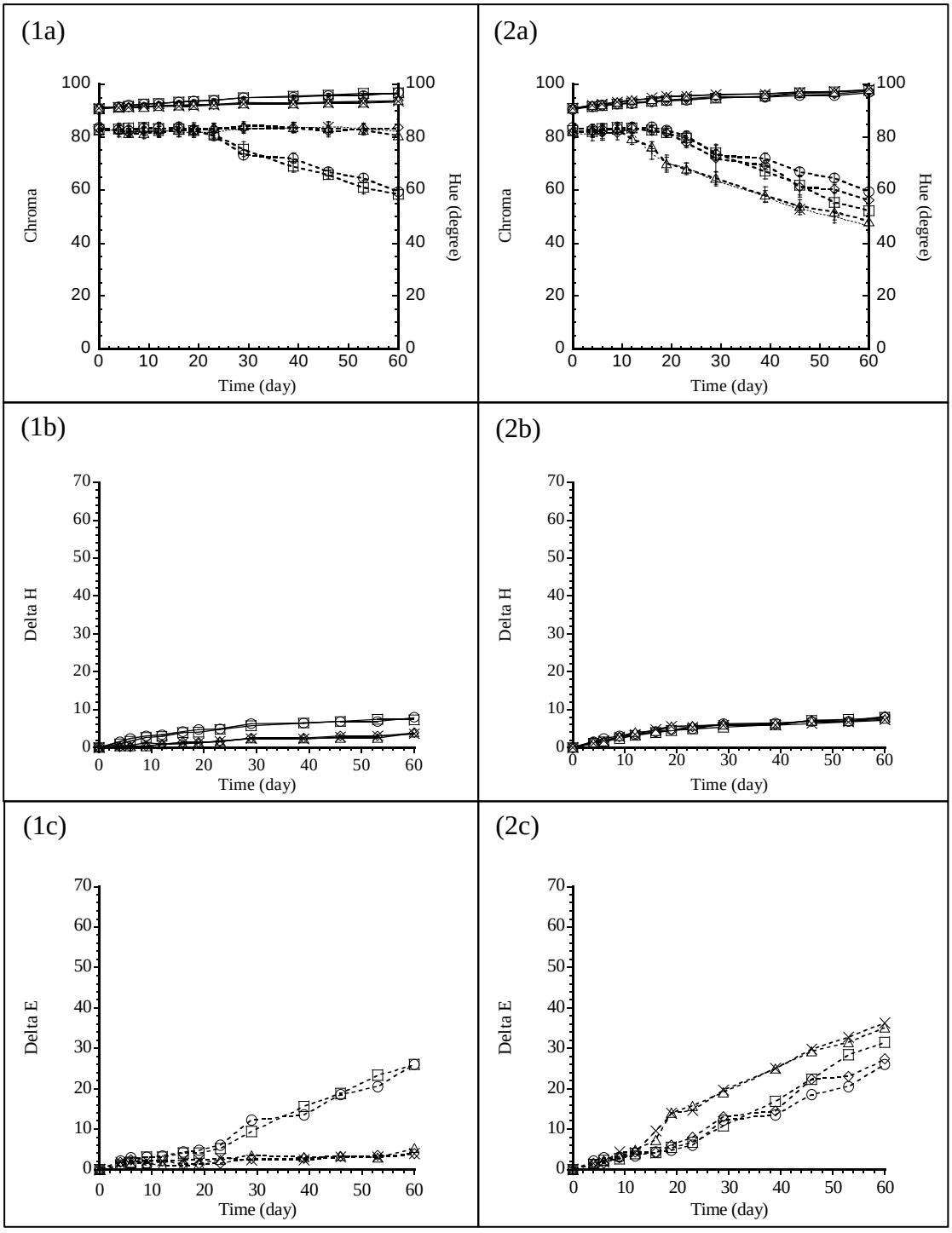

Figure 41 Chroma (a,–––), hue (a,—), Δ*H* (b), and Δ*E* (c) of gardenia dye in SDS (1) or Tween80 (2) micellar systems, i.e. phosphate buffer pH 7 (○), 1CMC (□), 5 CMC (◇), 10 CMC (△), and 15 CMC (×)

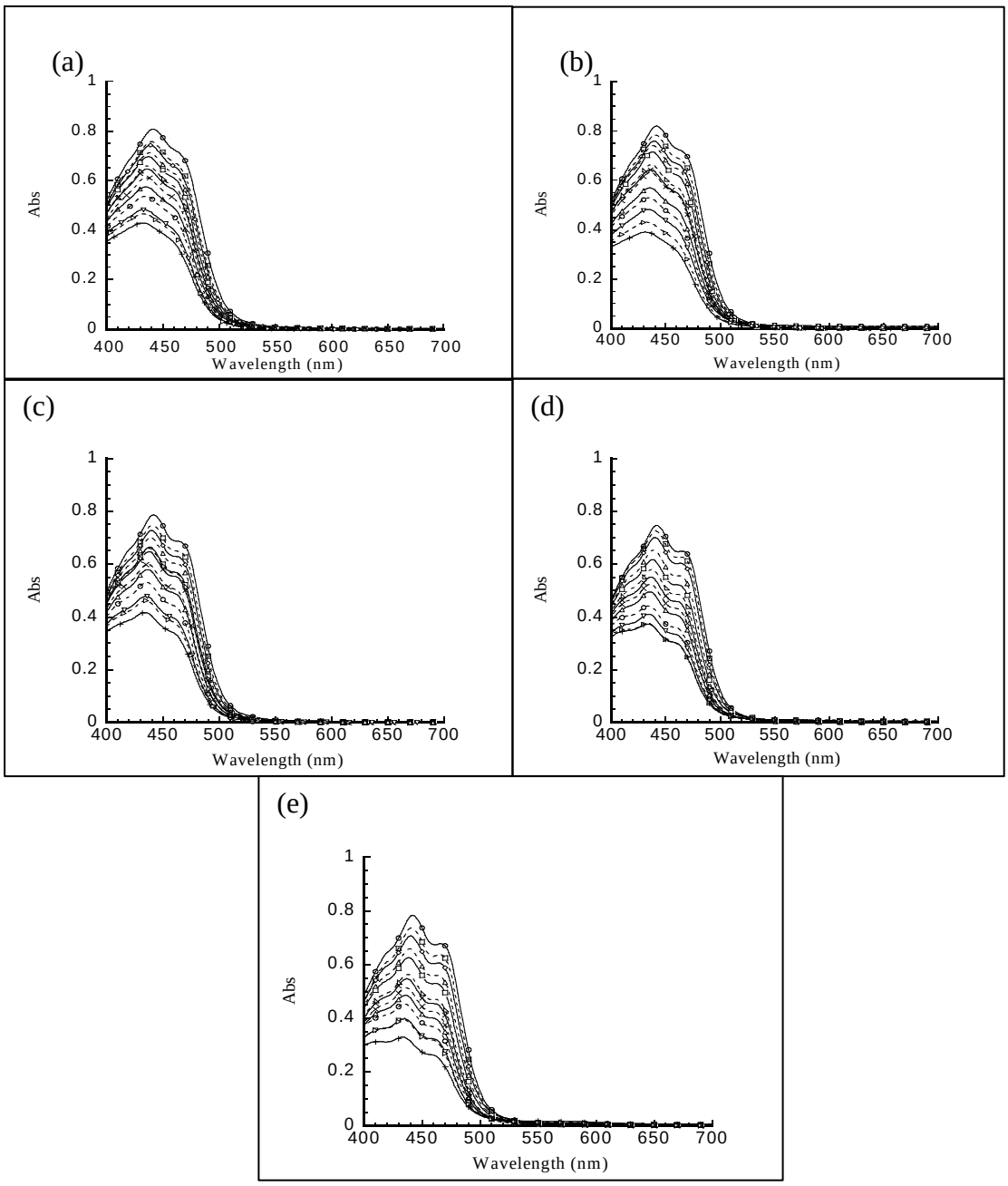

Figure 42 UV-visible spectra of gardenia color in phosphate buffer pH 7 in the absence or in the presence of Tween80 micellar system: no Tween80 (a), 1 CMC (b), 5 CMC (c), 10 CMC (d) and 15 CMC (e) at day 0 (○), day 4 (--□--), day 6 (◇), day 9 (--△--), day 12 (□), day 16 (--◺--), day 19 (×), day 23 (--×--), day 29 (△), day 39 (--○--), day 46 (▽), day 53 (--▷--), and day 60 (+)

results indicated that high concentrations of Tween80 facilitated fading of gardenia color.

CIELab values of gardenia color showed in Figure 41, 2a, 2b, and 2c. Chroma of gardenia color in the absence and in the presence of Tween80 at all concentrations were similar among other until day 9. After 9 days, chroma of gardenia color in the presence of Tween80 at concentrations of 10 and 15 CMC decreased over time. Chroma profiles of gardenia color in the absence and in the presence of Tween80 at concentrations of 1 and 5 CMC started decreasing after day 19. Hue profiles of gardenia color in the absence and in the presence of Tween80 micelles were superimposable throughout the experiment and increased from 90 to 100 over 60 days. These results indicated that at initial time, all gardenia dye solutions were yellow and its color turned pale yellow with decreasing in color intensity by the end of 60 days. The color change of gardenia solutions in the presence of Tween80 at concentrations of 10 and 15 CMC was observed as early as day 9 while color changes of the rest of solutions were observed later on. However, ΔH profiles illustrated that color changed over a period of time but the color change did not depend on Tween80 concentration since all ΔH profiles were not different from each other. Failure in depicting color change by ΔH profiles were probably because decreasing in b values and increasing a values, which were used in calculation of ΔH values in equation 11, were in the same extent. Thus, the two effects compensated each other leading to failure in depicting color change in this case.

Addition of surfactants in dye solutions may change shade of the solutions due to the interaction of surfactants and dye. Color of roselle solution visually changed from red to yellow over time in the absence and in the presence of surfactants. Higher absorbance and stronger chroma were observed when SDS was added in buffered solution of roselle. The order of roselle color fading was in: SDS micelles > Tween80 micelles > buffer. The more the SDS concentration were the great of the color fading. However, this color change did not depend on the concentration of Tween80.

In case of lac solution, the change of visualize color was obvious during the first few days of study which agree with both data from UV-spectroscopy technique and CIELab technique. The order of lac color changing was in: SDS micelles >

Tween80 micelles > buffer, which was presented by CIELab data but not seen in CI, CD, and % color remaining values. However, this color change did not depend on either SDS or Tween80 concentration. Moreover, roselle and lac dyes show physical incompatability with Tween80 micelles.

From both UV-spectroscopy data and CIELab data, color of the gardenia solution remained yellow over the experiment time. Color fading was observed in: Tween80 micelles > buffer > SDS micelles. However, the color change did not obviously depend on the concentration of Tween80.

Surfactants affect color stabilities at different extent depending on properties of the surfactants and the colors. SDS, a negative surfactant, is likely to have more influence on the colors than Tween80, a neutral surfactant. As a pseudophase micellar system, SDS micelles may provide microenvironment to facilitate the color fading such as fading of roselle and lac solutions or to deter the color fading in case of gardenia solution. Moreover, charged color molecules are likely to interact a charged surfactant at a greater extent than a neutral surfactant. Addition of Tween80 was found to accelerate the color fading for all dyes in this study. Tween80 micelles could provide a suitable microenvironment for color change. However, changes of dye solutions in a low concentration of surfactants (1 CMC) was not different from that in the solution without surfactants.

In this experiment, most of UV-spectroscopy data agreed with CIELab data. Chroma gives information comparables to color intensity while ΔE is similar to a mirror image along x-axis of % color remaining plot. There are advantages of CIELab technique over UV-visible spectroscopy technique in some aspects. Hue value gives better visualized color than UV-visible spectroscopy data. Although the samples were turbid, data analysis using CIELab technique do not require extra data treatment because a scanner works by measuring reflected light at surface of samples while UV-visible spectrophotometer works by mearsuring transmitted light of the samples. Therefore, using the data from CIELab do not need to normalize the data as spectroscopy technique. Visible spectrum gives more and obvious information regarding interaction between dye molecules and micelles than the CIELab technique. In contrary, spectroscopy cannot specify the visualized color of sample but the

CIELab system can. Moreover, some geometry parameters (such as color density) failed to depicted color change.

In this experiment, roselle and lac solutions became turbid when Tween80 was added into the solutions. These cloudy solutions caused more deviation of visible absorbance measurement than CIELab measurement. Sometimes ΔE is a more sensitive parameter than CI, CD, and % color remaining since this parameter is an addition of visual changes as seen in the lac stability study. The change of color will be better explained when both UV-visible spectroscopy technique and CIELab system were utilized together.

However, the color stability does not completely imply the chemical stability of components in the dyes. HPLC chromatogram of gardenia dye did not show the peaks of crocin, crocetin monogentiobiosyl monoglucosyl ester, crocetin monogentiobiosyl ester while color remaining was approximately 50% or more in all solutions because degradation products may have the same color.

CHAPTER V

CONCLUSIONS

This research aimed to study change and stability of roselle, lac, and gardenia colors in micellar systems, which were prepared from three surfactants (anionic (SDS), cationic (CTAB), and non-ionic surfactant (Tween80) at various micellar concentrations (0, 1, 5, 10, and 15 folds of CMC).

Percent yield of roselle, lac and gardenia from extraction process were 31.96 ± 0.32, 2.79 ± 0.23 and 13.66 ± 0.88%, respectively. The physical appearance of obtained lyophilized dyes from roselle, lac, and gardenia are pink-red bulky powder, dark red bulky powder and yellow flake, respectively. The maximum wavelengths (λ_{max}) of roselle and lac colors in succinate buffer pH 4.5 were 528 and 490 nm, respectively, while the maximum wavelength of gardenia in phosphate buffer pH 7 was 443 nm giving visualized color, i.e. pink, orange and yellow, respectively. The apparent pK_a of roselle and lac dyes were 3.00 ± 0.08 and 5.96 ± 0.15, respectively. The presence of each colorant in these dyes was identified by HPLC and LC-MS. Major coloring compounds in roselle is delphinidin-3-sambubioside (molecular weight of 597.2) and cyanidin-3-sambubioside (molecular weight of 581.1). Lac dye composed of laccaic acid A (molecular weight of 535.9), B (molecular weight of 494.9), and C (molecular weight of 537.9), while major colorant in gardenia dye was crocin (molecular weight of 976.4), crocetin monogentiobiosyl monoglucosyl ester (molecular weight of 814.3), and crocetin monogentiobiosyl ester (molecular weight of 652.2).

In stress conditions, roselle and lac dyes were very stable in acid medium while the least degradation of gardenia was observed in alkaline medium. The lac color fading was not obviously affected by elevated temperature. Light and oxidizing agent accelerated bleaching of roselle, lac, and gardenia dyes.

Stabilities of roselle, lac, and gardenia dyes were studied in three micellar systems; i.e. SDS, CTAB, and Tween80. CMC values of each system were determined in phosphate buffer pH 7 or succinate buffer pH 4.5 at 26 ± 1 °C by tensiometer equipped with a Wilhelmy plate. The CMC values of SDS, CTAB, and Tween80 in phosphate buffer pH 7 were $4.97 \times 10^{-4} \pm 1.55 \times 10^{-5}$, $2.05 \times 10^{-5} \pm 2.87 \times 10^{-6}$, and $1.85 \times 10^{-5} \pm 1.25 \times 10^{-6}$ M while the CMC values in succinate buffer pH 4.5 were $5.12 \times 10^{-4} \pm 2.82 \times 10^{-5}$, $2.05 \times 10^{-5} \pm 1.85 \times 10^{-6}$, and $1.84 \times 10^{-5} \pm 8.23 \times 10^{-6}$ M, respectively.

In this study, roselle, lac, and gardenia dyes in CTAB are turned to cloudy due to cloud point phenomena. Roselle color in the presence of SDS or Tween80 faded overtime and changed from pink to yellowish within 12 days. In case of lac color, lac dye in the presence of both micellar systems showed color change from orange to orange-red while gardenia dye in the presence of high SDS micellar concentrations remained yellowish but the rest of this dye were obviously faded within 60 days. The order of stabilities of roselle and lac dyes in micellar systems were buffer > Tween80 > SDS while the order of stability of gardenia dye in micellar systems was SDS > buffer > Tween80. However, roselle and lac dyes in some concentrations of Tween80 micelles turned to cloudy probably due to cloud point depression.

For color measurement, both spectroscopy data and CIELab techniques should be used for assessment the color change. Spectroscopy is a general method which analyzes physicochemical properties of substance while CIELab system is based on tristimulus theory and shows variables related with human eye-brain perception. Therefore, spectroscopy technique gives information on interaction between compounds. Such information is not available by using CIELab system. On the contrary, the spectroscopy can not characterize the observed color while CIELab system can be used to identify the color and to effectively monitor color changes. Each technique inherits advantages and disadvantages over the other.

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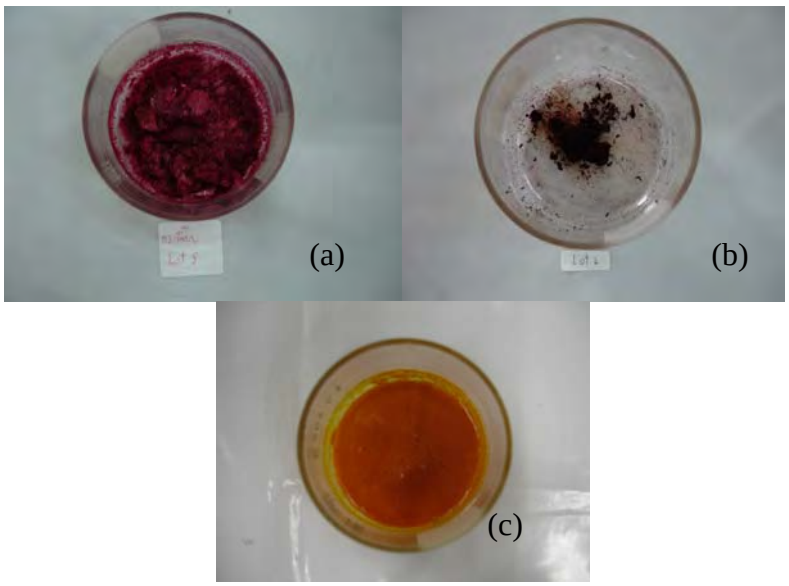
APPENDICES

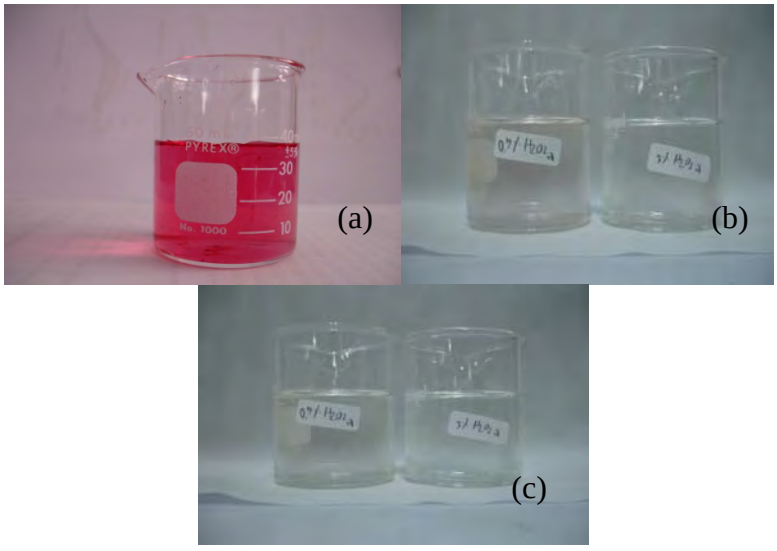
APPENDIX

A. Preliminary stability of roselle, lac, and gerdenia color

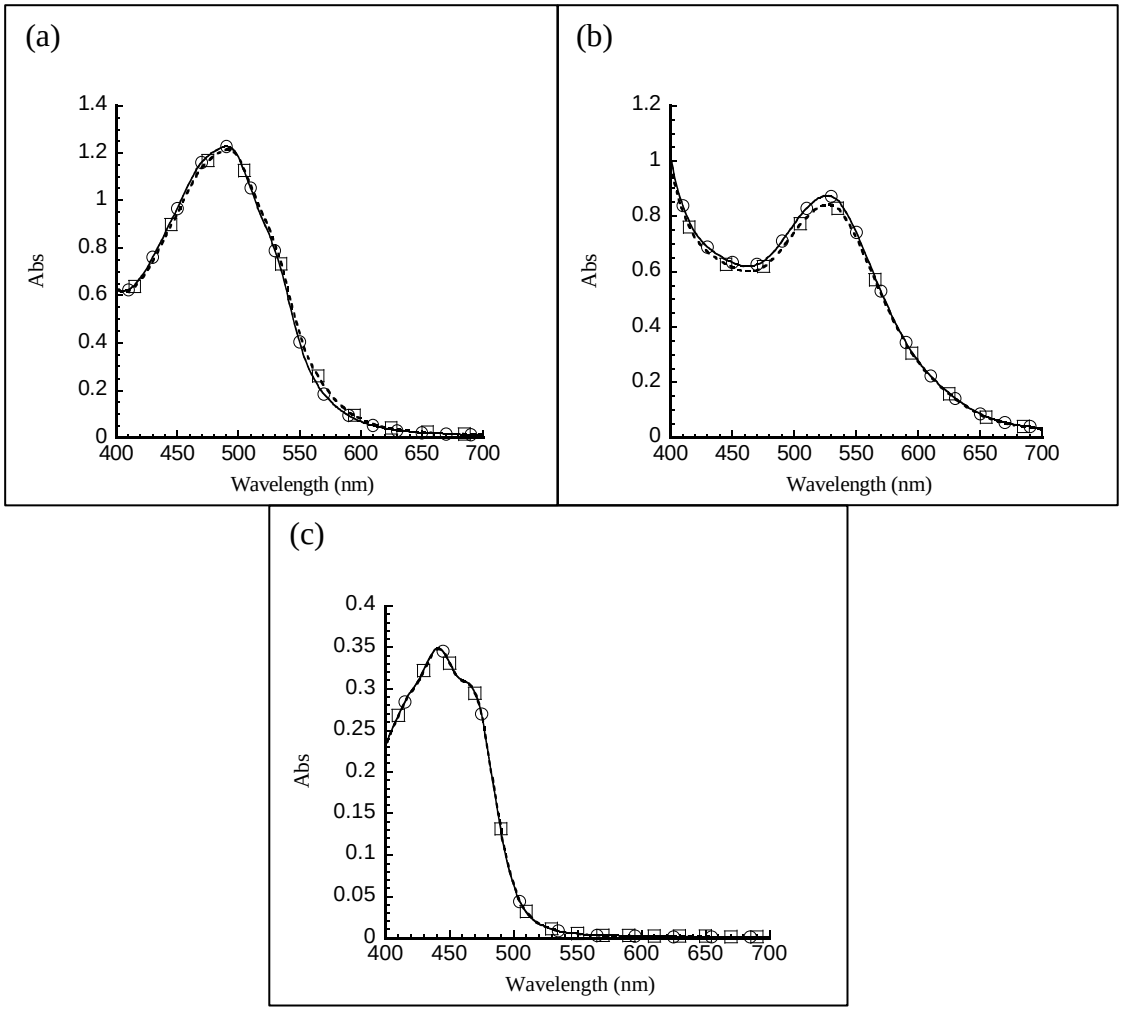
A.1 Lyophilized powder of roselle (a), lac (b) and gardenia (c) from water extraction (before extraction development)



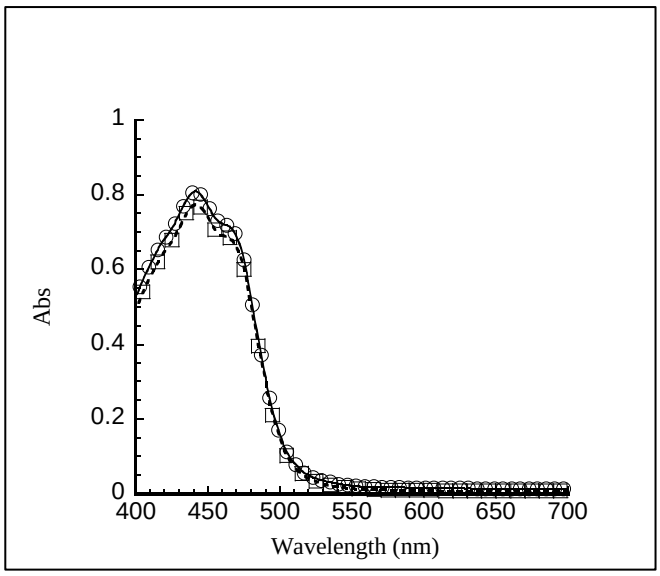
A.2 Roselle dye in 3% H_2O_2 and 0.5% H_2O_2 at 0 minute (a), 5 minutes (b), and 15 minutes (c)



A.3 The comparison visible spectra of roselle (a), lac (b), and gardenia (c) dyes between before (○) and after (□) lyophilization



A.4 The comparison visible spectra of gardenia dyes between before (○) and after (□) evaporation



B. Experimental data

B. 1 pKa of roselle and lac dye

Table B.1.1 The pKa value of roselle

No.	Ka	pK_a
1	0.00124	2.91
2	0.00096	3.01
3	0.00084	3.01
Mean	0.00101	3.00
SD	0.00021	0.08

Table B.1.2 pKa value of lac

No.	Ka	pK_a
1	7.13811E-07	6.15
2	1.40062E-06	5.85
3	1.17136E-06	5.93
Mean	1.09526E-06	5.96
SD	2.85505E-07	0.15

B.2 Surface tension of SDS, CTAB, and Tween80 in phosphate buffer pH 7 or succinate buffer pH 4.5

Table B.2.1 Surface tension of SDS in phosphate buffer pH 7

Conc (M)	surface tension			Mean	±	SD
	1st	2nd	3rd			
5.00E-06	56.421	54.072	54.510	55.001	±	1.249
1.00E-05	51.438	48.655	52.862	50.985	±	2.140
5.00E-05	46.350	44.993	44.941	45.428	±	0.799
1.00E-04	39.098	38.990	38.823	38.970	±	0.139
2.00E-04	32.193	32.529	32.797	32.506	±	0.303
5.00E-04	27.675	28.155	28.077	27.969	±	0.258
5.00E-03	27.829	27.925	28.179	27.978	±	0.181
0.01	29.462	29.456	29.384	29.434	±	0.043
0.05	29.863	29.727	29.875	29.822	±	0.082
0.08	29.950	29.846	29.944	29.913	±	0.058

Table B.2.2 Surface tension of SDS in succinate buffer pH 4.5

Conc (M)	surface tension 1st	2nd	3rd	Mean	±	SD
5.00E-06	59.005	56.961	53.143	56.370	±	2.975
1.00E-05	55.672	55.292	53.143	54.702	±	1.364
5.00E-05	48.337	47.676	48.089	48.034	±	0.334
1.00E-04	42.890	43.998	43.743	43.544	±	0.580
2.00E-04	37.388	36.750	36.371	36.836	±	0.514
5.00E-04	29.318	29.524	28.761	29.201	±	0.395
5.00E-03	30.311	29.742	29.581	29.878	±	0.384
0.01	30.585	30.65	30.591	30.609	±	0.036
0.05	30.890	31.115	30.803	30.936	±	0.161
0.08	31.179	31.141	31.129	31.150	±	0.026

Table B.2.3 Surface tension of CTAB in phosphate buffer pH 7

Conc (M)	surface tension 1st	2nd	3rd	Mean	±	SD
5.00E-07	57.219	59.008	58.482	58.236	±	0.919
1.00E-06	52.508	47.974	46.127	48.870	±	3.283
5.00E-06	43.259	41.004	39.379	41.214	±	1.949
2.00E-05	30.322	30.362	30.321	30.335	±	0.023
5.00E-05	30.920	30.410	30.368	30.566	±	0.307
8.00E-05	29.071	29.926	30.005	29.667	±	0.518
9.00E-05	30.097	30.120	30.003	30.073	±	0.062
1.00E-04	30.099	30.066	29.992	30.052	±	0.055
5.00E-04	29.889	29.801	29.876	29.855	±	0.048
0.001	29.816	29.799	29.834	29.816	±	0.018
0.01	31.830	32.022	32.027	31.960	±	0.112

Table B.2.4 Surface tension of CTAB in succinate buffer pH 4.5

Conc (M)	surface tension 1st	2nd	3rd	Mean	±	SD
5.00E-08	53.875	55.806	54.383	54.688	±	1.001
5.00E-07	55.290	58.594	57.302	57.062	±	1.665
1.00E-06	51.733	54.115	54.894	53.581	±	1.647
5.00E-06	38.535	37.997	38.284	38.272	±	0.269
2.00E-05	30.404	29.810	30.572	30.262	±	0.400
5.00E-05	29.450	29.362	29.446	29.419	±	0.050
8.00E-05	29.193	28.979	29.325	29.166	±	0.175
9.00E-05	29.411	29.360	29.196	29.322	±	0.112
1.00E-04	29.381	29.313	29.407	29.367	±	0.049
5.00E-04	29.516	29.465	29.522	29.501	±	0.031
0.001	29.631	29.557	29.597	29.595	±	0.037
0.01	31.640	31.624	31.655	31.639	±	0.016

Table B.2.5 Surface tension of Tween80 in phosphate buffer pH 7

Conc (M)	surface tension 1st	2nd	3rd	Mean	±	SD
5.00E-06	56.421	54.072	54.510	55.001	±	1.249
1.00E-05	51.438	48.655	52.862	50.985	±	2.140
5.00E-05	46.350	44.993	44.941	45.428	±	0.799
1.00E-04	39.098	38.990	38.823	38.970	±	0.139
2.00E-04	32.193	32.529	32.797	32.506	±	0.303
5.00E-04	27.675	28.155	28.077	27.969	±	0.258
0.005	27.829	27.925	28.179	27.978	±	0.181
0.01	29.462	29.456	29.384	29.434	±	0.043
0.05	29.863	29.727	29.875	29.822	±	0.082
0.08	29.950	29.846	29.944	29.913	±	0.058

Table B.2.6 Surface tension of Tween80 in succinate buffer pH 4.5

Conc (M)	surface tension			Mean	±	SD
	1st	2nd	3rd			
5.00E-06	59.005	56.961	53.143	56.370	±	2.975
1.00E-05	55.672	55.292	53.143	54.702	±	1.364
5.00E-05	48.337	47.676	48.089	48.034	±	0.334
1.00E-04	42.890	43.998	43.743	43.544	±	0.580
2.00E-04	37.388	36.750	36.371	36.836	±	0.514
5.00E-04	29.318	29.524	28.761	29.201	±	0.395
0.005	30.311	29.742	29.581	29.878	±	0.384
0.01	30.585	30.650	30.591	30.609	±	0.036
0.05	30.890	31.115	30.803	30.936	±	0.161
0.08	31.179	31.141	31.129	31.150	±	0.026

B. 3 *L*, *a*, and *b* values of roselle, lac, and gardenia dyes in micellar systems

Table B.3.1 *L*, *a*, and *b* values of roselle dye in succinate buffer pH 4.5

Time (day)	Mean ± SD								
	L			*a*			*b*		
0	38.63	±	2.60	54.42	±	1.21	9.75	±	0.99
0.17	38.83	±	2.08	55.79	±	1.25	11.00	±	1.41
0.42	40.46	±	2.60	52.29	±	1.43	10.38	±	1.28
2	41.04	±	2.31	50.08	±	1.64	12.88	±	0.90
3	41.96	±	2.31	46.71	±	1.40	17.17	±	1.17
4	44.79	±	2.26	43.00	±	1.22	22.54	±	1.28
5	47.50	±	2.15	37.67	±	0.87	30.04	±	1.43
6	46.96	±	2.29	35.75	±	0.99	35.42	±	1.61
7	49.00	±	2.25	33.08	±	1.61	37.17	±	2.37
8	49.71	±	2.39	32.42	±	0.72	41.29	±	1.97
9	50.04	±	2.58	31.29	±	0.81	45.79	±	2.13
12	52.29	±	2.42	25.33	±	0.70	51.83	±	1.81

Table B.3.2 *L*, *a*, and *b* values of roselle dye in the presence of SDS micelles at a concentration of 1 folds of CMC

Time (day)	Mean ± SD								
	L			*a*			*b*		
0	38.96	±	2.58	55.13	±	1.39	9.46	±	1.40
0.17	39.08	±	2.62	53.67	±	1.20	10.33	±	1.09
0.42	39.29	±	1.99	53.29	±	1.46	10.96	±	1.49
2	39.79	±	2.43	51.75	±	1.29	13.33	±	1.24
3	42.00	±	2.32	47.67	±	1.05	17.67	±	1.43
4	43.92	±	2.32	43.04	±	1.27	22.71	±	1.23
5	44.29	±	2.31	40.75	±	0.85	29.13	±	1.98
6	47.79	±	2.30	35.79	±	1.28	35.67	±	2.24
7	48.33	±	2.46	33.75	±	1.19	36.46	±	1.67
8	48.92	±	2.89	33.08	±	0.97	39.38	±	1.91
9	49.13	±	2.36	30.96	±	1.30	44.54	±	2.13
12	49.71	±	2.35	25.29	±	5.05	52.92	±	2.76

Table B.3.3 *L*, *a*, and *b* values of roselle dye in the presence of SDS micelles at a concentration of 5 folds of CMC

Time (day)	Mean ± SD								
	L			*a*			*b*		
0	35.50	±	1.93	58.67	±	1.71	12.38	±	1.74
0.17	36.17	±	1.95	59.29	±	1.49	12.96	±	2.37
0.42	37.5	±	2.21	59.04	±	1.55	11.42	±	1.53
2	38.17	±	1.90	57.17	±	1.58	13.54	±	1.25
3	38.29	±	2.42	50.29	±	1.33	17.79	±	1.50
4	41.46	±	2.34	48.75	±	1.48	22.58	±	1.44
5	43.92	±	2.69	44.38	±	0.82	28.29	±	1.78
6	46.67	±	2.48	38.92	±	1.02	35.92	±	1.32
7	46.79	±	2.19	36.79	±	1.06	39.42	±	1.56
8	47.25	±	2.51	35.71	±	1.33	40.79	±	1.59
9	47.38	±	2.12	33.75	±	0.68	44.13	±	2.33
12	50.46	±	2.67	27.38	±	0.88	51.75	±	1.82

Table B.3.4 *L*, *a*, and *b* values of roselle dye in the presence of SDS micelles at a concentration of 10 folds of CMC

Time (day)	Mean ± SD								
	L			*a*			*b*		
0	35.54	±	1.69	59.88	±	2.03	16.54	±	2.38
0.17	36.21	±	1.69	60.63	±	2.02	15.88	±	2.11
0.42	36.58	±	1.84	60.67	±	1.97	14.29	±	1.99
2	36.88	±	2.05	60.54	±	2.00	15.92	±	2.38
3	38.83	±	2.10	57.33	±	1.24	18.38	±	1.56
4	40.67	±	2.24	52.38	±	1.64	22.17	±	1.97
5	43.71	±	2.31	46.71	±	1.08	28.58	±	1.89
6	45.92	±	2.26	40.71	±	1.12	34.83	±	2.48
7	47.79	±	2.65	37.25	±	2.36	39.17	±	3.47
8	47.13	±	2.47	39.00	±	1.47	40.38	±	2.48
9	47.21	±	2.02	35.54	±	0.83	44.13	±	2.40
12	49.08	±	2.41	31.04	±	3.30	48.13	±	3.66

Table B.3.5 *L*, *a*, and *b* values of roselle dye in the presence of SDS micelles at a concentration of 15 folds of CMC

Time (day)	Mean ± SD								
	L			*a*			*b*		
0	35.58	±	1.84	59.79	±	2.06	21.71	±	2.76
0.17	35.50	±	1.67	59.96	±	1.40	22.46	±	1.79
0.42	35.38	±	1.69	59.96	±	1.90	21.75	±	1.82
2	36.29	±	1.94	60.17	±	1.95	22.00	±	1.18
3	36.17	±	2.04	58.42	±	1.91	22.21	±	2.50
4	39.79	±	1.91	56.04	±	1.57	23.42	±	2.15
5	41.42	±	2.39	51.54	±	4.19	32.21	±	3.27
6	45.42	±	2.43	42.54	±	1.67	38.42	±	2.95
7	45.83	±	2.24	40.79	±	2.54	40.08	±	2.78
8	46.13	±	2.19	39.83	±	2.57	41.04	±	2.39
9	47.71	±	2.74	36.04	±	3.90	46.92	±	3.37
12	49.54	±	2.11	30.33	±	1.81	52.63	±	2.00

Table B.3.6 *L*, *a*, and *b* values of roselle dye in the presence of Tween80 micelles at a concentration of 1 folds of CMC

Time (day)	Mean ± SD								
	L			*a*			*b*		
0	40.38	±	2.16	52.46	±	1.35	8.79	±	1.06
0.17	42.33	±	2.44	51.25	±	1.33	9.75	±	1.11
0.42	40.08	±	2.30	51.21	±	1.06	11.29	±	1.30
2	42.83	±	2.44	49.46	±	2.60	12.71	±	1.63
3	45.17	±	2.48	45.88	±	1.57	19.79	±	1.41
4	49.88	±	2.40	36.67	±	1.01	29.42	±	2.15
5	51.13	±	2.64	32.54	±	1.22	38.79	±	1.91
6	53.42	±	2.24	28.17	±	1.40	41.46	±	3.34
7	53.33	±	2.50	27.08	±	1.41	47.00	±	3.56
10	54.71	±	2.26	23.08	±	0.65	48.04	±	1.97
12	55.13	±	2.68	23.08	±	1.06	52.29	±	2.22

Table B.3.7 *L*, *a*, and *b* values of roselle dye in the presence of Tween80 micelles at a concentration of 5 folds of CMC

Time (day)	Mean ± SD								
	L			*a*			*b*		
0	41.04	±	2.26	52.25	±	1.29	9.38	±	1.41
0.17	41.21	±	2.69	52.13	±	1.08	10.5	±	1.59
0.42	41.58	±	2.38	50.58	±	1.14	10.5	±	1.35
2	42.71	±	2.39	49.50	±	1.02	12.79	±	1.22
3	45.96	±	2.33	43.92	±	1.10	19.29	±	1.40
4	50.29	±	2.68	35.17	±	1.01	30.83	±	1.20
5	51.79	±	2.86	32.83	±	2.50	38.00	±	2.32
6	54.38	±	2.52	28.13	±	1.26	39.46	±	1.53
7	55.17	±	2.68	25.46	±	0.72	41.96	±	1.71
10	55.5	±	2.40	23.17	±	1.05	45.25	±	1.39
12	56.38	±	2.52	22.13	±	0.80	50.79	±	2.17

Table B.3.8 *L*, *a*, and *b* values of roselle dye in the presence of Tween80 micelles at a concentration of 10 folds of CMC

Time (day)	Mean ± SD								
	L			*a*			*b*		
0	41.25	±	2.49	51.67	±	1.34	10.38	±	1.17
0.17	41.92	±	2.50	51.42	±	1.25	10.25	±	1.11
0.42	42.17	±	2.10	49.75	±	1.85	11.08	±	1.06
2	42.79	±	2.28	49.71	±	1.49	13.42	±	1.53
3	45.54	±	2.54	43.58	±	1.67	18.75	±	1.54
4	49.33	±	2.43	36.50	±	1.91	29.21	±	2.72
5	51.17	±	2.41	33.04	±	1.76	35.21	±	2.48
6	52.50	±	2.13	28.88	±	2.01	41.04	±	1.88
7	53.67	±	2.39	28.00	±	2.15	42.13	±	2.31
10	54.25	±	2.31	24.08	±	1.82	48.38	±	1.50
12	54.71	±	2.27	22.38	±	0.65	50.46	±	2.83

Table B.3.9 *L*, *a*, and *b* values of roselle dye in the presence of Tween80 micelles at a concentration of 15 folds of CMC

Time (day)	Mean ± SD								
	L			*a*			*b*		
0	39.92	±	2.52	52.17	±	0.96	10.88	±	1.33
0.17	42.08	±	2.47	50.46	±	1.06	10.25	±	1.11
0.42	41.42	±	2.28	50.71	±	1.00	11.21	±	1.06
2	43.67	±	2.65	48.17	±	1.31	12.29	±	1.53
3	44.79	±	2.45	43.54	±	0.72	18.63	±	1.54
4	50.46	±	2.60	35.00	±	0.78	28.88	±	2.72
5	51.83	±	2.26	32.42	±	1.25	33.00	±	2.48
6	54.25	±	2.51	28.29	±	1.00	38.04	±	1.88
7	53.17	±	2.48	28.83	±	0.92	39.75	±	2.31
10	55.50	±	2.54	24.25	±	0.61	46.13	±	1.50
12	55.75	±	2.88	22.42	±	0.78	50.67	±	2.83

Table B.3.10 *L*, *a*, and *b* values of lac dye in succinate buffer pH 4.5

Time (day)	Mean ± SD								
	L			*a*			*b*		
0	52.83	±	3.76	54.58	±	4.24	63.92	±	2.65
2	54.21	±	2.96	52.58	±	3.16	63.58	±	2.04
3	52.46	±	3.08	53.00	±	3.32	63.25	±	2.15
8	50.63	±	2.62	57.25	±	2.57	62.50	±	2.04
14	49.67	±	2.26	57.38	±	2.89	61.67	±	1.66
18	48.92	±	2.39	58.96	±	1.55	61.38	±	1.61
21	49.04	±	2.12	59.38	±	0.88	61.58	±	1.89
24	51.13	±	2.42	58.21	±	1.38	63.00	±	1.91
31	46.63	±	2.37	61.21	±	3.79	57.71	±	4.16
38	49.08	±	2.87	59.67	±	1.74	61.58	±	2.48
45	47.42	±	2.84	61.38	±	1.44	60.79	±	2.21
52	46.08	±	2.21	64.75	±	2.29	55.46	±	4.87
60	46.42	±	2.52	63.33	±	3.02	56.92	±	3.73

Table B.3.11 *L*, *a*, and *b* values of lac dye in the presence of SDS micelles at a concentration of 1 folds of CMC

Time (day)	Mean ± SD								
	L			*a*			*b*		
0	53.96	±	3.78	49.42	±	4.04	64.21	±	2.69
2	54.96	±	3.24	46.21	±	3.30	64.33	±	2.04
3	55.13	±	3.04	45.92	±	3.65	64.38	±	2.15
8	50.88	±	2.07	53.88	±	3.19	62.13	±	2.04
14	50.79	±	2.43	50.13	±	5.74	62.29	±	1.66
18	49.96	±	2.05	54.96	±	2.26	61.58	±	1.61
21	51.04	±	2.27	55.42	±	2.24	62.50	±	1.89
24	50.67	±	3.06	56.96	±	4.26	61.96	±	1.91
31	49.25	±	2.21	57.42	±	1.50	61.21	±	4.16
38	50.75	±	2.11	59.00	±	1.22	62.54	±	2.48
45	50.42	±	2.36	59.08	±	1.53	60.79	±	2.21
52	50.04	±	2.42	59.50	±	2.17	62.13	±	4.87
60	48.29	±	2.29	60.38	±	3.81	60.21	±	3.73

Table B.3.12 *L*, *a*, and *b* values of lac dye in the presence of SDS micelles at a concentration of 5 folds of CMC

Time (day)	Mean ± SD								
	L			*a*			*b*		
0	55.88	±	2.09	46.04	±	1.30	65.25	±	1.80
2	56.04	±	2.42	46.71	±	0.95	65.38	±	2.02
3	50.08	±	2.02	54.08	±	1.38	61.42	±	1.98
8	50.00	±	1.87	54.83	±	1.24	61.58	±	1.53
14	49.04	±	2.10	58.29	±	1.37	61.25	±	1.54
18	49.96	±	2.42	58.04	±	1.68	61.96	±	2.20
21	47.79	±	2.89	58.88	±	4.53	57.79	±	4.98
24	48.79	±	2.34	59.58	±	1.84	61.38	±	1.79
31	47.88	±	2.54	58.79	±	2.08	59.21	±	2.15
38	49.92	±	2.47	61.50	±	1.53	62.21	±	1.74
45	49.88	±	2.13	61.50	±	1.47	61.29	±	1.46
52	48.75	±	2.27	61.17	±	1.66	60.71	±	1.73
60	47.46	±	2.47	62.29	±	2.56	59.46	±	1.59

Table B.3.13 *L*, *a*, and *b* values of lac dye in the presence of SDS micelles at a concentration of 10 folds of CMC

Time (day)	Mean ± SD								
	L			*a*			*b*		
0	55.92	±	2.00	45.83	±	1.34	65.04	±	1.71
2	57.00	±	2.06	45.29	±	1.57	65.79	±	1.79
3	49.71	±	1.97	54.13	±	2.42	61.13	±	1.60
8	49.83	±	1.97	54.13	±	1.78	61.29	±	1.65
14	50.13	±	1.90	56.13	±	2.27	61.42	±	1.82
18	48.58	±	2.06	58.17	±	1.69	61.00	±	1.77
21	48.25	±	2.13	58.88	±	1.85	60.46	±	1.61
24	49.42	±	2.36	58.29	±	1.16	61.21	±	2.17
31	48.46	±	2.80	59.54	±	2.11	60.71	±	2.31
38	48.96	±	2.16	59.96	±	1.20	61.42	±	1.64
45	49.58	±	2.24	60.58	±	2.17	60.13	±	1.57
52	49.46	±	2.32	59.92	±	1.47	59.63	±	3.16
60	48.50	±	2.19	60.04	±	1.43	60.04	±	1.81

Table B.3.14 *L*, *a*, and *b* values of lac dye in the presence of SDS micelles at a concentration of 15 folds of CMC

Time (day)	Mean ± SD								
	L			*a*			*b*		
0	55.50	±	2.17	45.13	±	1.48	64.67	±	1.93
2	56.33	±	2.35	45.42	±	1.18	65.46	±	1.82
3	50.29	±	1.94	52.83	±	1.71	61.67	±	1.74
8	49.08	±	1.98	54.46	±	1.59	60.88	±	1.70
14	48.17	±	1.99	57.33	±	1.76	60.63	±	1.69
18	46.92	±	3.16	60.08	±	2.62	59.46	±	2.95
21	48.50	±	2.70	57.29	±	1.46	61.17	±	1.81
24	48.13	±	1.96	60.13	±	1.48	60.13	±	1.94
31	48.33	±	2.16	59.75	±	1.73	60.83	±	1.79
38	47.92	±	3.59	57.83	±	1.49	60.08	±	3.31
45	49.13	±	2.03	59.71	±	2.18	61.46	±	1.69
52	47.79	±	1.69	59.83	±	1.93	60.21	±	1.22
60	47.21	±	1.96	61.58	±	1.72	58.25	±	3.37

Table B.3.15 *L*, *a*, and *b* values of lac dye in the presence of Tween80 micelles at a concentration of 1 folds of CMC

Time (day)	Mean ± SD								
	L			*a*			*b*		
0	57.38	±	2.36	44.08	±	0.83	66.08	±	1.91
2	56.00	±	2.23	46.25	±	0.99	65.21	±	1.89
3	53.04	±	2.20	49.88	±	0.95	63.46	±	1.74
8	53.04	±	2.65	53.46	±	2.19	63.92	±	2.19
14	53.67	±	2.71	52.38	±	1.38	64.42	±	1.95
18	53.00	±	2.80	51.42	±	2.21	63.38	±	2.20
21	52.46	±	2.17	53.13	±	2.21	63.46	±	1.91
24	53.08	±	2.21	51.88	±	1.33	63.71	±	1.78
31	52.33	±	2.20	52.21	±	0.88	63.08	±	1.98
38	51.83	±	2.46	53.67	±	1.13	63.17	±	1.99
45	52.25	±	2.19	53.46	±	1.28	63.38	±	1.84
52	46.79	±	3.30	54.63	±	2.46	58.58	±	3.48
60	50.13	±	2.13	53.38	±	2.34	61.63	±	1.86

Table B.3.16 *L*, *a*, and *b* values of lac dye in the presence of Tween80 micelles at a concentration of 5 folds of CMC

Time (day)	Mean ± SD								
	L			*a*			*b*		
0	54.79	±	2.18	45.04	±	1.55	66.13	±	1.90
2	54.92	±	2.15	47.04	±	1.92	64.54	±	1.77
3	52.54	±	2.32	50.08	±	2.41	63.04	±	1.78
8	51.67	±	2.14	51.42	±	1.82	62.50	±	1.98
14	49.92	±	2.43	55.29	±	1.81	61.67	±	2.06
18	50.63	±	1.91	53.79	±	3.16	60.58	±	2.62
21	47.38	±	2.89	54.71	±	2.73	59.58	±	2.67
24	49.13	±	2.07	54.50	±	2.11	60.88	±	1.68
31	48.58	±	1.98	54.63	±	1.66	60.67	±	1.66
38	48.33	±	1.95	55.96	±	1.57	60.67	±	1.52
45	47.83	±	1.88	57.79	±	2.17	60.38	±	1.58
52	46.25	±	3.54	58.00	±	2.32	59.58	±	2.52
60	46.29	±	2.46	56.38	±	0.97	58.92	±	1.98

Table B.3.17 *L*, *a*, and *b* values of lac dye in the presence of Tween80 micelles at a concentration of 10 folds of CMC

Time (day)	Mean ± SD								
	L			*a*			*b*		
0	57.42	±	2.22	44.13	±	1.33	66.08	±	1.98
2	54.71	±	2.40	47.58	±	1.21	64.33	±	1.88
3	51.50	±	2.87	52.88	±	0.90	62.54	±	2.32
8	52.00	±	2.64	52.38	±	1.53	62.79	±	2.15
14	50.75	±	1.87	52.08	±	0.72	61.96	±	1.63
18	50.50	±	1.98	52.46	±	1.18	61.58	±	1.77
21	49.50	±	1.91	56.38	±	1.58	61.38	±	1.71
24	50.21	±	2.00	58.21	±	1.47	62.33	±	1.69
31	49.83	±	2.20	55.17	±	1.71	61.29	±	1.60
38	49.21	±	2.62	58.50	±	1.53	61.58	±	2.30
45	48.08	±	2.48	58.58	±	3.02	60.71	±	1.94
52	48.33	±	2.14	58.58	±	1.18	60.96	±	1.78
60	46.00	±	2.28	56.79	±	1.69	58.75	±	1.73

Table B.3.17 *L*, *a*, and *b* values of lac dye in the presence of Tween80 micelles at a concentration of 15 folds of CMC

Time (day)	Mean ± SD								
	L			*a*			*b*		
0	56.50	±	2.11	45.88	±	1.94	65.54	±	1.74
2	54.00	±	2.48	49.50	±	2.11	64.13	±	1.96
3	51.00	±	2.28	53.54	±	1.28	62.33	±	1.95
8	51.54	±	2.11	54.04	±	1.46	62.71	±	1.76
14	52.00	±	2.32	53.67	±	0.87	63.13	±	1.90
18	52.50	±	2.04	52.13	±	1.51	63.33	±	1.52
21	51.00	±	2.19	55.75	±	1.15	62.50	±	1.93
24	52.08	±	2.19	53.96	±	1.71	63.04	±	1.81
31	49.54	±	1.91	54.96	±	3.16	60.96	±	1.78
38	50.25	±	2.13	57.25	±	1.22	62.13	±	1.87
45	47.83	±	2.55	59.38	±	2.14	61.08	±	1.79
52	48.17	±	2.32	59.88	±	1.65	61.04	±	1.85
60	44.50	±	1.82	58.42	±	0.97	57.92	±	1.38

Table B.3.18 *L*, *a*, and *b* values of gardenia dye in phosphate buffer pH 7

Time (day)	Mean ± SD								
	L			*a*			*b*		
0	84.46	±	1.69	-0.63	±	0.58	83.75	±	1.33
4	83.54	±	1.98	-2.17	±	0.70	82.83	±	1.71
6	83.13	±	2.25	-2.92	±	0.88	82.50	±	1.87
9	84.38	±	2.10	-3.71	±	0.69	83.46	±	1.61
12	84.46	±	2.41	-4.00	±	0.78	83.50	±	2.02
16	85.21	±	2.36	-4.88	±	0.74	83.75	±	1.92
19	84.58	±	2.19	-5.29	±	0.62	82.63	±	1.38
23	83.71	±	2.27	-5.46	±	0.59	80.33	±	1.46
29	85.17	±	2.28	-6.42	±	0.50	73.08	±	1.86
39	86.79	±	2.21	-6.54	±	0.66	71.83	±	1.90
46	87.92	±	2.24	-6.63	±	0.49	66.63	±	1.44
53	87.54	±	2.11	-6.46	±	0.66	64.29	±	1.68
60	88.38	±	1.97	-7.13	±	0.61	58.96	±	1.49

Table B.3.19 *L*, *a*, and *b* values of gardenia dye in the presence of SDS micelles at a concentration of 1 folds of CMC

Time (day)	Mean ± SD								
	L			*a*			*b*		
0	83.46	±	2.02	-0.96	±	0.95	82.88	±	1.73
4	83.88	±	1.73	-1.96	±	0.69	83.04	±	1.33
6	83.42	±	1.79	-2.63	±	0.88	83.42	±	1.79
9	84.75	±	2.03	-3.63	±	0.82	83.63	±	1.76
12	84.17	±	2.57	-3.88	±	0.80	83.25	±	1.94
16	84.29	±	2.61	-4.88	±	0.74	82.96	±	1.97
19	83.71	±	2.49	-5.00	±	0.78	82.33	±	1.63
23	84.13	±	2.49	-5.63	±	0.71	80.71	±	1.23
29	84.71	±	2.40	-6.38	±	0.65	75.29	±	2.79
39	86.96	±	2.40	-6.63	±	0.49	68.83	±	2.37
46	87.54	±	2.34	-6.83	±	0.56	65.50	±	1.72
53	87.63	±	2.24	-7.04	±	0.55	60.71	±	2.46
60	88.46	±	2.55	-6.83	±	0.56	58.08	±	2.19

Table B.3.20 *L*, *a*, and *b* values of gardenia dye in the presence of SDS micelles at a concentration of 5 folds of CMC

Time (day)	Mean ± SD								
	L			*a*			*b*		
0	84.17	±	2.16	-1.88	±	0.80	83.42	±	1.82
4	82.83	±	2.50	-1.88	±	0.80	82.21	±	2.08
6	82.33	±	2.35	-1.75	±	0.74	81.88	±	1.94
9	83.46	±	2.84	-2.29	±	0.69	82.75	±	2.27
12	82.71	±	2.44	-2.67	±	0.76	82.08	±	1.98
16	84.46	±	2.43	-3.21	±	0.78	83.50	±	2.04
19	84.25	±	2.29	-3.25	±	0.90	83.17	±	2.04
23	84.33	±	2.78	-3.42	±	0.78	83.25	±	2.40
29	85.00	±	1.79	-4.46	±	0.83	83.50	±	1.53
39	85.21	±	1.77	-4.42	±	0.50	83.63	±	1.47
46	84.67	±	2.22	-4.83	±	0.64	82.17	±	2.81
53	85.79	±	2.19	-5.00	±	0.59	83.25	±	1.80
60	83.71	±	2.48	-5.75	±	0.74	83.63	±	1.47

Table B.3.21 *L*, *a*, and *b* values of gardenia dye in the presence of SDS micelles at a concentration of 10 folds of CMC

Time (day)	Mean ± SD								
	L			*a*			*b*		
0	83.25	±	2.38	-1.71	±	0.75	82.58	±	2.04
4	83.88	±	2.36	-1.92	±	0.65	83.17	±	2.06
6	82.17	±	2.16	-1.88	±	0.74	81.58	±	1.67
9	82.04	±	2.79	-1.96	±	0.75	81.58	±	2.17
12	82.67	±	2.41	-2.46	±	0.78	82.21	±	1.91
16	83.38	±	2.89	-2.71	±	0.69	82.58	±	2.24
19	83.17	±	2.20	-2.92	±	1.02	82.58	±	2.24
23	84.63	±	2.39	-3.25	±	0.61	83.58	±	1.84
29	85.38	±	2.06	-4.00	±	0.88	84.25	±	1.73
39	84.83	±	2.22	-4.08	±	0.65	83.67	±	1.81
46	85.33	±	2.41	-4.25	±	0.90	83.13	±	2.29
53	84.71	±	2.54	-4.25	±	0.85	82.67	±	1.93
60	86.25	±	2.61	-5.50	±	0.78	80.54	±	3.02

Table B.3.22 *L*, *a*, and *b* values of gardenia dye in the presence of SDS micelles at a concentration of 15 folds of CMC

Time (day)	Mean ± SD								
	L			*a*			*b*		
0	84.00	±	2.27	-1.38	±	0.58	83.25	±	1.98
4	82.79	±	2.57	-1.75	±	0.74	82.04	±	1.85
6	82.63	±	2.32	-1.92	±	0.78	82.00	±	2.00
9	82.58	±	1.95	-2.58	±	0.88	82.33	±	1.71
12	82.50	±	2.65	-2.25	±	0.90	82.21	±	2.32
16	82.46	±	2.28	-2.58	±	0.88	81.88	±	1.85
19	82.38	±	2.36	-2.54	±	0.83	81.88	±	1.92
23	82.29	±	2.85	-2.96	±	0.69	81.79	±	2.30
29	84.33	±	2.18	-3.71	±	0.69	83.29	±	1.85
39	84.63	±	2.43	-3.63	±	0.88	83.71	±	1.97
46	85.25	±	2.35	-4.17	±	0.82	83.88	±	2.03
53	84.67	±	2.12	-4.42	±	0.72	83.25	±	1.59
60	84.63	±	2.10	-4.92	±	0.72	81.75	±	2.71

Table B.3.23 *L*, *a*, and *b* values of gardenia dye in the presence of Tween80 micelles at a concentration of 1 folds of CMC

Time (day)	Mean ± SD								
	L			*a*			*b*		
0	82.92	±	2.70	-1.21	±	0.78	82.46	±	2.30
4	82.79	±	2.50	-2.29	±	0.81	82.04	±	1.94
6	82.63	±	2.32	-2.75	±	0.79	83.25	±	1.82
9	82.58	±	2.81	-3.67	±	0.92	83.63	±	1.76
12	82.50	±	2.36	-4.42	±	0.72	83.50	±	1.96
16	82.46	±	2.67	-5.17	±	0.76	82.46	±	1.77
19	82.38	±	2.23	-5.63	±	0.77	81.54	±	1.25
23	82.29	±	2.65	-6.00	±	0.72	79.38	±	2.73
29	84.33	±	2.12	-6.04	±	0.69	73.79	±	2.77
39	84.63	±	2.20	-6.42	±	0.65	66.92	±	2.95
46	85.25	±	2.23	-7.13	±	0.74	61.33	±	3.64
53	84.67	±	2.44	-6.92	±	0.50	55.00	±	3.88
60	84.63	±	2.57	-7.13	±	0.61	51.79	±	1.77

Table B.3.24 *L*, *a*, and *b* values of gardenia dye in the presence of Tween80 micelles at a concentration of 5 folds of CMC

Time (day)	Mean ± SD								
	L			*a*			*b*		
0	82.54	±	2.34	-0.92	±	0.78	82.08	±	2.00
4	82.63	±	2.10	-2.46	±	0.98	82.25	±	2.45
6	82.29	±	3.42	-2.42	±	0.93	81.67	±	2.87
9	82.29	±	3.10	-3.75	±	0.61	81.63	±	2.53
12	84.46	±	2.50	-4.50	±	0.59	83.50	±	1.84
16	83.67	±	2.30	-4.96	±	0.69	82.42	±	1.77
19	86.29	±	2.20	-5.58	±	0.58	81.46	±	1.82
23	86.46	±	2.32	-6.21	±	0.59	77.63	±	1.74
29	88.25	±	2.51	-6.54	±	0.51	71.71	±	5.26
39	86.71	±	2.49	-6.25	±	0.53	69.33	±	1.74
46	86.38	±	2.12	-6.83	±	0.56	60.83	±	3.89
53	86.00	±	2.30	-6.71	±	0.55	60.13	±	1.70
60	86.17	±	2.44	-7.42	±	0.50	55.79	±	1.10

Table B.3.25 *L*, *a*, and *b* values of gardenia dye in the presence of Tween80 micelles at a concentration of 10 folds of CMC

Time (day)	Mean ± SD								
	L			*a*			*b*		
0	82.75	±	2.52	-1.75	±	0.85	82.21	±	2.06
4	83.42	±	2.50	-3.04	±	0.75	82.75	±	2.07
6	83.92	±	2.50	-3.33	±	0.92	83.13	±	1.98
9	83.83	±	2.39	-4.67	±	0.82	82.79	±	1.86
12	83.25	±	2.47	-5.67	±	0.56	79.25	±	2.05
16	83.79	±	2.32	-5.79	±	0.59	76.21	±	1.77
19	87.21	±	2.23	-6.58	±	0.50	69.75	±	3.26
23	86.29	±	2.10	-6.71	±	0.55	67.71	±	2.14
29	87.21	±	2.28	-6.71	±	0.55	64.25	±	2.45
39	86.63	±	2.52	-6.50	±	0.51	57.92	±	2.98
46	85.71	±	2.22	-6.79	±	0.41	53.54	±	2.47
53	86.67	±	2.30	-6.50	±	0.59	51.42	±	3.12
60	86.46	±	2.26	-6.79	±	0.51	47.63	±	3.05

Table B.3.26 *L*, *a*, and *b* values of gardenia dye in the presence of Tween80 micelles at a concentration of 15 folds of CMC

Time (day)	Mean ± SD								
	L			*a*			*b*		
0	82.08	±	2.60	-1.88	±	0.74	81.54	±	2.15
4	81.13	±	2.59	-2.96	±	0.75	80.92	±	2.26
6	82.25	±	2.86	-3.71	±	0.75	81.67	±	2.22
9	84.42	±	2.99	-4.75	±	0.79	83.75	±	1.89
12	84.29	±	2.56	-5.42	±	0.72	81.50	±	1.02
16	84.42	±	2.62	-6.25	±	0.44	73.42	±	2.06
19	87.79	±	2.00	-6.75	±	0.53	69.79	±	2.45
23	85.58	±	2.34	-6.50	±	0.51	68.08	±	1.89
29	86.92	±	2.50	-6.71	±	0.55	63.17	±	1.88
39	86.71	±	1.99	-6.29	±	0.55	57.42	±	2.12
46	86.08	±	2.10	-6.29	±	0.55	52.42	±	2.00
53	85.29	±	2.33	-6.38	±	0.49	49.29	±	2.14
60	85.42	±	2.34	-6.58	±	0.65	45.71	±	2.10

VITA

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