เสถียรภาพต่อการเกิดออกซิเดชันของกรดไขมันโอเมกา-3 อิมัลชันที่เคลือบด้วยพอลิอิเล็กโทรไลต์มัลติเลเยอร์

นางสาวสมประสงค์ ศิระมาด

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
สาขาวิชาปิโตรเคมีและวิทยาศาสตร์พอลิเมอร์
คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2551

ลิขสิทธิ์ของจุฬาลงกรณ์มหาวิทยาลัย

OXIDATIVE STABILITY OF OMEGA-3 FATTY ACID EMULSIONS COATED WITH POLYELECTROLYTE MULTILAYER

Miss Somprasong Siramard

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science Program in Petrochemistry and Polymer Science
Faculty of Science
Chulalongkorn University
Academic Year 2008

Copyright of Chulalongkorn University

Thesis title	OXIDATIVE STABILITY OF OMEGA-3 FATTY ACID EMULSIONS COATED WITH POLYELECTROLYTE MULTILAYER
By	Miss Somprasong Siramard
Field of study	Petrochemistry and Polymer Science
Advisor	Luxsana Dubas, Ph.D.
Co-Advisor	Stephan T. Dubas, Ph.D.

Accepted by the Faculty of Science, Chulalongkorn University in Partial Fulfillment of the Requirement for the Master's Degree.

.....Dean of the Faculty of Science
(Professor Supot Hannongbua, Dr.rer.nat)

THESIS COMMITTEE

.....Chairman
(Assistant Professor Warinthorn Chavasiri, Ph.D.)

.....Advisor
(Luxsana Dubas, Ph.D.)

.....Co-Advisor
(Stephan T. Dubas, Ph.D.)

.....Examiner
(Assistant Professor Varawut Tangpasuthadol, Ph.D.)

.....External Examiner
(Wilailuk Chaiyasit, Ph.D.)

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

สมประสงค์ ศิระมาด: เสถียรภาพต่อการเกิดออกซิเดชันของกรดไขมันโอเมกา-3 ที่เคลือบด้วยพอลิอิเล็กโทรไลต์มัลติเลเยอร์. (OXIDATIVE STABILITY OF OMEGA-3 FATTY ACID EMULSIONS COATED WITH POLYELECTROLYTE MULTILAYER) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: อ.ดร. ลักษณา ดูบาส, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม: Stephan T. Dubas, Ph.D., 112 หน้า.

งานวิจัยนี้ทำการทดลองการเตรียมอิมัลชันระบบน้ำมันในน้ำที่เคลือบด้วยพอลิอิเล็กโทรไลต์สามและสี่ชั้น รวมทั้งการเพิ่มเสถียรภาพต่อการเกิดออกซิเดชันและการเกิดครีมของโอเมกา-3 อิมัลชัน โดยภาวะที่เหมาะสมในการเตรียมอิมัลชันได้แก่ เตรียมได้โดยการผสมน้ำมันทูนา 5% โดยน้ำหนักและ 95% โดยน้ำหนักของสารละลายโปรตีนเคซีนความเข้มข้น 1.5% โดยน้ำหนัก ที่ pH 6 ให้เป็นเนื้อเดียวกันเป็นเวลา 2 นาที ที่จำนวนรอบ 20, 000 รอบต่อนาที จำนวน 5 รอบ มัลติเลเยอร์อิมัลชันสามารถเตรียมได้โดยใช้เทคนิคการเคลือบชั้นต่อชั้นของสารพอลิอิเล็กโทรไลต์ (layer-by-layer assembly) ลำดับแรกอิมัลชันถูกเคลือบชั้นต่อไปด้วยพอลิไดอัลลิลไดเมทิลแอมโมเนียมคลอไรด์ (PDADMAC) หรือ ไคโทซาน (พอลิอิเล็กโทรไลต์ที่มีประจุบวก) เรียกว่าอิมัลชันเคลือบสองชั้น ต่อจากนั้นอิมัลชันเคลือบสามชั้นสามารถเตรียมโดยเคลือบอิมัลชันสองชั้นด้วยอัลจิเนต(พอลิอิเล็กโทรไลต์ที่มีประจุลบ) ลำดับสุดท้ายอิมัลชันเคลือบสี่ชั้นของ เคซีน-PDADMAC-อัลจิเนต-PDADMAC ในการเตรียมมัลติเลเยอร์อิมัลชันได้ศึกษาหาจุดสมมูลระหว่างอิมัลชันและพอลิอิเล็กโทรไลต์ที่มีประจุตรงกันข้ามโดยการวัดเปอร์เซ็นต์การส่องผ่านของแสงและค่าศักย์ซีต้า พบว่าอัตราส่วนน้ำหนักระหว่างอิมัลชันและสารพอลิอิเล็กโทรไลต์ของอิมัลชันที่เคลือบด้วยสองชั้นของ เคซีน-PDADMAC และ เคซีน-ไคโทซาน และอิมัลชันที่เคลือบด้วยสามชั้นของ เคซีน-PDADMAC-อัลจิเนต และ เคซีน-ไคโทซาน-อัลจิเนต และอิมัลชันที่เคลือบด้วยสี่ชั้นของเคซีน-PDADMAC-อัลจิเนต-PDADMAC เท่ากับ 3.2, 3.0, 0.24, 0.33 และ 0.77 ตามลำดับ เสถียรภาพต่อการเกิดออกซิเดชันของน้ำมันโอเมกา-3 ในน้ำมันทูนาในน้ำอิมัลชัน ศึกษาโดยใช้วิธี ferric thiocyanate ผ่านเทคนิค UV-Vis spectroscopy นอกจากนี้ยังพบว่าอิมัลชันซึ่งมีสารพอลิอิเล็กโทรไลต์หลายชั้นเคลือบบนหยดน้ำมันสามารถเพิ่มเสถียรภาพต่อการเกิดครีม รวมทั้งเสถียรภาพต่อการเกิดออกซิเดชันของน้ำมันได้ ความเสถียรต่อการเกิดออกซิเดชันในวันที่ 4 และ 5 ของอิมัลชันเคลือบสอง, สามและสี่ชั้นของทุกชนิด เพิ่มขึ้น 36-38% เมื่อเทียบกับอิมัลชันที่เคลือบด้วยชั้นเคซีนเพียงชั้นเดียว การที่ความหนาของชั้นที่มากขึ้นและประจุบวกที่ล้อมรอบอนุภาคน้ำมันในกรณีของอิมัลชันเคลือบสองชั้นและสี่ชั้น สามารถป้องกันอนุภาคน้ำมันจากตัวออกซิไดซ์ได้ นอกจากนี้ความเสถียรต่อการเกิดครีมในวันที่ 7 ของอิมัลชันเคลือบสอง, สามและสี่ชั้นเพิ่มขึ้น 29.78, 45.70 และ 65.38% ตามลำดับ เมื่อเทียบกับอิมัลชันที่เคลือบด้วยชั้นเคซีนเพียงชั้นเดียว

สาขาวิชา.....ปิโตรเคมีและวิทยาศาสตร์พอลิเมอร์.. ลายมือชื่อนิสิต.....สมประสงค์ ศิระมาด.....

ปีการศึกษา..2551..... ลายมือชื่ออ.ที่ปรึกษาวิทยานิพนธ์หลัก.....

ลายมือชื่ออ.ที่ปรึกษาวิทยานิพนธ์ร่วม.....

4972518623: MAJOR PETROCHEMISTRY AND POLYMER SCIENCE PROGRAM
KEYWORDS: POLYELECTROLYTE MULTILAYER / LAYER-BY-LAYER / EMULSION / OMEGA-3 / LIPID OXIDATION

SOMPRASONG SIRAMARD: OXIDATIVE STABILITY OF OMEGA-3 FATTY ACID EMULSIONS COATED WITH POLYELECTROLYTE MULTILAYER. ADVISOR: LUXSANA DUBAS, Ph.D., CO-ADVISOR: STEPHAN T. DUBAS, Ph.D., 112pp.

In this study, we demonstrated the first preparation procedure for oil-in-water emulsion coated with tertiary and quaternary polyelectrolyte layers and their improvement on the oxidation and creaming stability of omega-3 oil-in-water emulsions. Based on the study of the optimum preparation condition, primary emulsions were prepared by homogenizing a solution of 5 %w tuna oil and 95 %w of 1.5 %w casein in water at pH 6 for 2 minutes at 20,000 rpm and 5 homogenization cycles. Surface modification of the primary emulsion by polyelectrolytes was achieved by using the layer-by-layer self assembly technique. First, the primary emulsions were further coated with either poly(diallyl dimethyl ammonium chloride) PDADMAC or chitosan (polycationic) called secondary emulsion. Then, the tertiary emulsions were prepared by coating secondary emulsion with alginate (polyanonic). Last, the quaternary emulsion coated with casein-PDADMAC-alginate-PDADMAC could be synthesized. The equivalent point for multilayer emulsion preparation was investigated by measuring %transmission and zeta potential. The mass ratios of the emulsion to polyelectrolyte at the equivalent points for the preparation of secondary emulsion were 3.2 and 3.0 for casein-PDADMAC and casein-chitosan, respectively. The mass ratios for tertiary emulsions coated with casein-PDADMAC-alginate, casein-chitosan-alginate and quaternary emulsion coated with casein-PDADMAC-alginate-PDADMAC preparations were 0.24, 0.33 and 0.77, respectively. Oxidative stability of the omega-3 in tuna oil-in-water emulsions was measured by using UV-Vis spectroscopy via ferric thiocyanate method. Physical and oxidative stability of the multilayer emulsions were found to be improved when compared with primary emulsions. The oxidative stability of all multilayer emulsions were increased around 36-38% over primary emulsion at day 4 and 5 of storage. This is because the thicker layer of multilayer emulsions and the cationic charge on the droplet of secondary and quaternary emulsion can prevent the droplet from the oxidants. The creaming stability of secondary, tertiary and quaternary emulsion was also increased around 29.78, 45.70 and 65.38% compared to the primary casein emulsion at day 7 of storage.

Field of Study:...Petrochemistry and Polymer Science.. Student's Signature:.....Somprasong Siramard.....
Academic Year:...2008..... Advisor's Signature:.....
Co-Advisor's Signature:.....

ACKNOWLEDGEMENTS

I wish to express my deep gratitude to Dr. Luxsana Dubas and Dr. Stephan T. Dubas, my advisor and co-advisor, for their valuable guidance, advice and encouragement throughout this work.

I would like to acknowledge the committee, Assistant Professor Dr. Warinthorn Chavasiri, Assistant Professor Dr. Varawut Tangpasuthadol and Dr. Wilailuk Chaiyasit for their valuable suggestions and comments as committee and thesis examiners.

I gratefully acknowledge the generous access to research facilities and chemical support from Metallurgy and Materials Science Research Institute. I would like to thank the “Innovation for the improvement of food safety and food quality for new world economy” project for financial support. The tuna oil used in this study was kindly supported by T.C. Union Global Public Company Limited.

Finally, I would like to express my deepest appreciation to my family and all my friends for their love, encouragement and worthy moral support throughout my study at Chulalongkorn University.

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

CONTENTS

CHAPTER Page

CHAPTER Page

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

LIST OF TABLES

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

LIST OF FIGURES

FIGURE Page

FIGURE Page

FIGURE Page

LIST OF ABBREVIATIONS

PEL	:	Polyelectrolyte
PEM	:	Polyelectrolyte multilayer thin films
LbL	:	Layer-by-Layer technique
ESA	:	Electrostatic self - assembly
PDADMAC	:	Poly(diallyldimethylammonium chloride)
pI	:	Isoelectric point
pK_a	:	Acid dissociation constant
mM	:	milli Molar
μM	:	micro Molar
nm	:	nanometer
mV	:	milli Volt
%w	:	%weight per weight
%w/v	:	%weight per volume

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

CHAPTER I

INTRODUCTION

Omega-3 polyunsaturated fatty acids (PUFAs) are considered essential fatty acids. These long chain omega-3 fatty acids benefit for growth and development throughout the life cycle. Omega-3 fatty acids are found in fish oil, such as tuna oil and salmon oil, and other marine oils. From the fact that omega-3 cannot be synthesized by the body, thus human must obtain them from food. However, the addition of these oils into food is very difficult due to the highly susceptible to oxidation. Oxidation of these polyunsaturated fatty acids is a major cause of deterioration in the quality of products that affects many characteristics such as rancid flavor, change of color and loss of nutritive value, which has greatly limited their more widespread usage [1]. The nutritional benefits of omega-3 fatty acids make them excellent candidates as functional food ingredients if problems of oxidative rancidity can be overcome [2]. The prevention of lipid oxidation consists of preventing the formation of free radicals by limiting the amount of exposure to oxygen (vacuum packaging), retarding the rate of reaction by refrigerating or freezing, avoiding substances that catalyze the oxidation reaction, adding chelating agents to prevent metal ions, adding antioxidants that prevent the free radicals from propagating and microencapsulation [3].

The food industry is one of many industries which rely on the use of emulsion. Many food products such as milk, mayonnaise, butter, sauce and salad dressings are all emulsions. Food emulsion products can contain many kinds of components, including oils, emulsifiers, polymers, proteins and particles. The emulsifying agent at the liquid-liquid interface is essential to obtain stable emulsions under the environmental stresses by production of small oil droplets, lowering interfacial tension between oil and aqueous phase and prevention of droplet aggregation. Oil-in-water emulsions are prepared by homogenizing oil and aqueous

phase together in the presence of emulsifier. Proteins have been used as emulsifiers forming the membrane, interfacial membrane, around emulsions and produce small oil droplets during homogenization. Proteins also stabilize emulsions by imparting an electrical charge to the emulsion droplet at low or above p*I*, isoelectric point, of the proteins, which has repulsive force for prevention of droplet coalescence [4]. This interfacial membrane can be used to inhibit lipid oxidation by decreasing the interactions between the omega-3 fatty acids and transition metals such as Fe and Cu, which are the initially catalysis of the lipid oxidation reaction, in the aqueous phase [5]. Furthermore, cationic charge of protein can repel the metals away from the lipid in the droplets by electrostatic interaction [6].

Recently, the numerous studies have been published the new technique to prepare stable emulsion, called Layer-by-Layer deposition technique (LbL) or Electrostatic Self-Assembly technique (ESA), which give better both of physical stability to environmental stresses and oxidative stability of polyunsaturated fatty acids than single layer oil-in-water emulsions [7]. In the Layer-by-Layer deposition technique, an ionic emulsifier adsorbs to the surface of oil droplets during homogenization. Then, the primary emulsion containing small oil droplets is produced. An oppositely charged polyelectrolyte is added to the system, which is adsorbed to the droplet surfaces due to strong electrostatic attraction between the surface and oppositely charged polyelectrolyte molecules in solution forming the secondary emulsions. This procedure can be repeated to form oil droplets coated by interfaces containing three or more layers.

Earlier studies investigated the oxidative stability of primary emulsions and secondary emulsions. The studies showed better oxidative stability of secondary emulsions coated with cationic polyelectrolyte than primary emulsions coated with anionic emulsifier [8]. None of the research showed the oxidative stability of oil-in-water emulsions, which coated by more than two layers.

Therefore, our study aims to improve the oxidative stability of omega-3 fatty acids by preparing multilayer oil-in-water emulsions of tuna oil containing omega-3

fatty acid with the number of layers greater than two layers using Layer-by-Layer technique. The parameters controlling formation of multilayer emulsions such as number of coating layer, pH of emulsion, emulsifier and polyelectrolyte concentrations were studied to find the suitable condition to prepare stable multilayer emulsions. The study on oxidative stability of those emulsions was also evaluated.

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

CHAPTER II

THEORY AND LITERATURE REVIEW

2.1 Omega-3 fatty acids

Omega-3 fatty acids are a family of polyunsaturated fatty acids (PUFAs) that the first carbon-carbon double bond exists as the third carbon-carbon bond from the terminal methyl end (ω) of the carbon chain of the fatty acid. Omega-3 fatty acids are considered essential fatty acids. Important nutritional essential omega-3 fatty acids are α-linolenic acid (ALA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which the chemical structures were shown in Figure 2.1. These three polyunsaturated fatty acids have 3, 5 and 6 double bonds in a carbon chain of 18, 20 and 22 carbon atoms, respectively. The research indicates that omega-3 fatty acids reduce inflammatory responses and prevention risk factors associated with chronic diseases such as cardiovascular disease, cancer and arthritis. These essential fatty acids are also beneficial for brain (brain memory and performance), the central nervous, retinal and eye development of infants [9]. Omega-3 fatty acids were found in fish oil, such as tuna oil and salmon oil, and other marine oils.

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

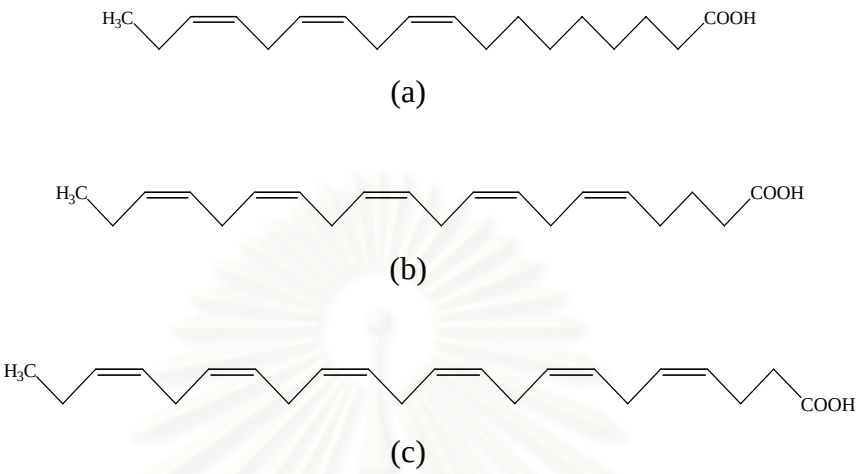


Figure 2.1 Chemical structures of (a) α-linolenic acid, (b) eicosapentaenoic acid and (c) docosahexaenoic acid.

2.2 Lipid Oxidation

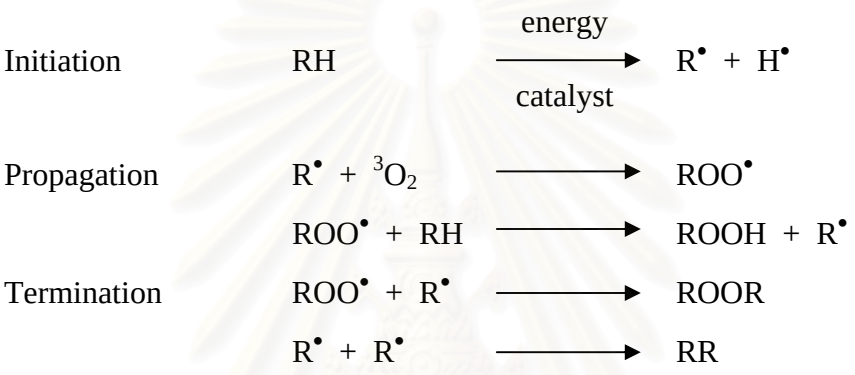
2.2.1 Mechanism of lipid oxidation

Oxidation of lipid is a major cause of deterioration in the quality of products that affects many characteristics such as rancid flavor, change of color, loss of nutritive value and production of toxic compounds, which has greatly limited their more widespread usage. The oxidation of oil is very important in terms of the palatability, nutritional quality and toxicity of lipids [8].

Different chemical mechanisms are responsible for the oxidation of lipids during processing and storage, depending upon the types of oxygen [10]. Two types of oxygen react with lipid. One is called atmospheric triplet oxygen, $^3O_2$, and the other is singlet oxygen, $^1O_2$. The important oxidation mechanisms in lipid are auto-oxidation and photosensitized oxidation.

Mechanisms of Autoxidation in lipid

Autoxidation is a free radical chain reaction, in which atmospheric triplet oxygen reacts with a lipid radical. Triplet oxygen is a radical compound with two unpaired orbitals in the molecules. It reacts with radical food compounds under normal reaction. Autoxidation of lipids includes the initiation, propagation and termination steps: [11]



In the presence of initiators, unsaturated lipids (RH) lose a hydrogen radical ($H^{\bullet}$) to form lipid alkyl radicals ($R^{\bullet}$). Heat, metal catalysts, UV and visible light can initiate and also accelerate free radical formation of fatty acid. The energy required to remove hydrogen from lipid is dependent on the hydrogen position in the molecules. A hydrogen atom adjacent to the double bond, especially hydrogen attached to the carbon between two double bonds, is removed easily.

The lipid alkyl radical ($R^{\bullet}$) reacts with diradical atmospheric $^3O_2$ and forms the lipid peroxy radical ($ROO^{\bullet}$). The reaction between the lipid alkyl radical and $^3O_2$ occurs very quickly at normal oxygen pressure. The lipid peroxy radical abstracts hydrogen from other lipid molecules and reacts with the hydrogen to form lipid hydroperoxide (ROOH) and other lipid alkyl radicals. These radicals catalyze the oxidation reaction. The rates for the formation of lipid peroxy radical and hydroperoxide depend only on oxygen availability and temperature. When radicals react with each other, nonradical species are produced and the reaction stops. Figure 2.2 shows the formation of hydroperoxide in the autoxidation of linoleic acid.

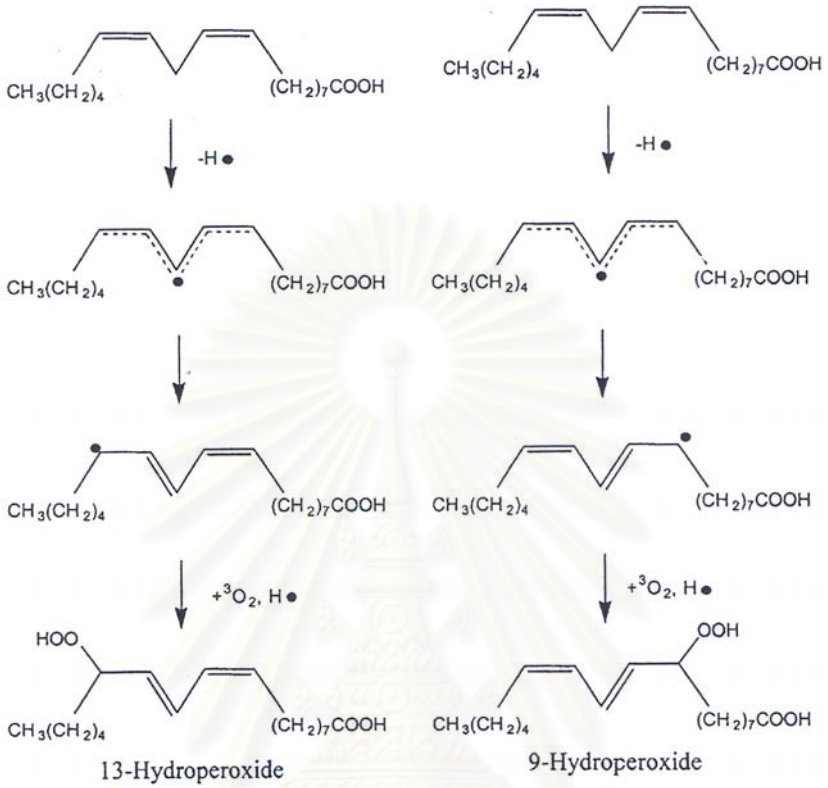


Figure 2.2 The formation of hydroperoxide in the auto-oxidation of linoleic acid. [10]

The primary oxidation products, lipid hydroperoxides, are relatively stable at room temperature and in the absence of metals. However, in the presence of metals or at high temperature they are readily decomposed to alkoxy radicals and then form secondary oxidation products, which are aldehydes, ketones, acids, esters, alcohols, and short-chain hydrocarbons. The most likely pathway of hydroperoxide decomposition is a hemolytic cleavage between oxygen and the oxygen bond, in which alkoxyl and hydroxyl radicals are produced (Figure 2.3). The ultimate secondary oxidation products of lipid are mainly low-molecular-weight aldehydes, ketones, alcohols and short-chain hydrocarbons. The time for secondary product

formation from the primary oxidation product, hydroperoxide, differs for different oils.

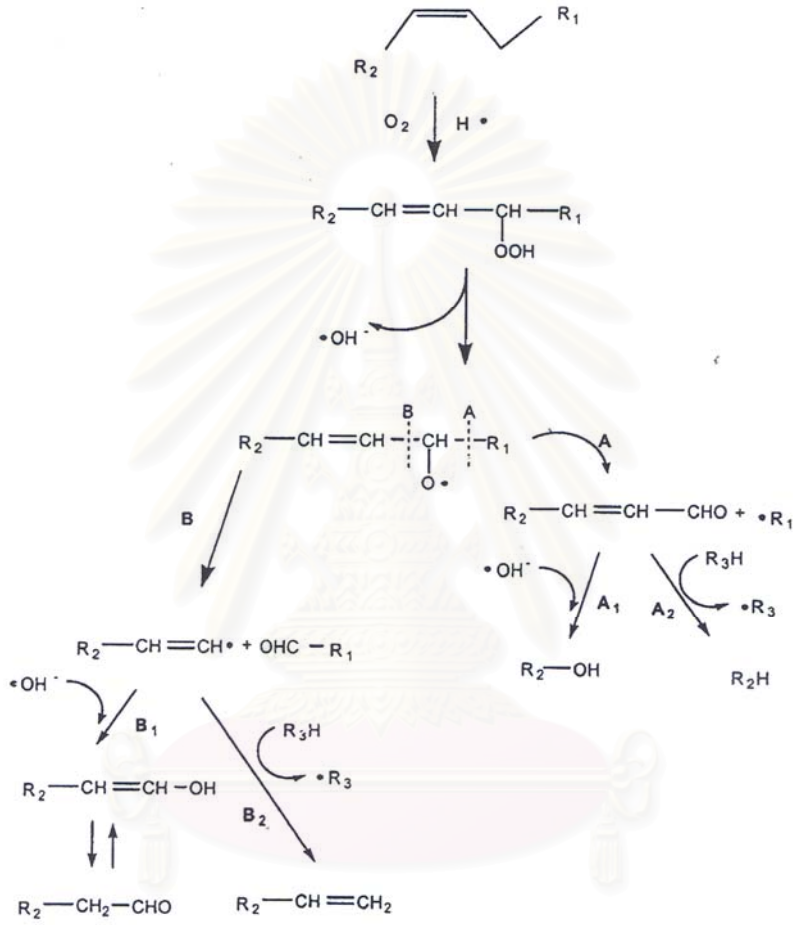


Figure 2.3 Mechanisms of hydroperoxide decomposition to form secondary oxidation compounds. [10]

Mechanisms for singlet oxygen formation and photosensitized oxidation in lipid

Photosensitized oxidation of lipids occurs in the presence of light, sensitizers and atmospheric oxygen, in which singlet oxygen is produced. Light accelerates the oxidation of oil, especially in the presence of sensitizers. Chlorophylls are common

sensitizers in edible vegetable oils. Sensitizers absorb light energy very rapidly, in picoseconds, to enter an excited singlet state (sens*) and then returned to the ground singlet state (sens) via the emission of light, internal conversion or intersystem crossing. Fluorescence and heat are produced by the emission of light and internal conversion, respectively. Intersystem crossing results in the excited triplet state of the sensitizers. An excited triplet sensitizer reacts with $^3O_2$ and produces $^1O_2$, then the sensitizer returns to its ground singlet state.

$$\text{sens} \xrightarrow{\text{light}} \text{sens}^*$$

$$\text{sens}^* + {}^3O_2 \longrightarrow \text{sens} + {}^1O_2$$

$^1O_2$ can react directly with high electron density double bonds and form hydroperoxides at the double bonds. The migration of the double-bond positions and trans fatty acid occurs when hydroperoxide is formed. This results in the production of both conjugated and nonconjugated hydroperoxides, which differs from autoxidation. Figure 2.4 shows the oxidation of linoleic acid by $^1O_2$. [11]

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

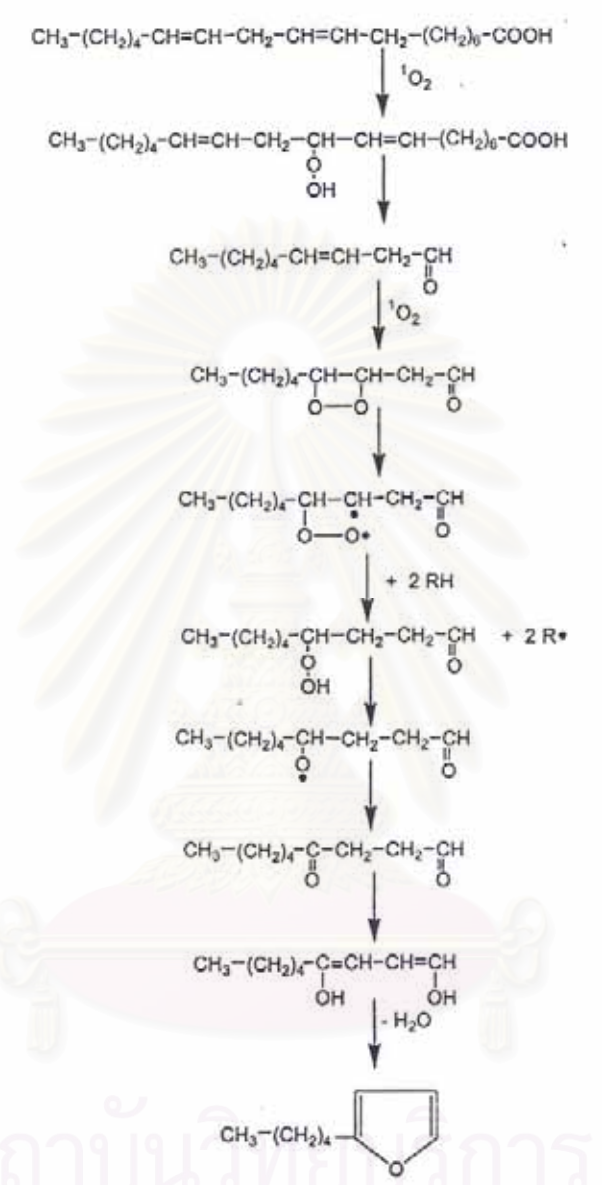


Figure 2.4 Mechanism of oxidation of linoleic acid by $^1O_2$. [10]

Hydroperoxides formed by $^1O_2$ oxidation are decomposed to secondary oxidation products by the same mechanisms for the hydroperoxides formed by $^3O_2$ in autoxidation.

In case of the oxidation of omega-3 which represented as α-linolenic acid, 16-hydroperoxide was formed as the primary oxidation product and propanal as the secondary oxidation product.

2.2.2 Factors affecting the oxidation of lipid

The oxidation of lipid is influenced by the composition of the lipid, energy in heat or light, the concentration and type of oxygen, transition metals and antioxidants. It is not easy to differentiate the individual effects of these factors because interactions exist among them. [10]

Composition of lipids

The autoxidation rate greatly depends on the rate of alkyl radical formation in the lipid, and the formation rate of fatty acid alkyl radical depends mainly on the types of fatty acid. Lipids that are more unsaturated oxidize more quickly than less unsaturated lipids. The conjugated double bonds are less reactive than non-conjugated double bonds. The higher number of double bonds can increase the oxidation rate also.

Storage Temperature and Light

The autoxidation of lipids and the decomposition of hydroperoxides increase as the storage temperature increases. The formation of autoxidation products is slow at low temperature.

The reaction temperature has little effect on $^1O_2$ oxidation. Light is much more important in $^1O_2$ oxidation [10].

Oxygen

The oxidation of oil can take place when oil, oxygen, and catalysts are in contact. Both concentration and type of oxygen affect the oxidation of oils. The

oxygen concentration in the oil is dependent on the oxygen partial pressure in the headspace of the oil. A higher amount of oxygen is dissolved in the oil when the oxygen partial pressure in the headspace is high. Oxidation of the oil increased with the amount of dissolved oxygen. The solubility of oxygen is higher in oil than in water, and in crude oils than in refined oils. The effect of oxygen concentration on the oxidation of oil increased at high temperature and in the presence of light and metals. However, the oil oxidation rate is independent of oxygen concentration at sufficiently high oxygen concentrations. The auto-oxidation rate of oil at > 4-5% oxygen in the headspace was independent of oxygen concentration and directly dependent on lipid concentration. However, the reverse was true at low oxygen pressure (< 4% oxygen) in the headspace [10].

Metal

Crude oil contains transition metals such as iron or copper, but the refining process reduces their concentrations. Edible oils manufactured without refining, e.g., extra virgin olive oil and sesame oil, contain relatively high amounts of transition metals. Metals increase the rate of oil oxidation due to metal can react directly with lipids to produce lipid alkyl radical. Lipid alkyl radical and reactive oxygen species accelerate the oxidation of oil.

$$Fe^{3+} + RH \longrightarrow Fe^{2+} + R^{\bullet} + H^{\bullet}$$

$$Fe^{2+} + {}^{3}O_2 \longrightarrow Fe^{3+} + O_2^{\bullet -}$$

$$O_2^{\bullet -} + O_2^{\bullet -} + 2H^{+} \longrightarrow {}^{1}O_2 + H_2O_2$$

Metals also accelerate autoxidation of oil by decomposing lipid hydroperoxides resulting the lipid alkyl radical species as shown below.

$$Fe^{2+} + ROOH \longrightarrow Fe^{3+} + RO^{\bullet} + OH^{-}$$

$$Fe^{3+} + ROOH \longrightarrow Fe^{2+} + ROO^{\bullet} + H^{+}$$

Antioxidants

Antioxidants are sometimes intentionally added to the oil to improve the oxidative stability. Antioxidants are the compounds that slow down the oxidation rate. Antioxidants inactivate free radicals, control transition metals, quench $^1O_2$, and inactivate sensitizers. Antioxidants (AH) can donate hydrogen atoms to free radicals and convert them to more stable nonradical products as shown below.

$$R^{\bullet} + AH \longrightarrow RH + A^{\bullet}$$
$$RO^{\bullet} + AH \longrightarrow ROH + A^{\bullet}$$
$$ROO^{\bullet} + AH \longrightarrow ROOH + A^{\bullet}$$
$$A^{\bullet} + A^{\bullet} \longrightarrow A\text{-}A$$
$$A^{\bullet} + RO^{\bullet} \longrightarrow A\text{-}OR$$

Prevention of lipid oxidation

The prevention of lipid oxidation consists of preventing the formation of free radicals by limiting the amount of exposure to oxygen (vacuum packaging), retarding the rate of reaction by refrigerating or freezing, avoiding substances that catalyze the oxidation reaction, adding chelating agents such as EDTA to prevent metal ions, adding antioxidants that prevent the free radicals from initiating and propagating and microencapsulation. The microencapsulation method by preparing the oil-in-water emulsion was used in the research for inhibition of lipid oxidation.

2.2.3 Methodology for determining the extent of lipid oxidation

The extent of oxidation can be determined by many methods. Peroxide value is one of the oldest and most commonly method used measurements of the extent of oxidation in oils [11]. Peroxide value can be measured by using 2 familiar methods.

1.) The standard iodometric procedure

The standard iodometric procedure can be measured by colorimetric or electrometric method, the iodine produced by potassium iodide added as a reducing agent to the oxidized sample. The liberated iodine is titrated with standard sodium thiosulfate.

$$ROOH + 2KI \longrightarrow I_2 + 2KOH + RO^-$$

$$I_2 + Na_2S_2O_3 \longrightarrow S_2O_3 + 2NaI$$

2.) The ferric thiocyanate method

The ferric thiocyanate method is based on the oxidation of ferrous (Fe^{2+}) to ferric (Fe^{3+}) ions, which are determined colorimetrically as ferric thiocyanate. This method is more sensitive and requires a smaller sample than does the iodometric method.

$$ROOH + Fe^{2+} \longrightarrow Fe^{3+} + RO^{\bullet} + OH^-$$

$$Fe^{3+} + 3(NH_4)SCN \longrightarrow Fe(SCN)_3 + 3NH_4^+$$

The determination of peroxide value is useful for bulk oils that can be analyzed directly. For foods, emulsions or muscle tissues, the lipid is extracted with mixtures of solvents that must be carefully removed by evaporation without decomposition of hydroperoxides. The ferric thiocyanate method is commonly applied to emulsions, which undergo oxidative deterioration at relatively low peroxide values. Thus, the ferric thiocyanate method was used for determining the extent of lipid oxidation in the research.

2.3 Emulsion

Emulsions are colloidal dispersions which is a mixture of two immiscible liquids. One liquid (the dispersed phase) is dispersed in a continuous liquid phase of different composition in the form of small droplets. In most emulsions, one of the

liquids is the aqueous while the other is hydrocarbon and referred to as oil. Emulsions are thermodynamically unstable, because energy is required to increase the surface area between the oil and water phases. The dispersion of one liquid into another is achieved by the energy obtained from vigorous or ultrasonic stirring, or from homogenization to create small oil droplets. However, on standing with time the dispersion will separate into layers of different densities. To prepare emulsions that are kinetically stable on standing for a reasonable time, it is necessary to use emulsifiers, also referred to as surfactants, which are surface-active molecules that absorb to the surface of freshly formed droplets during homogenization, reducing interfacial tension, forming a protective film or membrane that prevents the droplets from coming close enough together to aggregate. Emulsifiers are “amphiphilic” molecules (containing both hydrophilic and hydrophobic moieties) consisting of a polar group oriented in one phase, and a non-polar group oriented in the other phase. Emulsions can be classified into two types.

1.) Oil-in-water emulsions (O/W) in which the oil droplets are dispersed in the continuous water phase. This dispersed phase is stabilized by an emulsifier composed of a lipophilic part or hydrophobic part and a hydrophilic part. The lipophilic part consists of a long chain alkyl residue with good solubility in the oil phase. The hydrophilic part consists of a dissociable group or a number of hydroxyl groups with good solubility in the water phase. Common food emulsifiers include surface-active proteins (casein, whey, soy and egg), phospholipids (soy or egg lecithin), or surfactants (polyoxyethylene sorbitan monoesters named ‘Tweens’, sorbitan fatty acid esters named ‘Spans’ or fatty acids). Examples of oil-in-water food emulsions include milk, mayonnaise, salad dressing, cream and soups.

2.) Water-in-oil emulsions (W/O) in which water droplets are dispersed in the continuous phase. The lipophilic surfactant which is oil soluble is used to stabilize water-in-oil emulsions (examples: monoglycerides and Spans). Examples of water-in-oil food emulsions include butter, margarine and spreads.

Emulsifier

Emulsion can be considered to consist of three regions, i.e., the interior of a droplet, the continuous phase, and the interfacial membrane. The interfacial membrane which has a thickness of a few nanometers consists of a narrow region surrounding each emulsion droplet, consisting of a mixture of oil, water and emulsifier molecules. The various molecules in an emulsion partition themselves among these three different regions according to their polarity and surface activity. Nonpolar molecules are located predominantly in the oil phase, polar molecules in the aqueous phase, and surface-active molecules at the interface. Therefore, the nature of the emulsion droplet interfacial membrane would be expected to be extremely important in the lipid oxidation and stability of emulsion also. In the food industry, a variety of different emulsifiers can be used.

1.) Synthetic emulsifiers and surfactants

Numerous nonionic synthetic emulsifiers are used in the food industry. These materials are usually partial esters of medium or long-chain acids (C-12 to C-22) with polyhydric alcohols such as glycerol, propylene glycol, sucrose or sorbitan.

2.) Protein emulsifiers

Proteins are commonly used in food products to facilitate the formation and improve the stability of food emulsions. During homogenization, proteins are capable of rapidly absorbing to the surface of oil droplets, where they lower interfacial tension and inhibit droplet coalescence by forming protective membranes around the droplets. Proteins also stabilize oil-in-water emulsions by imparting an electrical charge to the emulsion droplet at pH values above or below pI (isoelectric point) of the proteins. This positive or negative electrical charge causes repulsive forces that inhibit droplet coalescence and flocculation, thus further stabilizing the emulsion.

3.) Phospholipid emulsifiers

Phospholipids, also commonly referred to as lecithins, which the chemical structures were shown in Figure 2.5. Lecithins are important surface-active substances in the production of food emulsifiers, and are generally derived

commercially either from soybean or egg yolk. Phospholipids consist of 2 parts, one is hydrophilic head part and the other is hydrophobic tail part. The hydrophilic head contains the negatively charged phosphate group, and may contain other polar group. The hydrophobic tail usually consists of long fatty acid hydrocarbon chains. This result allows phospholipids to play an important role in the emulsifier.

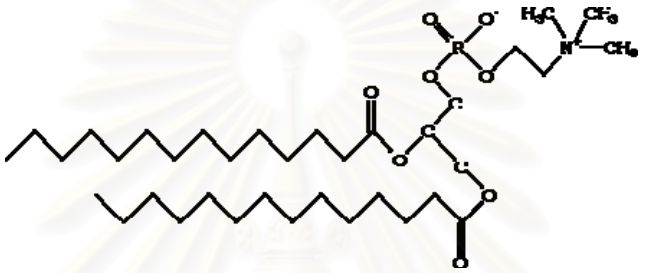


Figure 2.5 Chemical structure of lecithin

2.3.1 Emulsion stability

Most emulsions are not thermodynamically stable. Over time, emulsions tend to revert to the stable state of the phases comprising emulsions. The instability of emulsions can occur in four basic ways [12].

1.) Coalescence

Coalescence is when two or more droplets fuse together to form a single larger unit, which reducing the total surface area. This appearance makes the change in droplet size and size distribution. The formation of larger droplet in coalescence may lead to the phase separation or the breaking of emulsion.

2.) Aggregation or flocculation

Aggregation or flocculation is when two or more droplets clump together, possibly touching at some points, and with virtually no change in total surface area. In aggregation the species retain their identity but lose their kinetic independence

since the aggregate moves as a single unit. Aggregation of droplets may lead to coalescence and the formation of larger droplets until the phases become separated.

3.) Creaming or sedimentation

Creaming or sedimentation results from a density difference between the dispersed and continuous phases. This is not yet a destabilization of the dispersion, but produces two separate layers of dispersion that have different dispersed phase concentrations. One of the layers will contain an enhanced concentration of dispersed phase, which may promote flocculation.

4.) Breaking

Breaking is due to the combination of coalescence and creaming, the emulsions break into two layers, oil separates completely from the water and float at the top in a single continuous layer.

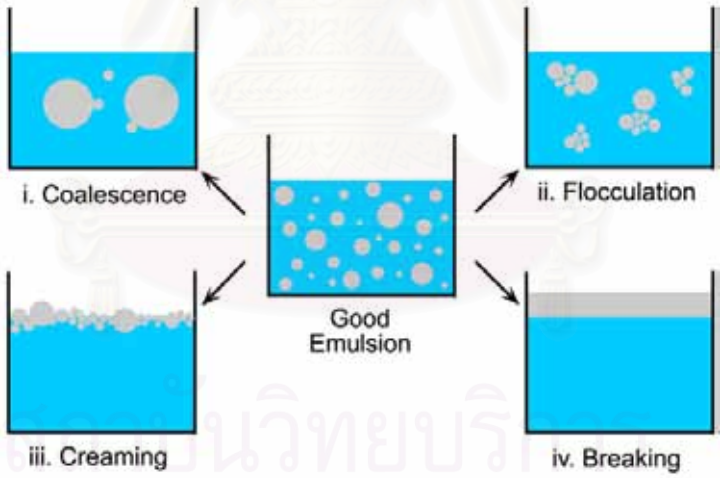


Figure 2.6 Illustrate the stability of emulsion [13]

Figure 2.6 clearly shows that flocculation and creaming leave the fine oil droplets intact while making them less well distributed throughout the water. Therefore, these two processes can be reversed by putting in a small amount of energy (i.e. moderate stirring or shaking). Brownian motion, within the water phase, can provide enough energy to keep exceptionally small droplets agitated and hence

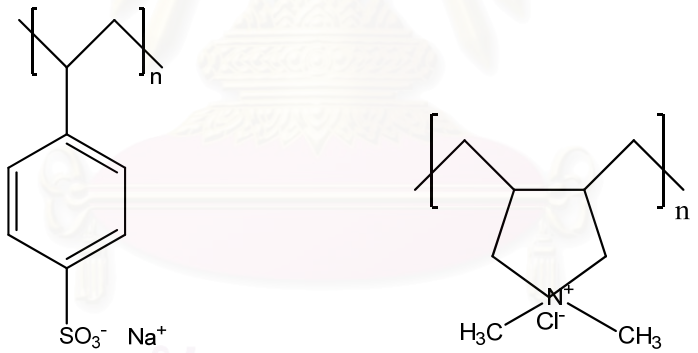
creaming is less [12]. Coalescence and breaking lead to large bodies of oil separating from the water and essentially result in the emulsion separating completely. To reverse this process the emulsion must be remade and this will require a lot of energy. As mentioned above, that most emulsions are thermodynamically unstable, meaning that they will eventually separate. However, emulsion can be stabilized when the repulsive forces between droplets must be dominant. Steric and electrostatic stabilization are the two main mechanisms for emulsion stabilization [14]. Steric stabilization involves the polymers added to the system adsorbing onto the particle surface and causing repulsion. At high polymer-dispersed species ratios, long-chain surfactants and high molecular-weight polymers can become adsorbed at the surfaces of dispersed species such that a significant amount of adsorbate extends out from the surfaces. If the adsorbed material extends out significantly from the particle surface, then an entropy decrease can accompany particle approach providing a short-range, volume-restriction, stabilization mechanism referred to as steric stabilization. The most important factor influencing the degree of steric stabilization is the thickness of the adsorbed layer in comparison with the size of the particles [14]. Electrostatic stabilization is based on the mutual repulsion of like electrical charges when two charged surfaces approach each other and their electric double layers overlap. The overlap causes a coulombic repulsive force acting against each surface, which will act in opposition to any attempt to decrease the separation distance.[12] The degree of repulsion between adjacent, similarly charged particles in dispersion can be indicated by using zeta potential.

Zeta potential is electric potential in the interfacial double layer at the location of slipping plane, which separates the mobile ion in diffuse layer from ion that remains attached to the charged surface (fixed layer), versus a point in the bulk fluid away from the interface. The zeta potential is determined by measuring the direction and velocity of droplet movement in a well-defined electric field [15]. Zeta potential value can be related to the stability of colloidal dispersions. For molecules and particles that are small enough, a high zeta potential will confer stability, i.e. the

solution or dispersion will resist aggregation. When the potential is low, attraction exceeds repulsion which colloids tend to flocculate resulting in the dispersion instability. So, colloids with high zeta potential (negative or positive) are electrically stabilized while colloids with low zeta potentials tend to coagulate or flocculate. A zeta potential value of 30 mV (positive or negative) can be taken as the value that separates low-charged surfaces from highly-charged surfaces [16].

2.4 Polyelectrolyte

The term “polyelectrolyte” is employed for polymer systems consisting of a macroion, i.e., a macromolecule carrying covalently bound anionic or cationic groups, and low-molecular counterions securing for electroneutrality. Examples of an anionic and a cationic polyelectrolyte (PEL) are presented in Figure 2.7.

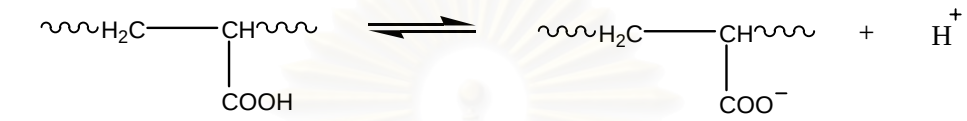


(a) (b)

Figure 2.7 Chemical structure of (a) sodium poly(styrene sulfonate) (PSS) and (b) poly(dilallylmethylammonium chloride) (PDADMAC).

Both Na-poly(styrene sulfonate) and poly(diallyldimethyl ammonium chloride) are dissociated into macroion and counterions in aqueous solution in the total pH range between 0 and 14 which is strong polyelectrolytes. Polymers like poly(acrylic acid) or poly(ethylene imine) are usually classified as polyelectrolytes,

in spite of the fact that they form a polyion-counterion system only in a limited pH range, and remain as an undissociated polyacid in the acid range or an undissociated polybase in the alkaline range as shown in Figure 2.8. This is a behavior typical for weak polyelectrolytes and quite analogous to weak low molecular electrolytes.



(a)

(b)

Figure 2.8 Dissociation equilibrium of the weak polyelectrolytes (a) poly(acrylic acid) and (b) poly(ethylene imine).

On the other hand, a polymer like cellulose capable of dissociating partially into cellulosate anions and counterions at extremely alkaline conditions (pH > 14) cannot be classified as a polyelectrolyte, as in the conventional pH range of dilute aqueous systems the OH groups of polymer are not ionized.

A special case of polyelectrolytes, the “polyampholytes”, carrying both anionic and cationic groups covalently bound to the macromolecule, are represented in nature by an abundant number of proteins but can also be obtained by various synthetic routes. An example is presented in Figure 2.9 as a typical polyampholyte, this copolymer carries cationic charged in an acid and anionic charges in an alkaline medium, while at the so-called “isoelectric point”, in the example pH 4, no free net charge exists at the macromolecule.

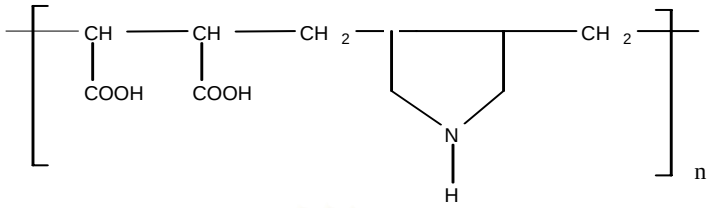


Figure 2.9 Chemical structure of a maleic acid-diallylamine copolymer.

In principle, any macromolecule can be transformed into a polyelectrolyte structure by covalently attaching the ionic groups to the polymer backbone, with linear or branched structures [17]. Limiting our further considerations to linear and branched structures, a vast number of polyelectrolyte classes are known today such as anionic and cationic polysaccharides and polysaccharidic derivatives, gelatin, nucleic acids, lignosulfonic acids, polyacrylic and polymethacrylic acid and its copolymer, maleic acid anhydride copolymers, polystyrene sulfonic acid, polyethylene imine, polyamines and polyamidamines, ionenes, poly(diallylmethylammonium chloride) and homo-and copolymers of cationic acrylic acid esters. This variability of polyelectrolyte chemical structures results from the tremendous number of polymer backbone structures. Today's commercial polyelectrolytes are predominantly obtained by a polymerization, polycondensation, or polyaddition process. Also numerous important PEL also originate from nature, such as gelatin, as a representative of the widespread class of proteins or pectins belonging to the group of anionic polysaccharides. Furthermore, some PEL of practical importance result from a chemical modification of nonionic natural polymers such as cellulose or starch.

In contrast to the huge variability of the polymer backbone structure, the number of different chemical structures of anionic or cationic sites responsible for the behavior of PEL in solution is rather small as shown in Table 2.1.

Table 2.1 Structures of ionic sites of PEL [17]

$-COO^-$	$-NH_3^+$
$-CSS^-$	$=NH_2^+$
$-OSO_3^-$	$\equiv NH^+$
$-SO_3^-$	$-NR_3^+$
$-OPO_3^{2-}$	

These ionic groups are usually classified as anionic and cationic; a further subdivision into weakly and strongly acid and basic groups is reasonable in analogy to “strong” and “weak” acids and bases of low molecular chemistry with the sulfonate, the sulfonate-half ester, and the tetraalkylammonium group being representative for the so-called “strong PEL”.

2.5 Layer-by-Layer deposition technique (LbL)

Layer-by-Layer deposition technique is currently used to modify the surface properties of materials by polyelectrolyte multilayer films formation. These polyelectrolyte based films are capable of self-organization. The self-organization process of polyelectrolyte films, also referred to as electrostatic self-assembly (ESA), has been well documented over the past ten years.

In the early 1990s, LbL electrostatic assembly research was revolutionized when Decher’s group demonstrated electrostatic multilayer adsorption of charged polymers onto solid surfaces. In this technique surface charge reversal was the basis of the assembling technique which did not require special equipment or procedures. In the late 1990s Caruso et al. extensively investigated the adsorption of polyelectrolytes onto colloidal particles using the LbL technique, which opened the way to many applications of this technique including encapsulation of enzymes and production of hollow nanoparticles.

In the LbL, electrostatic deposition technique a polyelectrolyte layer is formed on a charged surface due to electrostatic attraction between the surface and oppositely charged polyelectrolyte molecules in solution. The fact that the total number of charges on the adsorbed polyelectrolyte molecules is greater than that required to neutralize the opposite charges on the surface, means that charge reversal occurs. This overcompensation of the surface charge has two important consequences. First, it means that the adsorbing polyelectrolytes tend to form mono-layers because once the surface has been saturated with polyelectrolytes there is an electrostatic repulsion between it and the non-adsorbed polyelectrolytes that prevents further adsorption. Second, it means that further layers can be formed by adsorbing oppositely charged polyelectrolytes on top of the first layer. Repetition of both adsorption steps leads to the formation of multilayer structures. The polyelectrolyte that forms the outer layer usually determines the net charge on the overall interface.

For planar surfaces, the method of preparing multilayer structures, as shown in Figure 2.10, simply consists of consecutive immersion of the substrate into two or more coating solutions containing oppositely charged species.

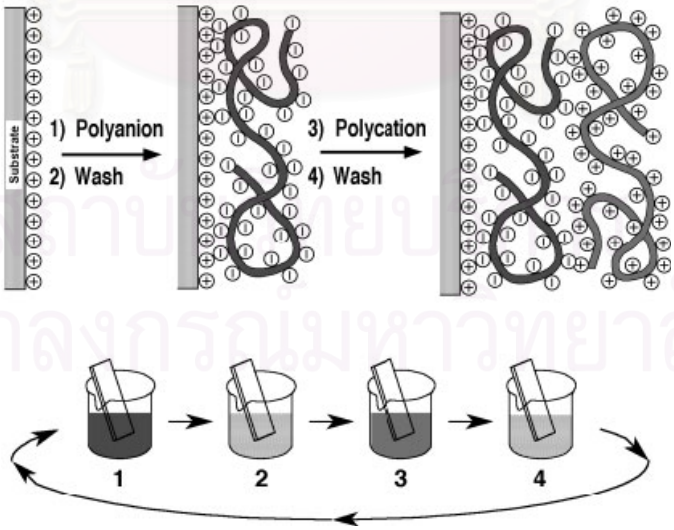


Figure 2.10 Schematic of Layer-by-Layer deposition technique. [18]

The LbL technology can simply be adapted to produce multilayer emulsions using a step-by-step method that should be fairly easy, fast and cheap. The basic method of producing multilayer emulsions is shown in Figure 2.11. In this method a primary emulsion is prepared by homogenizing oil and water phases in the presence of a positive or negatively charged emulsifier. The resulting primary emulsion is then mixed into an oppositely charged polyelectrolyte solution to create a secondary emulsion. The secondary emulsion is then mixed into another solution containing polyelectrolytes that have an opposite charge to the previous one to create a tertiary emulsion, and so on. The multilayer oil-in-water emulsion is formed. For the production of multilayer oil-in-water emulsion, it may be necessary to remove any excess polyelectrolyte between each adsorption step, although this can often be avoided by selection of an appropriate initial polyelectrolyte concentration. Otherwise, it will interact with the oppositely charged polyelectrolytes in the next solution, which may interfere with the formation of the multilayers around the particles.

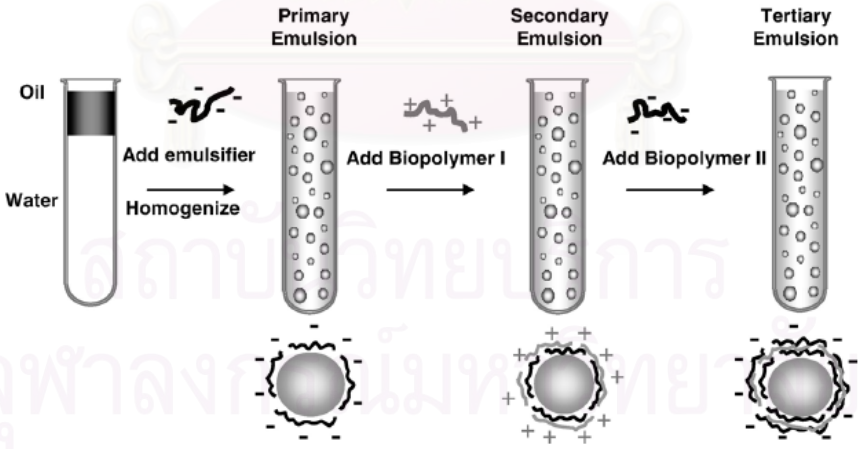


Figure 2.11 Schematic of Layer-by-Layer technique for the production of oil-in-water emulsions. [19]

A number of preparation strategies that have been developed to produce stable multilayer systems without promoting droplet aggregation due to the presence of excess free polyelectrolyte: [19]

1.) *Saturation method*. It is possible to add just enough polyelectrolyte to completely coat all of the particles present in the system, so that there is little free polyelectrolyte remaining in the aqueous phase. The saturation concentration for a particular system has to be determined empirically such as zeta potential.

2.) *Centrifugation method*. In this method a solution that contains excess polyelectrolyte is added to a colloidal suspension. Any excess non-adsorbed polyelectrolyte molecules are then removed by centrifuging the colloidal suspension, collecting the particles, and re-suspending them in an appropriate buffer solution. This procedures can be repeated a number of times to ensure that all of the free polyelectrolyte has been removed, before the next polyelectrolyte solutions is added. The main problem with this method is that it can promote particle aggregation during the centrifugation step because the particles are forced into close proximity.

3.) *Filtration method*. In this method a solution that contains excess polyelectrolyte is added to the colloidal suspension. However, in this case the excess non-adsorbed polyelectrolyte molecules are removed by membrane filtration of the colloidal suspension. A filter is used that allows the polyelectrolyte molecules to pass through, but not the colloidal particles. The colloidal suspension is put under pressure, which forces the aqueous phase containing the excess polyelectrolyte through the filter. At the same time, a buffer solution can be added to the colloidal suspension to keep the overall volume of the system constant. In this way, the colloidal particles are never forced into close proximity, which reduces the amount of particle aggregation that occurs in the system. Another advantage of this method is that it is not necessary to have a density difference between the particles and the surrounding liquid.

For all of the methods mentioned above one must carefully control the system composition and preparation conditions to form stable multilayer colloidal

particles. For example, it is important to ensure that there is sufficient polyelectrolyte present to cover all of the surfaces area and there is not too much excess polyelectrolyte in solution. These processes influence to the droplet aggregation during the preparation of multilayer emulsions.

2.5.1 Applications of multilayer emulsions

Multilayer emulsions may have a number of potential applications in the food industry. For example, thick highly charged multilayer interfaces may be useful in protecting droplets against aggregation or in preventing lipid oxidations. On the other hand, multilayer interfaces that change their properties in a controlled fashion in response to some environmental condition could be used for controlled or triggered release of active ingredients. Some of the potential applications of multilayer technology in the food industry can be described as follow.

2.5.1.1 Improved stability against environmental stresses

Food emulsions experience a variety of different environmental stresses during their manufacture, storage, transport and utilization. Some parameters that can effect the stability such as pH, salt concentration, thermal processing, etc. Multilayer can help improving the stability by controlling these parameters:

(1) *pH*

The aqueous solution surrounding the oil droplets in food oil-in-water emulsions may vary from acidic to slightly alkaline depending on the nature of the product. In addition, the aqueous phase pH may vary during the production, storage or utilization of the product. It is often important to ensure that the oil droplets do not aggregate when they are exposed to variations in pH. Multilayer interfaces are normally produced using weak polyelectrolytes, and so their thickness, structure and electrical characteristics are strongly dependent on solution pH. By manipulating the type of polyelectrolytes used to prepare multilayer emulsions it is therefore possible to control the influence of pH on droplet aggregation [20].

(2) *Salt*

The type and concentration of ions in food emulsion may vary considerably depending on the nature of food, the purity of the functional ingredients, and the hardness of water used to prepare the emulsion. In many situations it is important to ensure that the presence of ions does not promote emulsion instability, e.g., by screening electrostatic interactions or binding to oppositely charged groups. The presence of salts can alter interfacial and emulsion properties through a variety of physicochemical mechanisms, including changing the amount of polyelctrolyte adsorbed, altering the structure of the interfacial layer, or modulating the strength and range of the various colloidal interactions between the droplets. The influence of salts on the properties of multilayer emulsions depends on the electrical characteristics of the emulsifier-coated droplets and polyelectrolytes involved. A number of studies have shown that emulsions containing multilayer-coated droplets are more stable to high salt concentrations than those containing single-layer-coated droplets [21].

(3) *Thermal processing*

Many food emulsions undergo some form of thermal processing during their production, storage or utilization, e.g., pasteurization sterilization or cooking. It is usually to important that an emulsion is capable of withstanding these thermal treatments without breaking down due to droplet flocculation or coalescence. Many emulsifiers are unsuitable for creating droplets that are resistant to thermal processing because they undergo changes in their ability to prevent droplet aggregation with temperature. The studies have shown that wrapping a natural polyelectrolyte around an emulsion droplet may be able to improve its stability to thermal processing [22].

(4) *Chilling and freezing*

There are many potential applications for oil-in-water emulsions that can be chilled or frozen during storage and then warmed prior to use in the food industry. Cold storage is often used to protect product quality by retarding microbial

growth and undesirable chemical reactions, such as lipid oxidation. Nevertheless, many oil-in-water emulsions become physically unstable when they are chilled and/or frozen, and rapidly break down after reheating. It is therefore important to have technologies improve the stability of food emulsions to chilling, freezing and thawing. Multilayer emulsion may be able to stabilize the emulsion to freeze-thaw cycling because the thick interfacial membrane is resistant rupture by oil or fat crystals, or because the repulsive colloidal interactions generated by the thick electrically charged interfacial membranes are sufficiently large to overcome any attractive colloid interactions or mechanical forces that tend to push the droplets together during the freezing process [23].

(5) *Lipid oxidation*

There is considerable interest in the incorporation of polyunsaturated fats (such as omega-3 fatty acids) into food products because of their potential health benefits. Nevertheless, incorporation of these oils into food products is problematic because they are highly susceptible to oxidative degradation resulting in rancid off-flavors, which has greatly limited their more widespread usage. The multilayer technology provides the food industry with a powerful tool for altering the electrical charge and thickness of the interfacial layer surrounding the lipids, and thereby improving the stability of the encapsulated lipids to oxidation [8].

(6) *Dehydration*

Oil-in-water emulsions are often converted into a powdered form in the food industry to increase their shelf life, reduce transport costs and/or facilitate their utilization. The microencapsulation process is normally carried out by evaporating the majority of water from the emulsion using a suitable dehydration method, such as spray drying or freeze drying. Dehydration often promotes emulsion instability by adversely affecting the properties of the interfacial layers surrounding the oil droplet, e.g., by physically disrupting them or by promoting cross-linking of emulsifiers adsorbed onto different droplets. After dehydration it is important that the resulting powder has good functional characteristics, remains physically and

chemically stable during storage. In the research has shown a much better stability to droplet aggregation of multilayer emulsion than primary emulsion [8].

2.5.1.2 Controlled release

Delivery systems can be used to encapsulate, stabilize and deliver a variety of functional food component, e.g., flavors, bioactive lipids, enzymes, antimicrobials and antioxidants. Oil-in-water emulsions, used in either their wet or dehydrated states, can be used as delivery systems, because they are capable of containing oil-soluble, water-soluble and amphiphilic functional agents in a single system. The multilayer technique has the major advantage that wall characteristic, such as thickness, charge and permeability, can be finely tuned by careful selection of polyelectrolytes and preparation conditions. Moreover, multilayer emulsions can be made to detach from the droplet surfaces in response to alterations in pH, ionic strength or temperature. It may be possible to use this approach to encapsulate one or more charged functional components between the interfacial layers, so that they can be released in response to specific enviraonmental trigger [24].

2.5.1.3 Hollow (nano) capsules

Hollow capsules can be produced by adsorbing layers of polymers onto a colloidal template, which can then be removed by chemical or physical methods. Complete removal of the core yielded hollow polymer capsules of micrometer dimensions. The capsule porosity was found to be influenced by the selection of polyelectrolytes used, the wall thickness and the ambient conditions. The pores of the shell wall can be selectively opened and closed by chemically modifying or adjusting the ambient conditions [25].

จุฬาลงกรณ์มหาวิทยาลัย

2.6 Literature reviews

These studies focused on the increasing of the oxidative stability of tuna oil by using polyelectrolyte multilayer technique for multilayer emulsions preparation. Hereafter is described some works already published.

Min Hu and co-workers studied the oxidative stability of monolayer protein coated oil-in-water emulsion (primary emulsion) [26, 27]. Casein, whey protein isolate (WPI) and soy protein isolate (SPI) were chosen to stabilize the emulsions. The oil-in-water emulsions were controlled at pH3, which was below the isoelectric point (*pI*) resulting in the cationic oil-in-water emulsion droplets [27]. Similarly, whey protein isolate (WPI), sweet whey (SW), ß-lactoglobulin (ß-Lg) or α-lactoglobulin (α-Lg) stabilized emulsions at pH 3 were chosen [26]. Lipid oxidation was measured by monitoring both lipid hydroperoxide and hexanal formation. The result showed that casein coated oil-in-water emulsion can stabilize oil-in-water emulsions better than the others, whey protein isolate (WPI) and soy protein isolate (SPI). One of the reasons is casein can form a thick interfacial layer around dispersed oil droplets up to 10 nm compared to 1-2 nm whey proteins. So casein can decrease the interaction between the omega-3 and transition metal better than whey protein [27]. They also studied on the influence of pH on lipid oxidation in WPI-stabilized emulsions. Their results showed that formation of lipid hydroperoxides and propanal was much lower at pH below the protein's isoelectric point (p*I*), at which the emulsion droplets were positively charged. These data indicated that cationic protein coated emulsion can decrease the interaction between the omega-3 and transition metal by electrostatically repelling metals away from the lipids in the emulsion droplet core. These results indicate that it was possible to engineer emulsions with improved oxidative stability by producing protein-stabilized emulsions under acidic conditions.

Demet Guzey and co-workers [28] studied the impact of the electrostatic interactions on formation and stability of emulsions containing oil droplets coated by

ß-lactoglobulin-pectin complexes. They found that interfacial protein-polysaccharide complexes can be used to improve the physical stability of oil-in-water emulsions. Moreover, the impact of ionic strength on the formation and stability of oil-in-water emulsions containing polysaccharide-protein coated droplets was also examined. The result showed that polysaccharide-protein coated droplets (secondary emulsions) had a much improved stability to salt induced flocculation than protein coated droplets (primary emulsions). Secondary emulsions were found to increase the oxidative stability as compared to primary emulsions. Lauren A. Shaw and co-workers studied the oxidative stability of emulsion of oil-in-water where the oil droplets were coated by bi-layer of protein-polymer (secondary emulsion) using layer-by-layer technique [29]. Lecithin used as the emulsifier results in the anionic primary oil-in-water emulsion droplets. The secondary emulsion was prepared by adding chitosan. The result showed that tuna oil-in-water emulsion droplets coated by lecithin and chitosan were more oxidatively stable than emulsions coated by lecithin alone. Chelating effect on the oxidative stability of oil was studied by adding ethylenediaminetetraacetic acid (EDTA) to the secondary oil-in-water emulsion as well. They found that the oxidative stability of oil was increased when compared with secondary emulsion.

Tertiary emulsion of oil-in-water emulsion was prepared by Yeun Suk Gu and co-workers [30]. The influence of environmental stresses such as pH, salt and temperature on the properties and stability of oil-in-water emulsions containing oil droplets surrounded by one-, two- or three-layer were studied. The droplets stabilized by ß-Lactoglobulin, *i*-Carrageenan and gelatin as the primary, secondary and tertiary respectively. They found that multilayer emulsions had better stability to environmental stresses than primary emulsions under certain environmental conditions such as pH of solution, salt and temperature. Similarly, Demet Güzey and David J. McClements [31] studied the stability of corn oil-in-water emulsions stabilized by ß-lactoglobulin, pectin and chitosan as the primary, secondary and tertiary emulsion respectively. The result showed that primary emulsions were

unstable at all pH, salt concentrations, and thermal treatments compared to multilayer oil-in-water emulsions.

In this work, the improvement of the oxidative stability of omega-3 fatty acids by preparing multilayer oil-in-water emulsions of tuna oil containing omega-3 fatty acid using Layer-by-Layer technique was studied. The effect of number of layers coated around the droplets (1-4 layers) on lipid oxidation rate was investigated, which is different from the previous studies that the lipid oxidation of emulsion coated by only two layers was studied. Moreover, none of the researches could prepare the emulsion coated more than three layer and investigate the oxidative stability of emulsion as well. The saturation method was used to prepare the multilayer oil-in-water emulsions. The extent of lipid oxidation was measured using the ferric thiocyanate method.

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

CHAPTER III

EXPERIMENTAL

3.1 Chemicals

Poly(diallydimethyl ammonium chloride) or PDADMAC (medium molecular weight, 20 %w in water, typical M_w 200,000 – 350,000), chitosan (M_w 800,000, 84% deacetylation) and alginic acid, which is a weak anionic polyelectrolyte, were purchased from Aldrich. Casein, sodium salt was purchased from Sigma which contained 98% of purity. Cumene hydroperoxide (Molecular weight 152.5 g/mol) and 1-butanol were purchased from Sigma. Tuna oil was donated from T.C. Union Global Company which contained eicosapentaenoic acid (EPA) 7.08% and docosahexaenoic acid (DHA) 28.26%. Glacial acetic acid and hydrochloric acid were purchased from Lab-Scan. Sodium acetate trihydrate, sodium hydroxide, isooctane, methanol and 2-propanol were purchased from Merck. Analytical grade ammonium thiocyanate, ferrous sulphate and barium chloride was purchased from JT Baker. All chemicals were analytical grade, and were used without further purification. Distilled water was used for the preparation of all solutions.

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

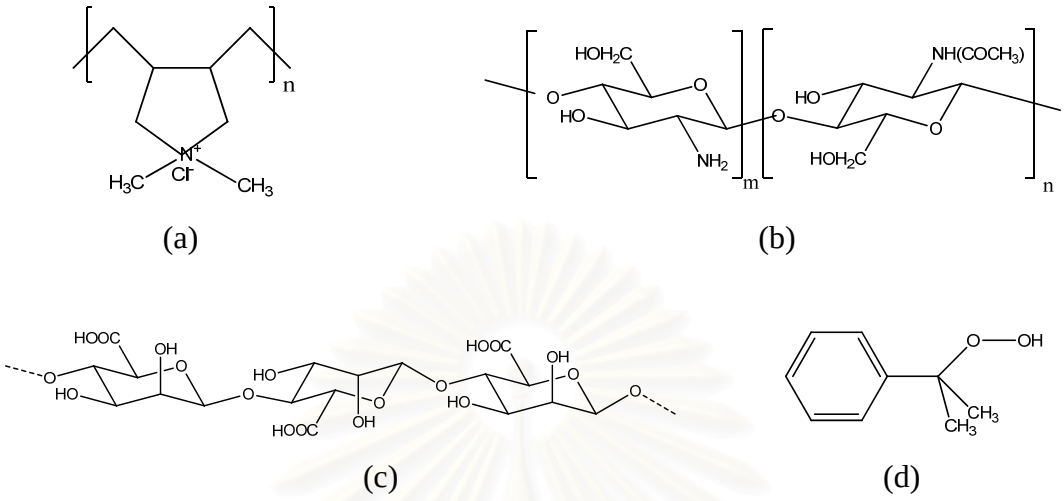


Figure 3.1 Chemical structure of (a) poly (diallyldimethylammonium chloride) (PDADMAC) (b) chitosan (c) alginic acid and (d) cumene hydroperoxide.

3.2 Equipments

Homogenizer model PT3100 from Polytron, vortex model Fine vortex from FINEPCR and the microcentrifuge model 260D from Denville were used in the research.

3.3 Solution preparation

3.3.1 Acetic-sodium acetate buffer solution

Buffer solutions of pH 3 and 6 were prepared by mixing acetic acid 100mM with sodium acetate 100 mM with the proportional ratio to get the required pH.

3.3.2 Emulsifier solution

Sodium caseinate was used as emulsifier in this experiment. Stock solution of sodium caseinate was prepared at both pH 3 and 6 by stirring 5 grams of sodium caseinate with 100 mM acetic-sodium acetate buffer solution at pH 3 and 6, respectively. The emulsifier solution prepared with acetic-sodium acetate buffer pH3 was adjusted to pH 3 with 100 mM HCl, whereas the emulsifier solution prepared with acetic-sodium acetate buffer pH 6 was adjusted to pH 6 with 100 mM NaOH. Then, added acetic-sodium acetate buffer at each pH to obtain 100 grams of solution. The stock solution at pH 6 was used for all experiments, while the stock solution at pH3 was used in the study of the effect of pH.

3.3.3 Polyelectrolyte solution

3.3.3.1 Poly(diallydimethyl ammonium chloride) (PDADMAC) solution

Poly(diallydimethyl ammonium chloride) (PDADMAC) was used as polycationic polyelectrolyte for multilayer emulsion preparation. Stock solution of 100mM PDADMAC was prepared by diluting 7.7 mL of concentrated PDADMAC (20 %w in water) (MW of monomer is 161.5 g/mol) with acetic-sodium acetate buffer solution pH 6. The pH of PDADMAC solution was adjusted to pH 6 by adding 100 mM NaOH, and then acetic-sodium acetate buffer pH 6 was added to obtain the total volume equal 100 mL. In the final step, the pH of solution was checked which the obtained pH was 6.

3.3.3.2 Chitosan solution

Chitosan was used as polycationic polyelectrolyte for secondary emulsion preparation. Stock solution which contained 1 %w/v chitosan was prepared by adding 1M acetic acid 5 mL into chitosan 1 gram. Then, when the chitosan solution turned clear, add water around 10 mL and stirred overnight. The pH of chitosan was adjusted to pH 6 by adding 100 mM of NaOH and then adjusted the final volume to 100 mL with water. The 0.1 %w/v chitosan could be prepared by diluting 1 %w/v chitosan 10 times using acetic-sodium acetate buffer solution at pH 6. In the final step, the pH of solution was checked which the obtained pH was 6.

3.3.3.2 Alginate solution

Alginate was used as polyanionic for multilayer emulsion preparation. Alginate concentration of 1 %w/v was prepared by dissolving alginate 1 gram with acetic-sodium acetate buffer solution pH 6. The pH of alginate solution was adjusted to pH 6 by adding 100 mM NaOH, and then acetic-sodium acetate buffer pH 6 was added to obtain the total volume equal 100 mL. The 0.1 %w/v alginate could be prepared by diluting 1 %w/v alginate 10 times using acetic-sodium acetate buffer solution at pH 6. In the final step, the pH of solution was checked which the obtained pH was 6.

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

3.3.4 Solution for lipid oxidation measurement

3.3.4.1 Cumene hydroperoxide standard solution

Cumene hydroperoxide was used as the standard compound to prepare the calibration curve for determining the hydroperoxide concentration of lipid oxidation measurement. Stock solution of cumene hydroperoxide which

contained 2.943 mM cumene hydroperoxide was prepared by dissolved 50.00 μL of cumene hydroperoxide, which contained 88% purity, into the mixture solvent of isooctane/2-propanol (3:1, v/v) to obtain total volume equal 100 mL. Then the cumene hydroperoxide standard solutions was prepared by diluting stock solution as 25.00, 50.00, 100.00, 200.00 and 500.00μL with the mixture solvent of isooctane/2-propanol (3:1, v/v) to obtain each total volume of 50mL. The prepared standard cumene hydroperoxide concentration was 1.47, 2.94, 5.89, 11.8 and 29.4 μM, respectively.

3.3.4.2 Ammonium thiocyanate solution

The 50.00 mL of 3.970 M ammonium thiocyanate solution was prepared by dissolving 15.11xx grams of ammonium thiocyante with distilled water.

3.3.4.3 Ferrous iron solution

Ferrous iron solution was prepared in situ by mixing 0.1320 M of barium chloride with 0.144 M of ferrous sulphate in one to one ratio [32]. The 50.00 mL of 0.132 M barium chloride was prepared by dissolving 1.612x grams of barium chloride with 0.4 M HCl. While 50.00 mL of 0.1440 M ferrous sulphate was prepared by dissolving 2.002x grams of ferrous sulphate with 0.4 M HCl. The ferrous sulphate solution had to be covered with aluminum foil to prevent the auto-oxidation of ferrous ion to ferric ion.

3.4 Experimental procedures

3.4.1 Study on the optimized conditions for the primary emulsion preparation condition

Tuna oil-in-water emulsions which used casein as the emulsifier can be prepared by homogenizing 5 %w tuna oil and 95 %w emulsifier solution (casein) at pH6 (except the study of the effect of pH) with a homogenizer at 20,000 rpm for 2 min per cycle in the ice bath as shown in Figure 3.2. The following parameters were investigated to find the suitable conditions for stable primary emulsions. The obtained primary emulsions were characterized by using optical microscope, %transmission measurement at wavelength of 550 nm, particle size diameter and zeta potential measurement. The stability to creaming of primary emulsion was also investigated by creaming stability measurement. All measurements were repeated two times on three different samples.

Figure 3.2 The set up of homogenizing station.

3.4.1.1 Study on the suitable number of homogenization cycle for the preparation of primary emulsion

Stable primary emulsion prepared using the homogenizer, so the number of homogenization cycle on the preparation of primary emulsion was the important parameter to investigate. The number of homogenization cycle was varied from 1-10. The emulsions had to wait for 5 min for next cycle of homogenization. The obtained primary emulsions were characterized by optical microscope and %transmission measurement. The oil-in-water emulsions were diluted 200 times for %transmission measurement. The suitable number of homogenization cycle was used in the later study.

3.4.1.2 Study on the effect of pH on the preparation of primary emulsion

Because the charge density of casein, which is used as the emulsifier, depends on pH, the effect of pH of casein solution on the primary emulsion preparation needs to be studied. The pH of primary emulsion which contain 1.5 %w casein was varied to be value of 3, 4, 5, 6 and 7. Primary emulsions at pH 3 and 6 were prepared then using 100 mM HCl to adjust primary emulsion from pH 6 to pH 5 and 100 mM NaOH to adjust primary emulsion from pH 3 to 4 and pH 6 to 7. The suitable pH obtained from this study would be used in the later study.

3.4.1.3 Study on the effect of casein concentration on the preparation of primary emulsion

The emulsifier concentration, also, affects the droplet size and the stability of emulsion, so the effect of casein concentration on the primary emulsion preparation was studied. Casein concentrations at the value of 0.5 %w, 1

%w, 1.5 %w, 2 %w and 2.5 %w were used in this study. The 85, 75, 65, 55 and 45 grams of acetic-sodium acetate buffer pH6 was added to 10, 20, 30, 40 and 50 grams of 5%w sodium caseinate (stock solution), respectively. Then, 5 grams of tuna oil was added to each solution of sodium caseinate solution to obtain 5 %w tuna oil and 95 %w casein. The concentration of casein in the emulsifier solution were 0.5 %w, 1 %w, 1.5 %w, 2 %w and 2.5 %w, respectively. The suitable casein concentration found in this experiment would be used to prepare multilayer emulsion in the later study.

3.4.2 Preparation of multilayer emulsions

Multilayer emulsions can be prepared by adding emulsions into oppositely charged polyelectrolytes solution. In this research, up to 4 layer emulsions were prepared. Because the surface droplet charge of primary emulsion was anionic, cationic polyelectrolyte such as PDADMAC and chitosan were used for secondary emulsion preparation. Anionic polyelectrolyte, alginate, was used for tertiary emulsion preparation. PDADMAC was used for quaternary emulsion preparation. The optimum volume ratio between emulsions and oppositely charged polyelectrolytes has to be quantified for the preparation of stable multilayer emulsions.

Multilayer emulsions can be prepared by mixing the emulsions with the oppositely charge polyelectrolyte, while homogenizing the 20 mL of polyelectrolyte solution at 20,000 rpm, for 2 min (1 cycle). For %transmission measurement, the prepared multilayer emulsion solutions were centrifuged at 4000 rpm for 10 min to separate the precipitant out of the solution. Then, the prepared multilayer emulsions were diluted 200 times. The particle size diameter and zeta potential of secondary emulsions were characterized by using Zetasizer which the analyzed solutions were diluted 50 times. All measurements were repeated two times on three different samples.

3.4.2.1 Investigate the optimum volume ratio between primary emulsions and cationic polyelectrolyte for secondary emulsion preparation

Secondary emulsions were prepared by titrating primary emulsions with either PDADMAC or chitosan which their pH values were maintained at pH 6. For PDADMAC, we also study the concentration effect on the equivalent point between primary emulsion and PDADMAC concentration. The 1, 5 and 10mM PDADMAC were used for this study. The ranges of volume ratio between primary emulsion and PDADMAC solutions were varied as shown in Table 3.1.

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

Table 3.1 The volume ratio between primary emulsion and PDADMAC solution for study on the effect of PDADMAC concentration

1 mM PDADMAC		5 mM PDADMAC		10 mM PDADMAC		30 mM PDADMAC	
Volume of 10 times diluted primary emulsion (mL)	Volume ratio	Volume of 10 times diluted primary emulsion (mL)	Volume ratio	Volume of 10 times diluted primary emulsion (mL)	Volume ratio	Volume of concentrated primary emulsion (mL)	Volume ratio
2.00	0.010	10.00	0.050	20.00	0.10	4.00	0.20
4.00	0.020	20.00	0.10	40.00	0.20	8.00	0.40
6.00	0.030	30.00	0.15	60.00	0.30	12.00	0.60
7.00	0.035	35.00	0.18	70.00	0.35	14.00	0.70
8.00	0.040	40.00	0.20	75.00	0.38	16.00	0.80
8.50	0.042	45.00	0.22	80.00	0.40	18.00	0.90
9.00	0.045	50.00	0.25	85.00	0.42	20.00	1.0
10.00	0.050	75.00	0.38	90.00	0.45	22.00	1.1
15.00	0.075			100.00	0.50	24.00	1.2
				150.00	0.75	26.00	1.3
						30.00	1.5
						36.00	1.8
						40.00	2.0

* volume ratio is the volume ratio between concentrated emulsion and polyelectrolyte solution

For secondary emulsion prepared with 0.1 %w/v chitosan, the effect of pH of chitosan on the equivalent point between primary emulsion and 0.1 %w/v chitosan was also evaluated. The pH 5.5, 6.0 and 6.5 were used for secondary emulsion preparation. The ranges of volume ratio between primary emulsion and chitosan solutions were varied as shown in Table 3.2.

Table 3.2 The volume ratio between primary emulsion and 0.1 %w/v chitosan solution under pH study effect

pH 5.5		pH 6.0		pH 6.5	
Volume of primary emulsion (mL)	Volume ratio	Volume of primary emulsion (mL)	Volume ratio	Volume of primary emulsion (mL)	Volume ratio
1.00	0.05	0.50	0.03	0.50	0.03
2.00	0.10	1.00	0.05	1.00	0.05
3.00	0.15	2.00	0.10	2.00	0.10
4.00	0.20	3.00	0.15	2.25	0.11
4.50	0.22	4.00	0.20	2.50	0.13
5.00	0.25	4.25	0.21	2.75	0.14
5.25	0.26	4.50	0.22	3.00	0.15
5.50	0.28	5.00	0.25	3.25	0.16
6.00	0.30	6.00	0.30	3.50	0.18
7.50	0.38	7.50	0.38	4.00	0.20
				5.00	0.25

3.4.2.2 Investigate the optimum volume ratio between secondary emulsion and anionic polyelectrolyte for tertiary emulsion preparation

Tertiary emulsions were prepared by titrating secondary emulsions with 20 mL of 1 %w alginate at pH 6 for secondary emulsions coated with PDADMAC while secondary emulsions coated with chitosan can be prepared by titrating with 20 mL of 0.1 %w alginate. The ranges of volume ratio between secondary emulsion and alginate solution were varied as shown in Table 3.3.

Table 3.3 The volume ratio between secondary emulsions and alginate solution

Secondary emulsion coated with PDADMAC with 1 %w/v alginate		Secondary emulsion coated with chitosan with 0.1 %w/v alginate	
Volume of secondary emulsion (mL)	Volume ratio	Volume of secondary emulsion (mL)	Volume ratio
4.00	0.20	2.00	0.10
8.00	0.40	4.00	0.20
12.00	0.60	6.00	0.30
14.00	0.70	8.00	0.40
16.00	0.80	9.00	0.45
18.00	0.90	10.00	0.50
20.00	1.0	11.00	0.55
22.00	1.1	12.00	0.60
24.00	1.2	14.00	0.70
26.00	1.3	16.00	0.80
28.00	1.4	18.00	0.90
32.00	1.6	20.00	1.0

3.4.2.3 Investigate the optimum volume ratio between tertiary emulsion and cationic polyelectrolyte for quaternary emulsion preparation

Quaternary emulsion was prepared by titrating tertiary emulsions coated with casein-PDADMAC-alginate and casein-chitosan-alginate, with 20 mL of 40 mM PDADMAC and 0.1 %w/v chitosan at pH 6, respectively. The range of the volume ratio between tertiary emulsion and PDADMMAC was varied as shown in Table 3.4.

Table 3.4 The volume ratio between tertiary emulsion and PDADMAC or chitosan solution

40 mM PDADMAC		0.1 %w/v chitosan	
Volume of concentrated tertiary emulsion coated with casein-PDADMAC-alginate (mL)	Volume ratio	Volume of concentrated tertiary emulsion coated with casein-chitosan-alginate (mL)	Volume ratio
4.00	0.20	2.00	0.10
10.00	0.50	4.00	0.20
14.00	0.70	6.00	0.30
16.00	0.80	8.00	0.40
18.00	0.90	9.00	0.45
20.00	1.0	10.00	0.50
22.00	1.1	11.00	0.55
24.00	1.2	12.00	0.60
26.00	1.3	14.00	0.70
30.00	1.5	16.00	0.80
34.00	1.7	18.00	0.90
40.00	2.0	20.00	1.0

3.4.3 Emulsion characterization

3.4.3.1 %Transmission measurement

Percent transmission is measured to investigate either the stability of emulsion or the multilayer emulsion preparation. %transmission can be measured at 550 nm by using UV-Vis Spectrophotometer as shown in Figure 3.3 [33]. The creaming stability can be measured the changing in %transmission or turbidity of emulsion after storage. The emulsion which diluted 200 times with acetic-sodium acetate buffer to obtain the 3 mL (30 mm high) of total volume was transferred into plastic spectrophotometer cuvette which covered with parafilm and then stored at room temperature which was measured every day of 1 week. The light beam passed through the emulsions at a height that was 10 mm from the cuvette bottom, i.e., about 33% of the emulsion's height. The formation of clear layer at the bottom of the cuvette exhibited the unstable to creaming stability. The increase in %transmission of the emulsion indicated the fact that serum layer had risen to at least 33% of the emulsion's height.

For the study of the effect of pH on the preparation of primary emulsion experiment, the acetic-sodium acetate at each pH was used as the background. The others experiments, the acetic-sodium acetate buffer pH 6 was first introduced and recorded as a background. All measurements were repeated two times on three different samples.

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

Figure 3.3 UV-Vis Spectrophotometer (SPECORD S100, Analytikjena)

3.4.3.2 The particle size diameter and zeta potential of emulsions

The particle size diameter and electrical charge (ζ, zeta potential) of particles in the emulsions were determined using commercial instrument capable of dynamic light scattering measurements and electrophoresis as shown in Figure 3.4.

The emulsions were prepared and stored in the closed vial at room temperature for 24 h prior to the analysis. The emulsions were diluted 50 times by using the acetic-sodium acetate buffer pH 6 for avoiding the multiple scattering effects. All measurements were repeated two times on three different samples.

Figure 3.4 Zetasizer Nano ZS, Malvern Instruments

3.4.3.3 Lipid Oxidation Measurement

Lipid oxidation measurement can be analyzed by measuring the extent of lipid hydroperoxide which is the primary product from lipid oxidation reaction. Lipid hydroperoxide can be measured by using the ferric thiocyanate method [34]. The process of this method is summarized as follow.

All multilayer emulsions (10 mL) which diluted to the same oil content with acetic-sodium acetate buffer pH6 were placed in sealed screw-cap test tubes and allowed to oxidize at ambient temperature in the dark. Lipid hydroperoxides were measured by mixing 0.2 mL of emulsions with 1 mL of isooctane/2-propanol (3:1, v/v) by vortexing, then the organic solvent phase was separated by centrifugation at 1000g for 2 min. The organic solvent phase (200 μL) was added to 2.8 mL of methanol/1-butanol (2:1, v/v), followed by 15 μL of 3.94 M ammonium thiocyanate and 15 μL of ferrous iron solution (prepared by mixing 0.132 M $BaCl_2$ and 0.144 M $FeSO_4$ in acidic solution). The absorbance of the solution was measured by using UV-Vis Spectrophotometer, as shown in Figure 3.6, at 510 nm. The blank solution could be prepared follow the above steps except the isooctane/2-propanol was used to instead the emulsions. Hydroperoxide concentrations were determined using a standard curve made from cumene hydroperoxide [35]. All measurements were repeated two times on three different samples.

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Study on the optimized conditions for the primary emulsion preparation condition

In order to prepare the suitable primary emulsion, optimized preparation parameters: homogenization cycles, pH of emulsifier solution, and casein concentrations were investigated. The stability of emulsion was monitored by measuring %transmission of diluted emulsion according to the creaming stability measurement in the previous studies [23, 33, 35]. If extensive droplet aggregation and creaming occurred, the %transmission would increase which indicated the instability to creaming. The droplet size and surface potential were also measured to determined the physical properties of emulsion.

4.1.1 Study on the suitable number of homogenization cycle for the preparation of primary emulsion

The number of homogenization cycle is one of parameters which affect stability of emulsions. In the research, the effect of the number of homogenization cycles on %transmission of the primary emulsion was studied. The increase in %transmission was attributed to extensive droplet aggregation and also exhibited the significant increase in mean particle diameter [35].

From Figure 4.1, %transmission was decreased with the increase in the number of homogenization cycles, which indicated the decreasing of droplet size. It was found that the number of homogenization cycle was around 5 where the %transmission was not significantly different change with any further increase in the homogenization cycle. Moreover, all casein concentrations also exhibited the same

profiles which the 5-homogenization cycle was the optimized number. Thus the 5-homogenization cycle was chosen for further study.

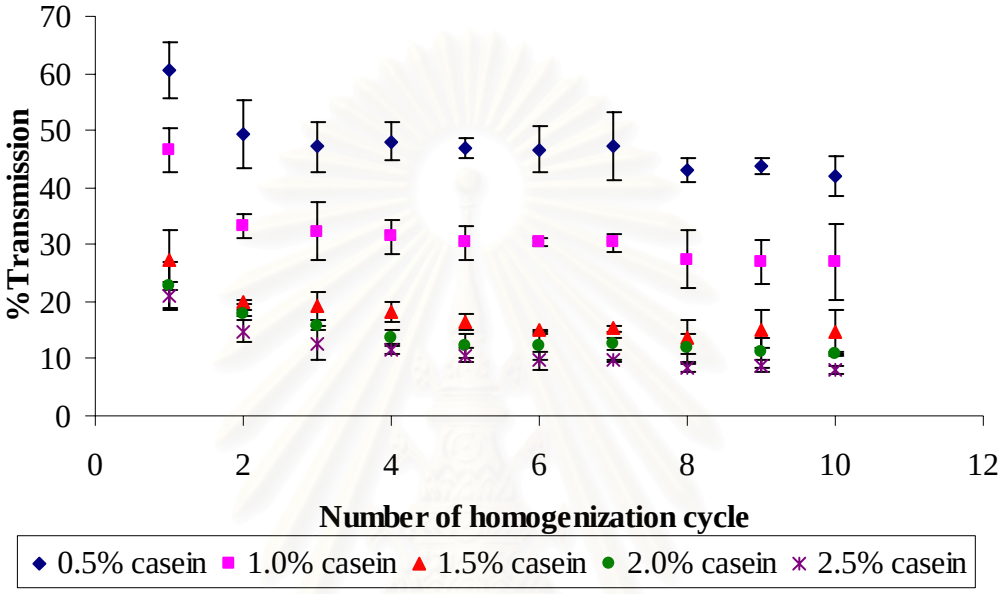


Figure 4.1 Effect of the number of homogenization cycle on %transmission of primary emulsion at pH 6 which varied casein concentration.

4.1.2 Study on the effect of pH on the preparation of primary emulsion

Casein was used as protein emulsifier in this experiment. The charge of casein can stabilize the emulsion by imparting an electrical charge to the emulsion droplet, resulting in the repulsive forces that inhibit droplet coalescence and flocculation. Its charge depends on the pH of casein solution whether above or below its isoelectric point (pI) of casein which is 4.6 [36].

In this study, the effect of amount and type of charges on the droplet size and emulsion stability was investigated. The pH range of 3-7 was chosen because casein exhibited either cationic or anionic charge in this range.

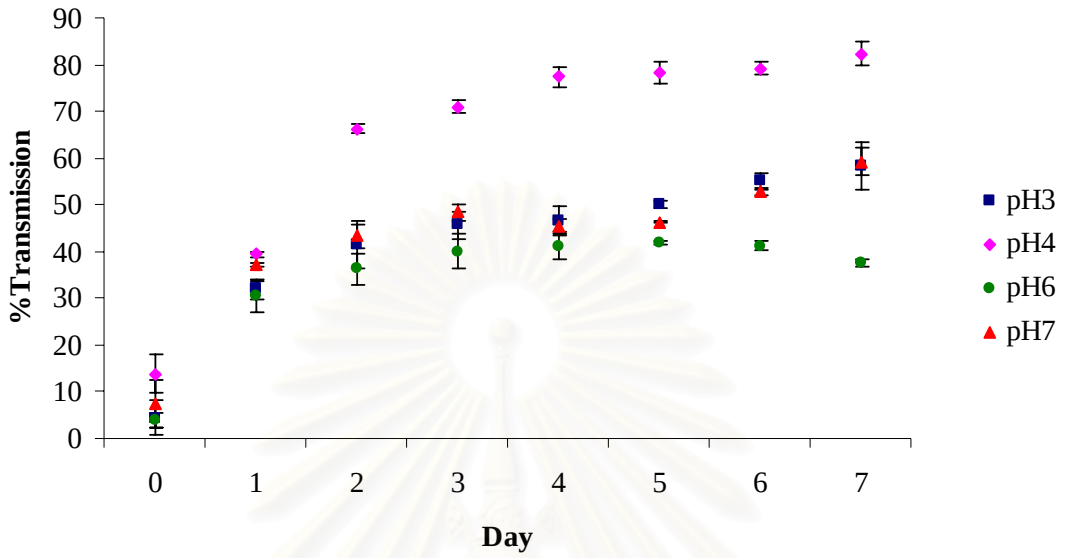


Figure 4.2 Effect of pH on %transmission of primary emulsion which contained 1.5 %w casein and 5 %w tuna oil prepared under the condition of 5-homogenization cycle.

The changed pH influences the charge density of casein which emulsified the droplet of emulsion that affect to the stability of emulsion. The effect of pH on stability of emulsion was investigated by measuring %transmission of emulsion every day for 1 week storage.

From Figure 4.2, comparison between pH values, primary emulsion prepared using pH 3, 6 and 7 emulsifier solutions exhibited the quite similar %transmission values and changing pattern that %transmission increased with storage days. This meant that the emulsion stability decreased with the longer storage time. For any storage day, the emulsion prepared by using pH 4 emulsifier solution showed the lowest stability compared to the other pH values. Furthermore, we observed dramatically increased in %transmission after the first day indicating that the stability of the emulsion at this pH was quite poor.

At pH 4, the emulsion exhibited very poor stability because this pH was close to the isoelectric point value (pI) of casein. Thus, emulsions which prepared using

casein at pH 4 would show small net electrical charge of positive and negative, respectively. Hence, the repulsive forces of those emulsions were not sufficient enough to prevent the coalescence and flocculation of emulsions, which result in the unstable primary emulsion.

At pH 5, we observed the precipitation of casein in solution because at pH 5 was close to pI of casein. Therefore we did not perform any further investigation under this condition.

The purpose of our study is to prepare multilayer emulsion. It is important to consider working at the pH that delivered the best stability and the pH compatibility of primary emulsion with further preparation condition. Even though, the most stable primary emulsion could be prepared at pH 3, 6 and 7. However, we chose to work at pH 6 for further coating layer with positive polyelectrolytes due to the limit pH working range of available cationic polyelectrolytes. The primary emulsion prepared at pH 6 showed the best stability over period of time. Therefore, in our study, the pH of emulsifier solution will be held at pH 6 unless stated.

4.1.3 Study on the effect of casein concentration on the preparation of primary emulsion

Casein concentration was the important parameter for primary preparation because protein concentrations are known to influence the droplet size and storage stability [26].

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

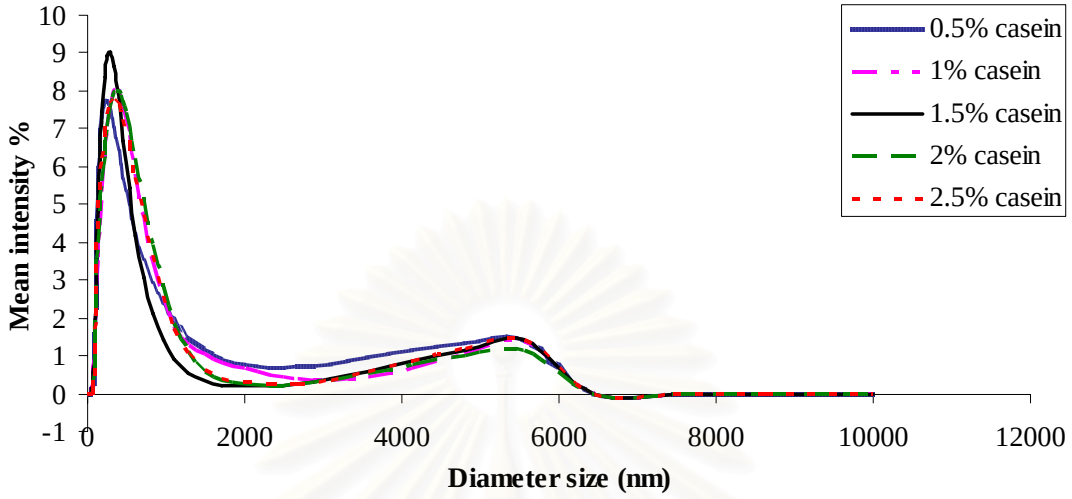


Figure 4.3 Effect of casein concentration on size distribution of primary emulsions which diluted 50 times at pH 6 prepared under the condition of 5-homogenization cycle. Primary emulsion was measured after 24 hours of storage.

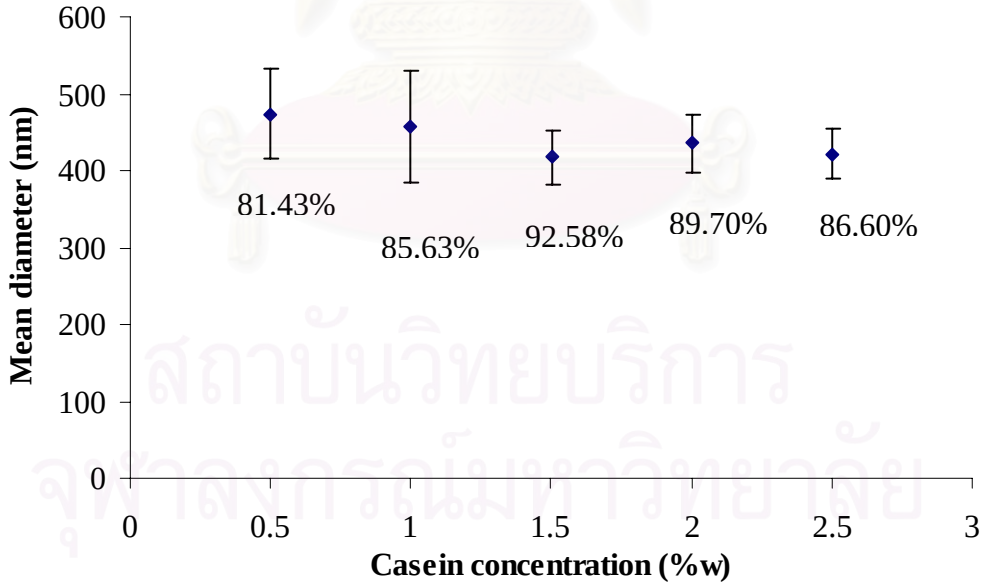


Figure 4.4 Effect of casein concentration on mean particle size and the intensity of primary emulsion which diluted 50 times at pH 6 of the first peak prepared under the condition of 5-homogenization cycle. Primary emulsion was measured after 24 hours of storage.

First, we have to state that for all of our studies, the particle size distribution of all types of emulsions were polydispersed, and had which bimodal distribution as shown in Figure 4.3 as an example. The first peak composed of smaller droplet size that exhibited the higher population, which was more than 90% for most cases except in the study on the effect of casein concentration on the preparation of primary emulsion section, where will be discussed below. The second peak contained larger droplet size. Unfortunately, because of the limit of instrumentation in our laboratory, we could not improve the size distribution of our emulsion. In order to obtain the good representative for our emulsion, the mean diameter of the first peak was, therefore, used to represent the droplet size in the prepared emulsions. It is because the high population of this first peak.

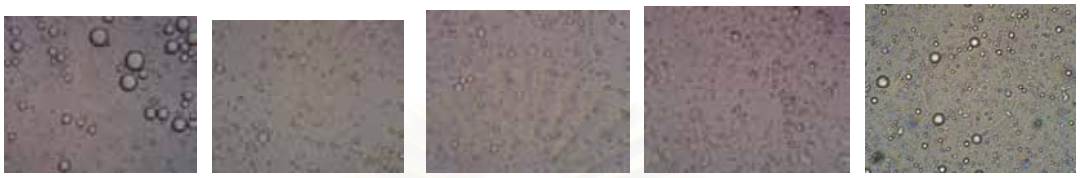
From Figures 4.3-4.5, our primary emulsion composed of the small droplets which the mean diameter was around 400 nm and larger droplet size with mean diameter of 3500 nm.

Primary emulsion contained 0.5 and 1 %w casein concentration exhibited the larger peak width and larger mean droplet sizes of the first peak compared to the emulsion prepared using higher %casein. The populations of the first peak for these concentration were lower than 90% because low casein concentration was insufficient to emulsify the oil droplets. Therefore, the droplets had a wider size range. The micrograph as shown in Figure 4.5 clearly shown that the average droplet size at low casein concentration (0.5-1 %w casein) were obviously larger than the others and the widest droplet size distribution was shown.

At the emulsion prepared with emulsifier solution containing 1.5 %w casein showed the narrowest peak width and the smallest mean droplet sizes, which one could see in the figure 4.5 (c).

However, while increasing the casein concentrations to 2-2.5 %w, the the mean droplet size distribution became wider. It may be the result of the excess of casein concentration in the emulsion causing the aggregate of casein molecule and droplets.which could clearly seen the bigger droplet size in figure 4.5 (e).

Interestingly, the mean droplet sizes of the first peak were independent of % casein in the emulsifier solution.



0.5% casein (a) 1% casein (b) 1.5% casein (c) 2% casein (d) 2.5% casein (e)

Figure 4.5 Micrographs of primary emulsion at pH 6 with different casein concentration obtained by optical microscopy prepared under the condition similarly to Figure 4.4.

From Figure 4.6, it showed that the charges on primary emulsions, which has casein as the emulsifier at pH6, was negative charges. The surface charges of the oil-in-water emulsions stabilized with casein were around -13 mV for all casein concentrations. Increasing protein concentration had no effect on the zeta potential of the emulsion droplets, which indicated that the droplet surfaces were saturated with casein even at the lowest casein concentration was used.

The zeta potential values in this study were lower than the reported value for good stable emulsion (30 mV) [12, 16]. This number indicated that the charges around oil droplets were small. In the other word, there were less electrostatic repulsion forces between oil droplets resulting in less stability emulsion. Due to the limit of instrument for preparation of emulsion, our work will be carried with this condition.

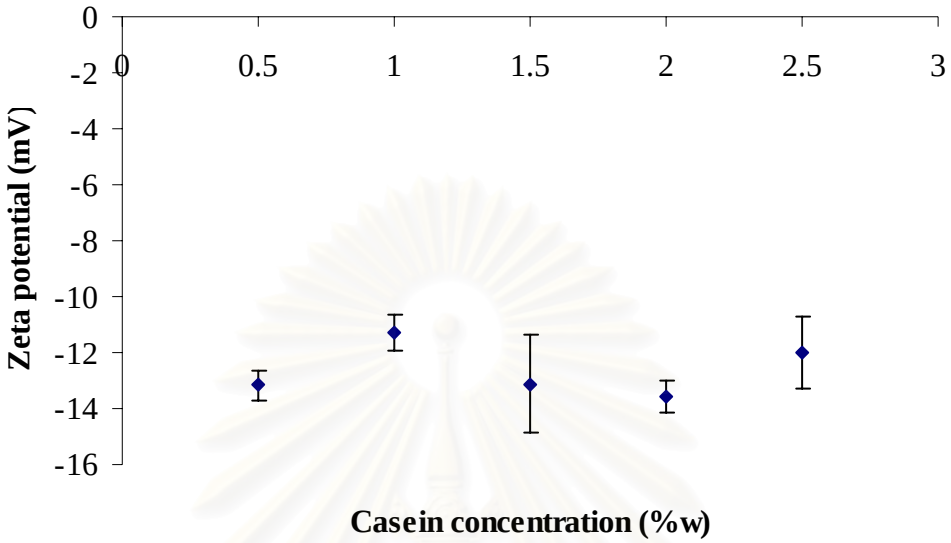


Figure 4.6 Effect of casein concentration on zeta potential of primary emulsion which diluted 50 times at pH 6 prepared under the condition of 5-homogenization cycle. Primary emulsion was measured after 24 hours of storage.

As mentioned above that protein concentration influence to the emulsion stability. Thus, the creaming stability of primary emulsion which varied casein concentration was also investigated to confirm the stability of primary emulsion. The %transmission of diluted emulsions was measured to indicate the creaming stability according to the previous studies [23, 33, 35]. The increase in %transmission exhibited the creaming instability which the serum layer had increased 33% of the emulsion's height.

Figure 4.7 showed the creaming stability of primary emulsion at different casein concentration at the first, third, fifth and seventh day, respectively. The changing of %transmission of the emulsions during the storage of the emulsions was shown. For every day, emulsions prepared with 0.5 %w casein concentration exhibited the least stable emulsion compared to the other concentrations. For emulsions prepared with higher % casein (1-2.5 %w casein) showed similar stability after the first day storage. However, the stability of emulsion prepared with 1 %w

casein clearly decrease; % transmission clearly increased with longer storage time compared to the higher concentration as we could see the forming of thicker layer on the top of emulsion solution. For emulsion prepared with 1.5-2.5 %w of casein concentration showed no significant differences in stability among these concentrations. Furthermore, the stability to aggregating when standing with time for 1.5-2.5 %w casein concentration was not significantly different. The characteristic of cream was shown in Figure 4.8 which the cream layer was more opaque.

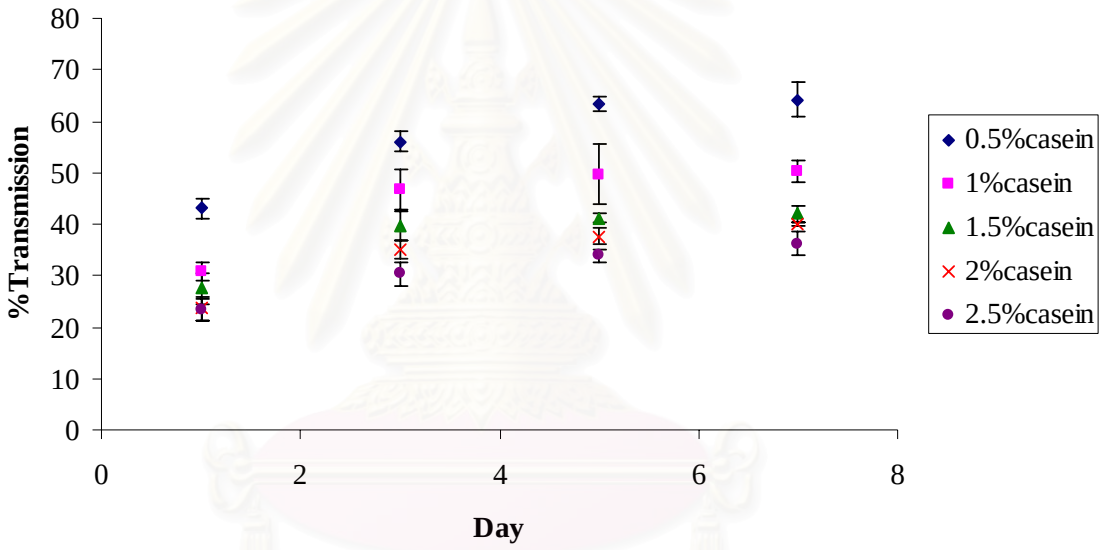


Figure 4.7 The aggregation stability of primary emulsion at pH 6 which varied casein concentration prepared under the condition similarly to Figure 4.6. The emulsions were diluted 200 times before storage.

Figure 4.8 The cream layer at the top of the bottle.

Based on what we have shown, casein concentration of 1.5 %w was the best for preparation of primary emulsions because this primary emulsion exhibited good physical properties and stability as compared to the others.

Hence, in our study, the primary emulsion was prepared by homogenizing 5 %w oil and 95 %w of pH 6 emulsifier solution containing 1.5 %w casein at 20,000 rpm for 2 minutes/cycle for 5 homogenization cycles.

4.2 Preparation of multilayer emulsions

Multilayer emulsion can be prepared by mixing emulsion coated with charged emulsifier and oppositely charged polyelectrolyte together. The main challenge in the preparation of multilayer emulsions is to find the suitable ratio between the volume of emulsion and opposite-charged polyelectrolyte to obtain stable multilayer emulsion. For secondary, tertiary and quaternary emulsion at pH 6, polycationic and polyanionic polyelectrolytes were successively coated onto the emulsion droplet as shown in Figure 4.9. In the research, PDADMAC and chitosan

were used as polycationic polyelectrolytes and alginate was used as polyanionic polyelectrolyte.

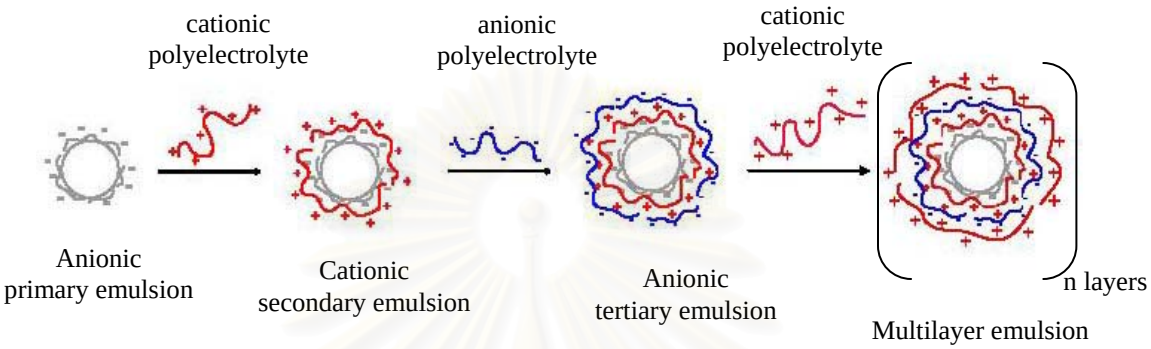


Figure 4.9 Schematic of multilayer emulsion preparation

4.2.1 Investigate the optimum volume ratio between primary emulsions and cationic polyelectrolyte for secondary emulsion preparation

Secondary emulsions can be prepared by mixing primary emulsion with oppositely charged polyelectrolyte. First, the optimum volume ratio between emulsion and oppositely charged polyelectrolyte need to be investigated. The optimum volume ratio is the equivalent point between emulsion and polyelectrolyte. At this point, there is no any excess of polyelectrolyte to cause the depletion flocculation in solution, resulting in the instability of emulsion [21]. For this research, primary emulsion contained 1.5 %w casein at pH 6, which showed anionic charged droplet, was used as primary emulsion for multilayer emulsion preparation. Thus, cationic polyelectrolyte had to be used for multilayer preparation.

Secondary emulsions preparation with PDADMAC

PDADMAC is a strong cationic polyelectrolyte which is pH independent. In Figure 4.10 showed the titration curve of primary emulsion and 10 mM PDADMAC solutions.

We could see that graph was separated into 3 sections. At the first part (volume ratio of 0.050-0.30), solution became more opaque where % transmission decreased gradually with increasing the volume of emulsion. At the volume ratio to 0.20 to 0.30, the lowest %transmission was observed. After this section we observed a precipitation in the solution which resulted in the increase of %transmission (volume ratio of 0.35-0.40). For the last section, the precipitation of complex in a solution was observed resulting in the clear solution (volume ratio of 0.45-0.60). After this point the %transmission was found to decrease again.

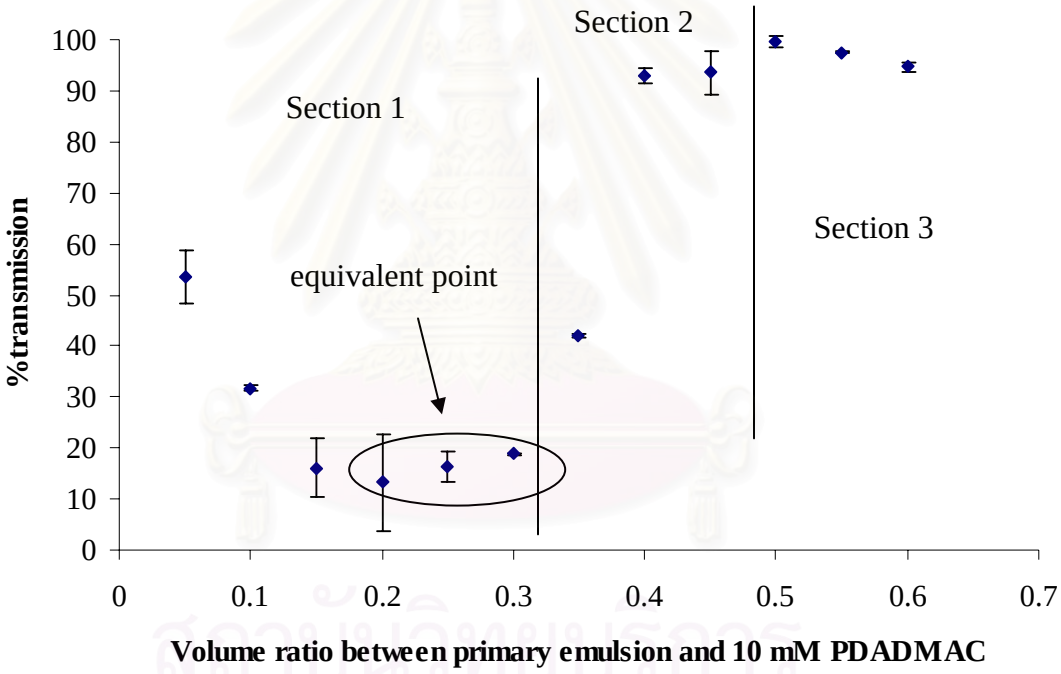


Figure 4.10 The titration curve between primary emulsion and 10 mM PDADMAC at pH 6 which varied the volume ratio between primary emulsion and PDADMAC on %transmission.

Figure 4.11 The characteristic of the mixing solution of secondary emulsion preparation which varied the volume ratio between primary emulsion and PDADMAC solution.

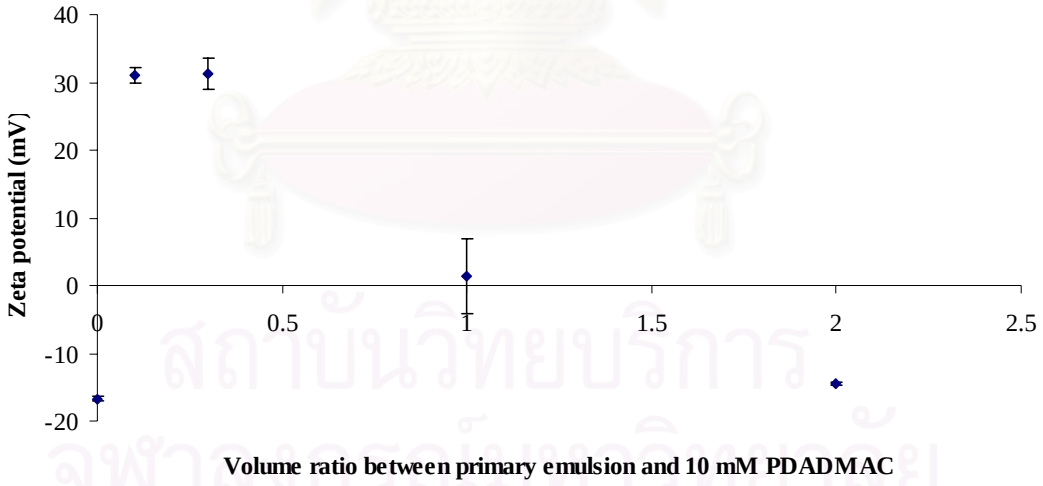


Figure 4.12 Effect of ratio between volume of primary emulsion and 10 mM PDADMAC at pH 6 on zeta potential. The mixture solution was measured after 24 hours of storage then diluted 50 times for the measurement.

To understand the formation mechanism of secondary emulsion coated with casein-PDADMAC, zeta potential is the best of the evidences. Zeta potential showed the charge of emulsion droplet. Figure 4.12 showed the zeta potential of emulsion droplet as the function of the ratio between volume of primary emulsion and 10 mM PDADMAC. We chose to work at the volume of 0.10 (section I), 0.3 (the lowest % transmission), 1.0(section III) and 2.0 (after section III). It found that zeta potential was changed from negative, primary emulsion coated with casein at pH 6, to positive values at the volume of 0.10 and 0.30. These changes in zeta potential suggested the cationic PDADMAC molecules adsorbed to the surfaces of anionic casein-coated emulsion droplets. Thus, secondary emulsion occurred at volume ratio of 0.10 and 0.30 and had the similar surface charges between these two ratios.

At volume ratio of 1.0, we observed very small zeta potential of the droplets in the solution implied that there were very small charges on the droplet. This might be the result of the formation of complex between anionic charged droplet of primary emulsion and cationic charged droplet of secondary emulsion. This observation was consistent with the physical appearance of emulsion, which we observed a precipitate out of the complex and left the clear solution.

At the volume ratio of 2.0, zeta potential was changed to negative values again, which suggested, that excess of primary emulsion present in the solution.

From zeta potential data, we can confirm the mechanism of the formation of secondary emulsion when the volume ratio between primary emulsion and PDADMAC was varied.

We can suggest the mechanism for secondary emulsions preparation as displayed in Figure 4.13, which related to Figure 4.10 at the ratio before, near and after the precipitation.

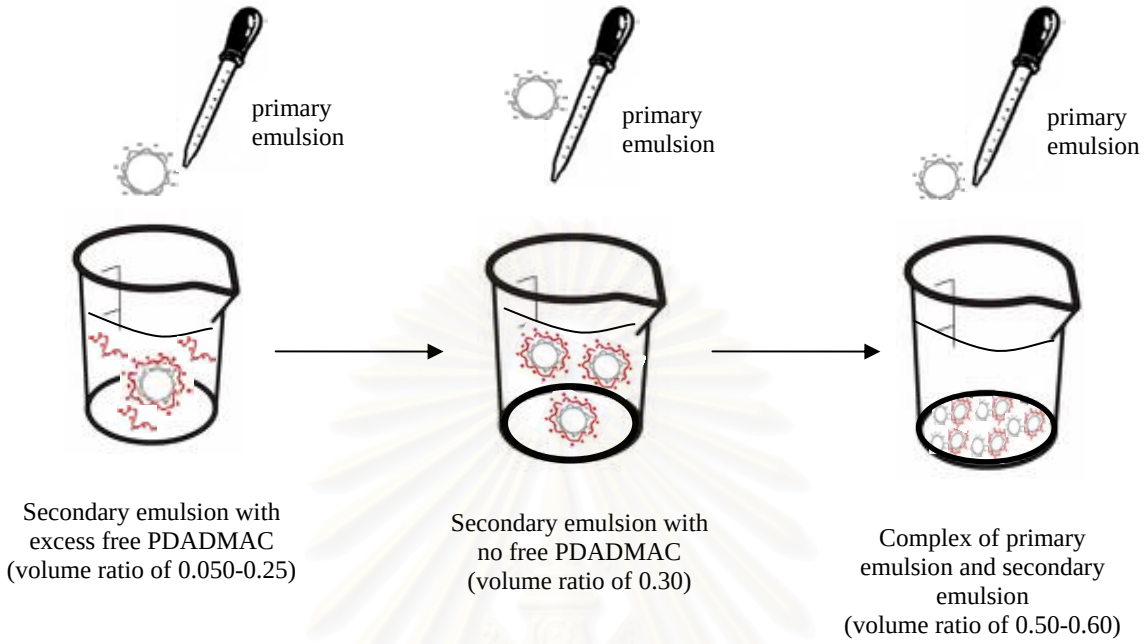


Figure 4.13 Mechanism for preparation of secondary emulsion.

Therefore, the lowest % transmission is the equivalent point between primary emulsion and PDADMAC. Although, the observed equivalent point were in the range volume ratio of 0.20-0.30 we chose to work on the volume of 0.30 because at this volume ratio should has smaller amount of PDADMAC excess in the solution. The excess of PDADMAC in the solution can be the problem for further preparation because it can form complex with anionic polyelectrolyte.

Thus, secondary emulsions coated with 10 mM PDADMAC can be prepared by mixing the 6 mL primary emulsion and 20 mL of 10 mM PDADMAC (volume ratio 0.30). To be able to work with different concentration, the mass ratio between mass of emulsion and mass of polyelectrolyte, PDADMAC, of this point is 2.8.

In Figure 4.14, the particle size distribution of the secondary emulsion prepared using primary emulsion and 10 mM PDADMAC as the function of volume ratio was shown. As we have shown above, the secondary emulsion can be prepared by mixing the primary emulsion and 10 mM PDADMAC at the volume ratio of 0.10 and 0.30. At volume ratio of 0.30, we observed the best particle size distribution of

secondary emulsion. Prepared secondary emulsion using volume ratio 1:10 gave larger size distribution at the first peak than volume ratio of 0.30, which may be due to the result of the depletion flocculation was appeared from the excess of PDADMAC for secondary emulsion at volume ratio 0.10 as has been reported in the research of Aoki [23]. The depletion flocculation occurs when the free polyelectrolyte in the continuous phase generates an attractive osmotic force that is strong enough to overcome the various repulsive forces. The origin of this osmotic force is the exclusion of polyelectrolyte molecules from a narrow region surrounding the droplet surfaces [19].

For the volume ratio of 1.0 and 2.0, the first peak of particle size distribution was the same range as primary emulsion profile which showed the existence of primary emulsion in these volume ratios. Moreover, at the volume ratio of 1.0, the wider peak width of the second peak supported that there was the aggregation of the complex between anionic primary emulsion droplets and cationic secondary emulsion droplets.

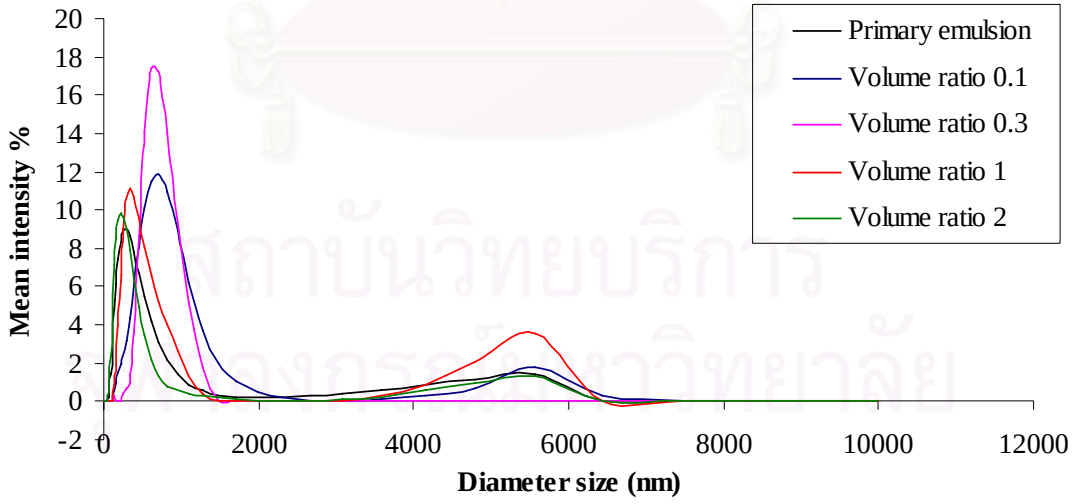


Figure 4.14 Effect of ratio between volume of primary emulsion and 10 mM PDADMAC at pH 6 on particle size distribution. The mixture solution was measured after 24 hours of storage then diluted 50 times for the measurement.

Figure 4.15 showed the particle size at the first peak of emulsion droplets which varied the ratio between volume of primary emulsion and 10 mM PDADMAC. The droplet population of the first peak of solution at volume ratio of 0.10, 0.30, 1.0 and 2.0 were 94.93, 95.03, 92.17 and 91.25%, respectively. The particle sizes of emulsion prepared using ratio of 0.10 and 0.30 were around 503.4 and 565.7 nm which was larger than primary emulsion (diameter around 422.4 nm). At volume ratio of 1.0 and 2.0, the particle sizes were around 412 and 376 nm, respectively, which was smaller than that of secondary emulsion but closer to the primary emulsion.

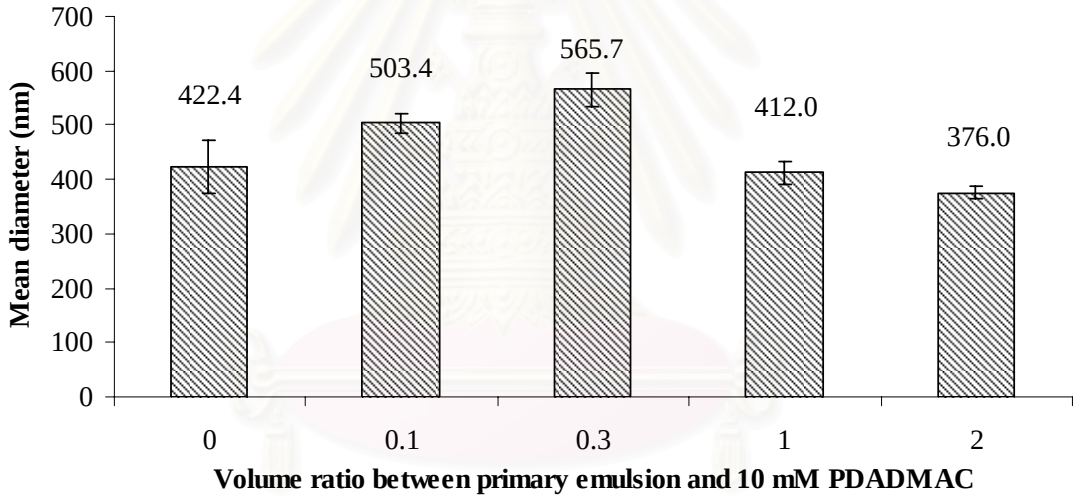


Figure 4.15 Effect of ratio between volume of primary emulsion and 10 mM PDADMAC at pH 6 on particle size of the first peak of size distribution curve shown in Figure 4.14. The mixture solution was measured after 24 hours of storage then diluted 50 times for the measurement.

The titration between primary emulsion and PDADMAC solutions with varied PDADMAC concentration was also studied as shown in Figure 4.16. All PDADMAC concentration (1, 5 and 10 mM) gave the same secondary preparation

profile as mentioned above. We would like to note that the data of 30 mM were not shown here. When PDADMAC concentration was increased, the volume of added primary emulsion to reach the equivalent point for secondary emulsion formation increased also. The volume ratio at equivalent point of each concentration was approximately similar. The linear relationship between PDADMAC concentration and the volume of emulsion at equivalent point was observed with R^2 of 0.997 (Figure 4.17). The mass ratios at equivalent point for 1, 5, 10 and 30 mM PDADMAC were 3.7, 3.3, 3.3 and 3.1, respectively. Therefore, the average mass ratio of secondary emulsion coated with casein-PDADMAC preparation was 3.2.

The obtained secondary emulsion coated with casein-PDADMAC at pH 6 containing 2.5 %w tuna oil, 0.75 %w casein and 15 mM PDADMAC was produced from the mixing of primary emulsion contained 5 %w tuna oil, 1.5 %w casein with 30 mM PDADMAC at volume ratio of 1.0 for tertiary emulsion preparation in the later study.

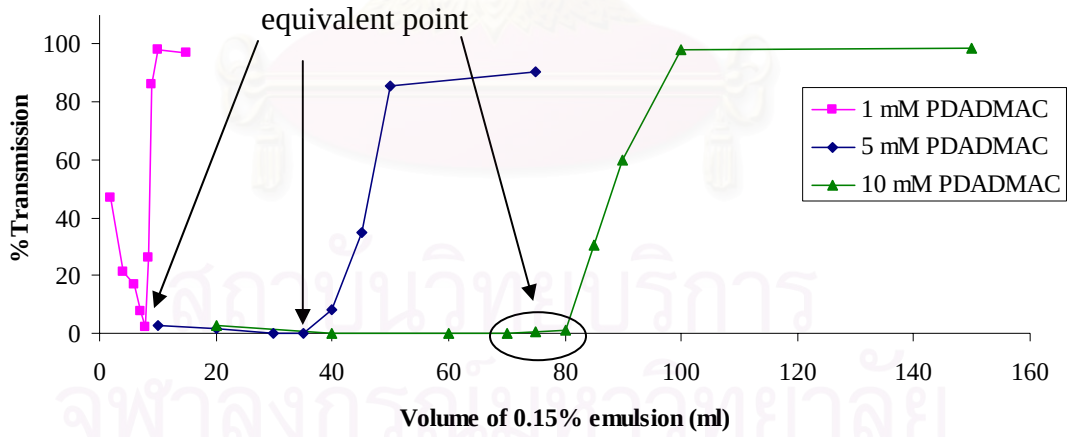


Figure 4.16 The titration curve between primary emulsion and PDADMAC solution: 1 mM, 5 mM and 10 mM (data of 30 mM are not shown here).

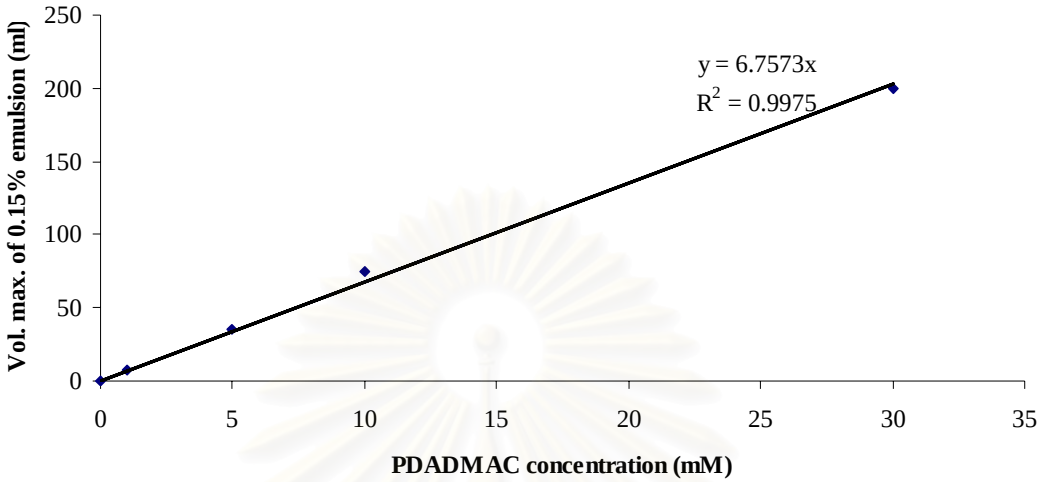


Figure 4.17 The relation between PDADMAC concentrations with volume at the equivalent point of the emulsion.

Secondary emulsions preparation with chitosan

Chitosan is a biopolymer composed of D-glucosamine (deacetylated unit) and N-acetyl-D-glucosamine (acetylated unit) which the number of deacetylated unit is more than acetylated unit. The amino group of chitosan has a pK_a value of ~6.5, thus, chitosan is positively charged at low pH and soluble in acidic solution with a charge density dependent on pH. Thus, the effect of pH on the formation of secondary emulsion with 0.1 %w/v chitosan was tested. In Figure 4.18, the titration curves between primary emulsion and 0.1 %w/v chitosan which varied pH on %transmission were shown. Similar curve shapes were observed.

The mass ratios between primary emulsion and chitosan were 4.1, 3.2 and 2.6 for the chitosan pH 5.5, 6.0 and 6.5, respectively. At pH 5.5, the volume ratio between primary emulsion and 0.1 %w/v chitosan to reach the equivalent point was the maximum value because chitosan at this pH had the most of charge density as compared to the other. While at pH 6.5, the volume ratio between primary emulsion and 0.1 %w/v chitosan was lowest. Because chitosan at high pH exhibited low charge density as compared to low pH so, the volume of primary emulsion used at

high pH was smaller than low pH. Moreover, the instability of secondary emulsion at pH 6.5 was shown that the precipitation of chitosan was observed due to the low charge density of chitosan at pH 6.5. However, primary emulsion at pH 5.5 was less stable than at pH 6 which the higher cream layer and the precipitation of casein of primary emulsion pH 5.5 were observed. Thus, the primary emulsion at pH 5.5 was not suitable for secondary emulsion preparation. Thus, pH 6 of chitosan was used to prepare the secondary emulsion.

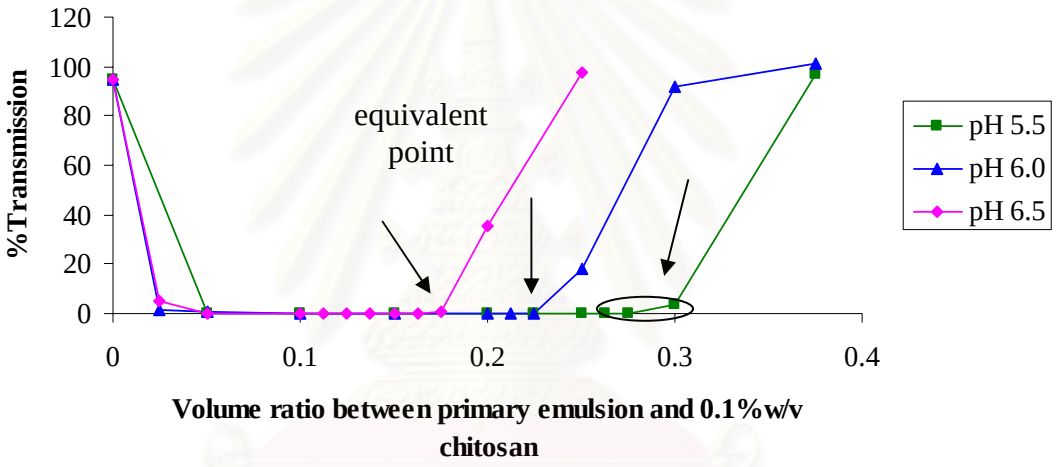


Figure 4.18 The titration curve between primary emulsion and 0.1 %w/v chitosan as a function of pH.

Secondary emulsions prepared with 0.1 %w/v chitosan gave the same profile as PDADMAC. In Figure 4.19, the relationship between %transmission and the volume ratio of primary emulsion to 0.1 %w/v chitosan at pH 6 was shown. Based on our observation in the titration between primary emulsion and chitosan solution, we can conclude that the mixing volume ratios of 0.20 appears to be the equivalent point of secondary emulsion preparation with using 1.5 %w casein as the primary emulsion. This equivalent point was less than the equivalent point in Figure 4.18 (0.23) because the studied range of the volume ratio in this experiment was wider

than the experiment in Figure 4.18. However, the equivalent point of both experiments exhibited nearby value of volume ratio. In this profile, the decreasing of %transmission was appeared after all secondary emulsions were precipitated because there was the excess primary emulsion in the solution.

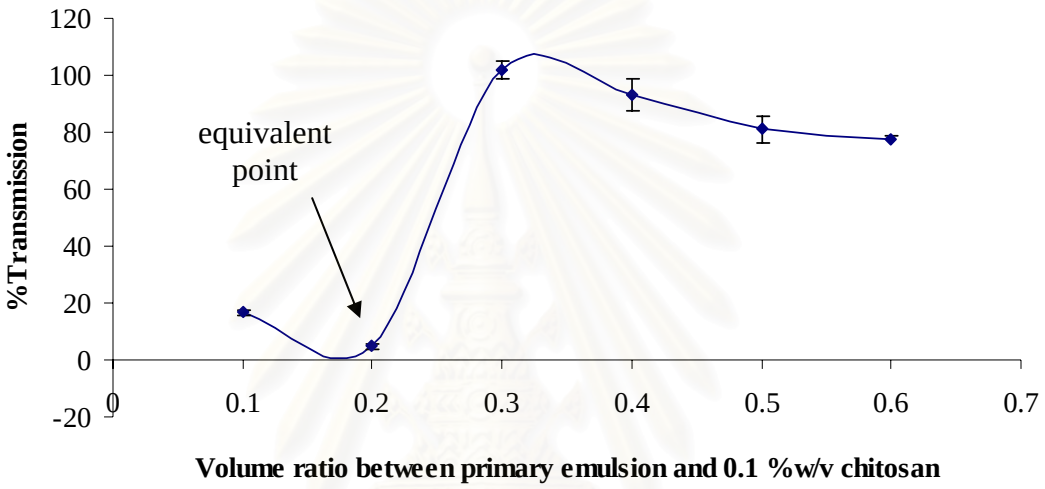


Figure 4.19 The titration curve between primary emulsion and 0.1 %w/v at pH 6 which varied the volume ratio between primary emulsion and chitosan on %transmission.

Zeta potential of particle or emulsion droplets of secondary emulsion prepare with 0.1 %w/v chitosan was measured as shown in Figure 4.20 As we have demonstrated that the primary emulsion coated with casein at pH 6 posses negative charge, which the zeta potential was around -13 mV. At volume ratio of 0.10 and 0.20, zeta potential values were changed to around +18 mV which exhibited the coating of chitosan onto the anionic emulsion droplets. Thus, secondary emulsion completely occurred around volume ratio of 0.10 and 0.20. For volume ratio of 0.50, zeta potential value changed to small negative charges. This change in zeta potential suggests the cationic chitosan molecules could be adsorbed to the surfaces of anionic casein-coated emulsion droplets at pH 6. The small zeta potential exhibited less

electrostatic repulsion of the droplets. Therefore, the complex between anionic charged droplet of primary emulsion and cationic charged droplet of secondary emulsion could be formed.

From the titration curve and zeta potential data, the volume ratio between primary emulsion and 0.1 %w/v chitosan is 0.20, which the mass ratio of emulsion to chitosan was 3.0, was suitable for secondary emulsion coated with casein-chitosan preparation using 0.1 %w/v chitosan.

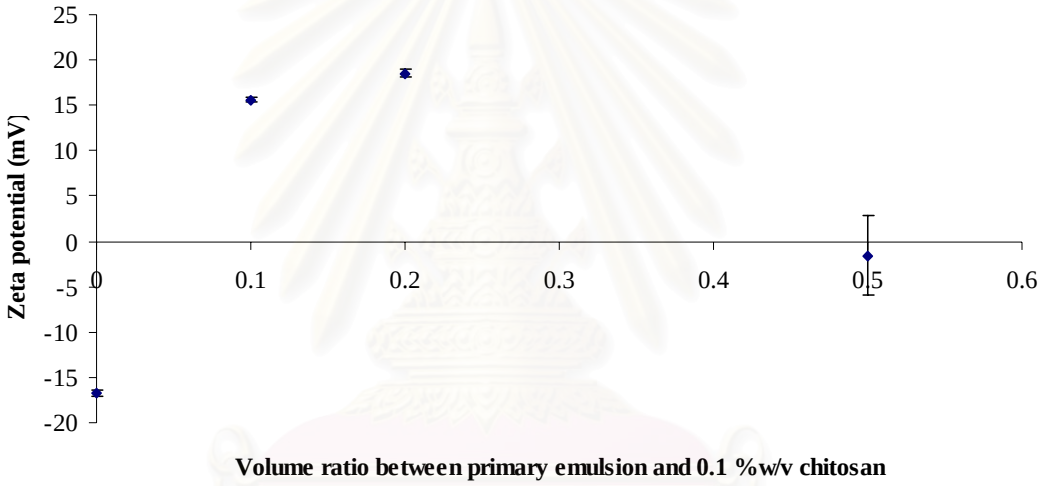


Figure 4.20 Effect of volume ratio between primary emulsion and 0.1 %w/v chitosan at pH 6 on zeta potential. The mixture solution was measured after 24 hours of storage then diluted 50 times for the measurement.

The particle size distribution of secondary emulsion preparation with 0.1 %w/v chitosan was shown in Figure 4.21. Secondary emulsion prepared with the volume ratio between primary emulsion and 0.1 %w/v chitosan of 0.10 and 0.20 have shown the similar size distribution pattern with very close mean size diameter range. While at volume ratio of 0.50, the particle size and its distribution were similar to the distribution profile of primary emulsion. Therefore, it confirmed the existence of primary emulsion in the solution under this condition.

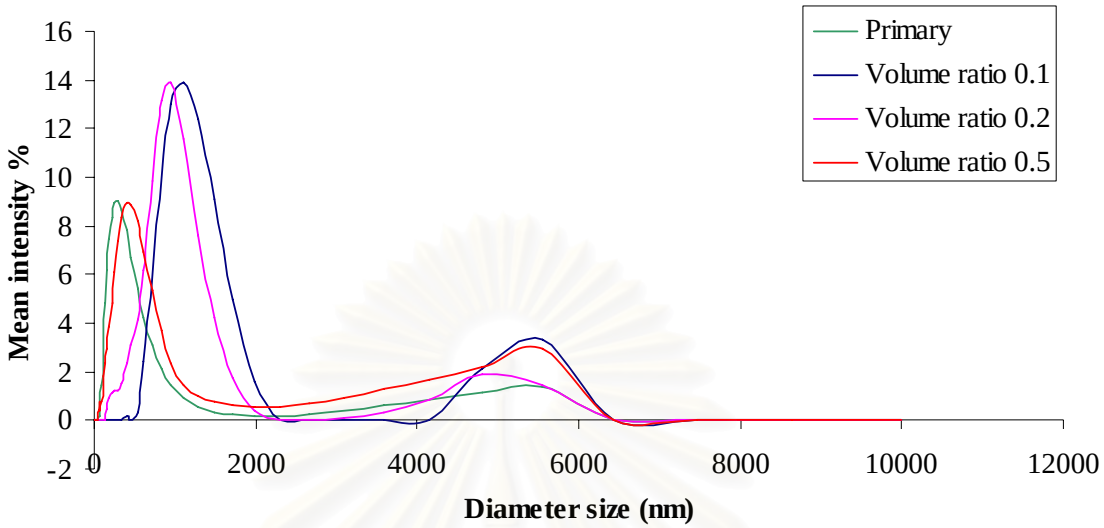


Figure 4.21 Effect of ratio between volume of primary emulsion and 0.1 %w/v chitosan at pH 6 on particle size distribution. The mixture solution was measured after 24 hours of storage then was diluted 50 times for the measurement.

The particle size of emulsion droplets of the first peak at the volume ratio of 0.10, 0.20 and 0.50 are shown in Figure 4.22. It was found that particle sizes of secondary emulsion at ratio of 0.10 and 0.20 were larger than primary emulsion (diameter around 422nm). At volume ratio of 0.50, mean particle size of the first peak was smaller than the volume ratio of 0.20, secondary emulsion, but its size was close to the size of primary emulsion, which confirmed the existence of primary emulsion in this ratio.

Thus, the volume ratio between primary emulsion and 0.1 %w/v chitosan equals to 0.20 suitable for secondary emulsion preparation. The mass ratio of this equivalent point was 3.0.

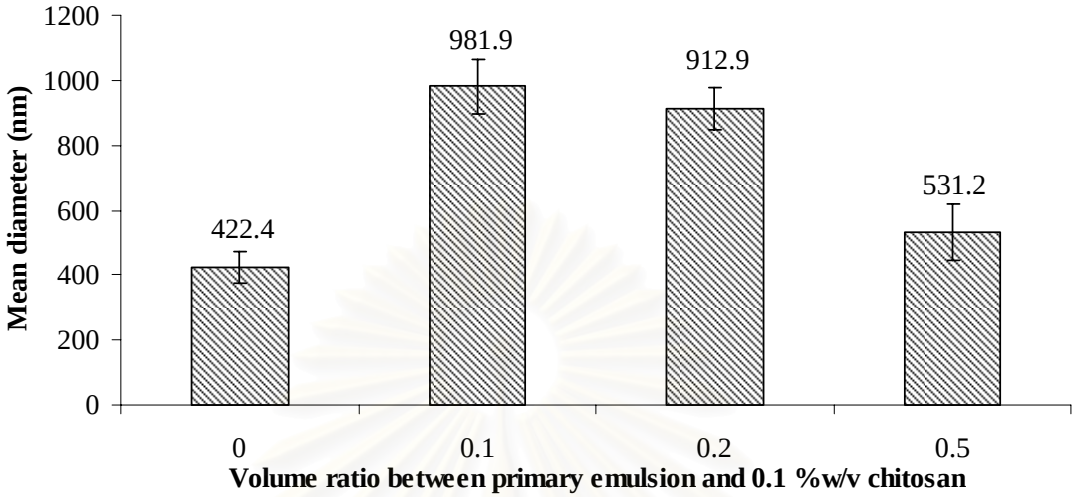


Figure 4.22 Effect of volume ratio between primary emulsion and 0.1 %w/v chitosan at pH 6 on particle size of the fist peak. The mixture solution was measured after 24 hours of storage then was diluted 50 times for the measurement.

The secondary emulsion coated with casein-chitosan at pH 6 containing 0.83 %w tuna oil, 0.25 %w casein and 0.083 %w/v chitosan was produced from the mixing of primary emulsion contained 5 %w tuna oil and 1.5 %w casein with 0.1 %w/v chitosan at volume ratio 0.20 for tertiary emulsion preparation in the later study.

Interestingly, the mean particle size of secondary emulsion prepared by PDADMAC was smaller than the one prepared by chitosan. This observation might be the result of the charge density of chitosan. Because chitosan has less charge density compared to PDADMAC per molecule, it should require more molecules of chitosan to coat around casein coating around the oil droplets compared to PDADMAC resulted in the bigger droplet size.

4.2.2 Investigate the optimum volume ratio between secondary emulsion and anionic polyelectrolyte for tertiary emulsion preparation

Tertiary emulsion was prepared by mixing secondary emulsion with oppositely charge polyelectrolyte as same as secondary emulsion preparation. In this research secondary emulsions were coated by either PDADMAC or chitosan, so anionic polyelectrolytes had to be used in this experiment. Alginate, an anionic polyelectrolyte at pH 6, was selected for tertiary emulsion preparation. The volume ratio between secondary emulsion and this polyelectrolyte was investigated. In Figure 4.23 showed the titration curve between secondary emulsion coated with PDADMAC and 1 %w/v alginate on %transmission, which showed the similar profile as secondary emulsion coated with PDADMAC preparation. As reported earlier, the equivalent point was the volume ratio which showed the lowest %transmission. We found that at volume ratio of 1.0, which the mass ratio between secondary emulsion and alginate was 0.24, was the lowest point. Therefore it indicated the equivalent point for the titration between secondary emulsion and alginate solution. While the volume ratio was larger than 1.0, increasing in %transmission was shown, where we observed the precipitation of anionic alginate and cationic secondary emulsion droplets coated with casein-PDADMAC membrane.

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

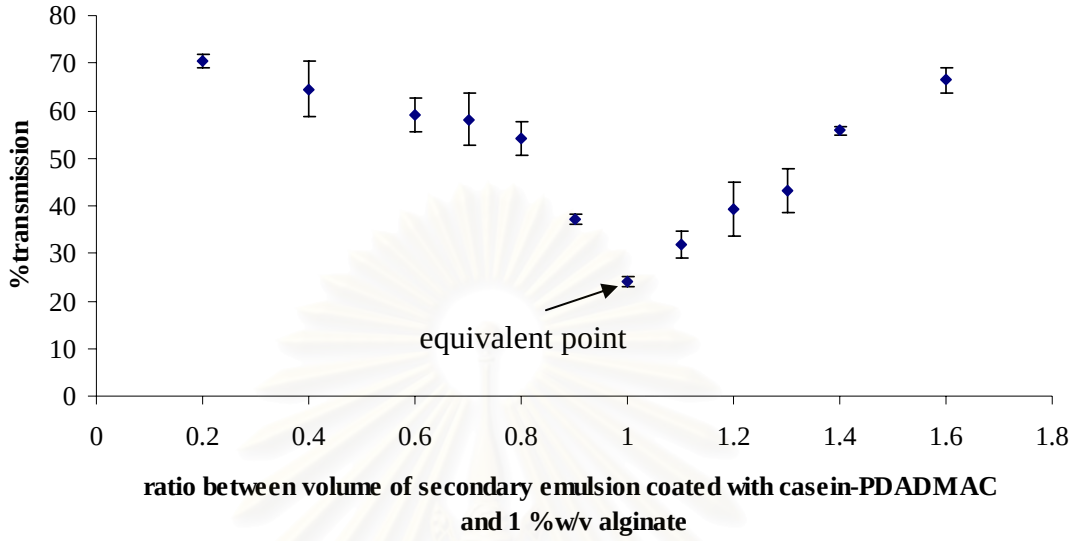


Figure 4.23 The titration curve between secondary emulsion coated with casein-PDADMAC and 1 %w/v alginate at pH 6.

The tertiary emulsion containing casein-chitosan-alginate membrane was produced at pH 6. The titration curve between secondary emulsions coated with casein-chitosan and 0.1 %w/v alginate on %transmission for tertiary emulsion preparation was shown in Figure 4.24. Similar result as secondary emulsion coated with PDADMAC preparation was found. As mentioned above, the volume ratio of 0.40, which the mass ratio between secondary emulsion and alginate was 0.33, exhibited as the equivalent point for tertiary emulsion contained casein-chitosan-alginate membrane. When the volume ratio passed this point, the precipitation of anionic alginate and cationic secondary emulsion droplets was observed.

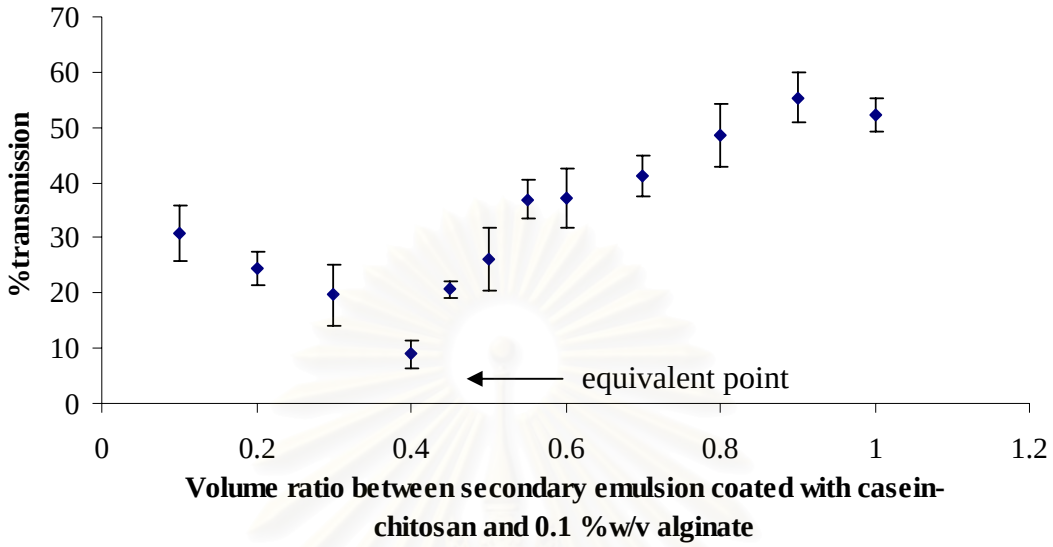


Figure 4.24 The titration curve between secondary emulsion coated with casein-chitosan and 0.1 %w/v alginate at pH 6.

The particle size distribution, particle size diameter and zeta potential of these tertiary emulsions were also studied. The particle size distribution of each tertiary emulsion was shown in Figure 4.25 and 4.26. The mean diameter of the first peak and zeta potential of each tertiary emulsion were exhibited in Table 4.1.

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

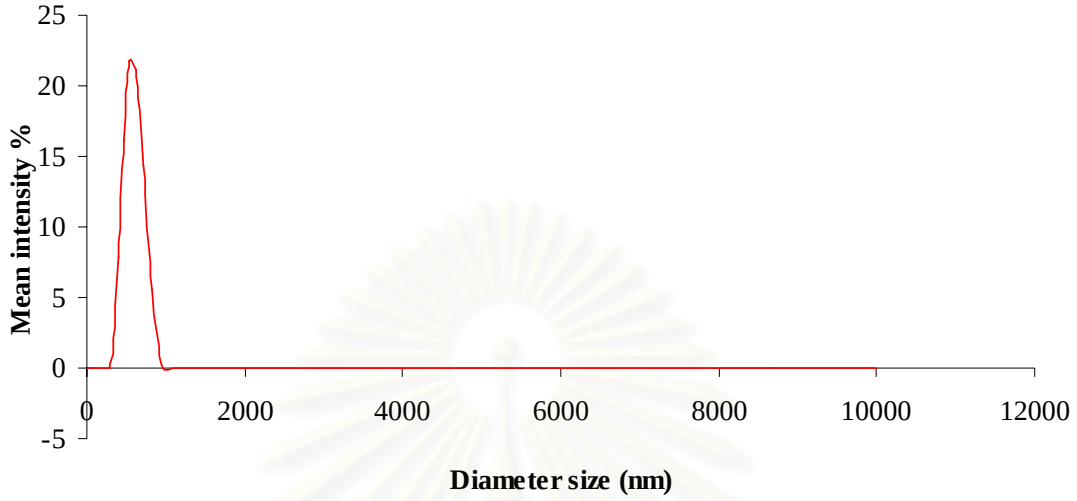


Figure 4.25 The particle size distribution of tertiary emulsion coated with casein-PDADMAC-alginate at pH 6. The mixture solution was measured after 24 hours of storage then diluted 50 times for the measurement.

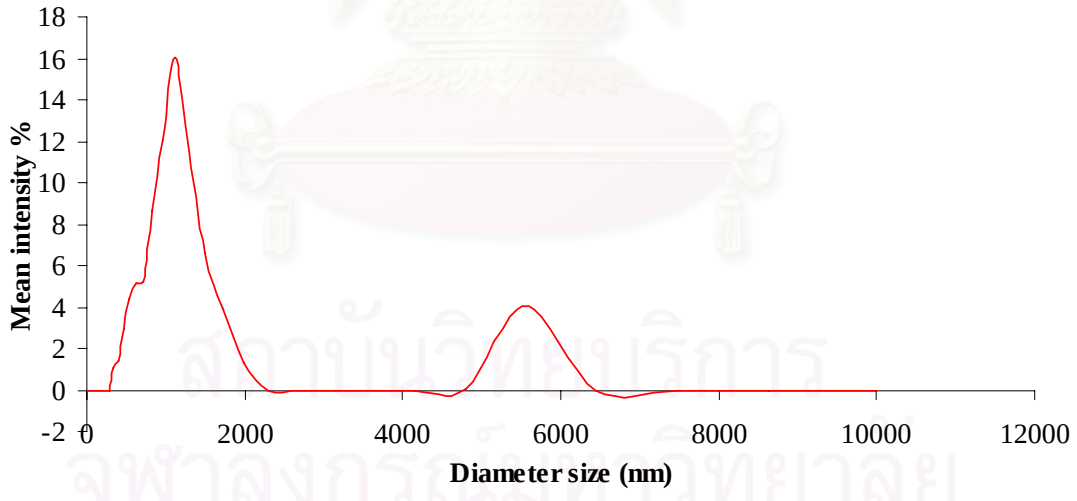


Figure 4.26 The particle size distribution of tertiary emulsion coated with casein-chitosan-alginate at pH 6. The mixture solution was measured after 24 hours of storage then diluted 50 times for the measurement.

Table 4.1 The particle size mean diameter and the intensity at the first peak and zeta potential of each tertiary emulsion at pH 6. The mixture solution was measured after 24 hours of storage then diluted 50 times for the measurement.

Emulsion	Concentration of cationic polyelectrolyte	Volume ratio (mass ratio)	Mean diameter* (nm)	Intensity (%)	Zeta potential (mV)
Tertiary emulsion coated with PDADMAC-Alginate	1.0%w/v	1.0 (0.24)	589.5 (±74.1)	98.7 (±1.1)	-24.2 (±1.3)
Tertiary emulsion coated with Chitosan-Alginate	0.1%w/v	0.40 (0.33)	1092.7 (±65.7)	91.0 (±2.7)	-31.6 (±1.4)

* data calculated from the first peak

The particle sizes of the tertiary emulsion coated with casein-PDADMAC-alginate were nearly the same as the sizes of secondary emulsions coated with casein-PDADMAC (diameter around 565 nm). The characteristic of particle size which did not increase when increasing the number of coating layer was observed in the previous study [34]. It has been reported that the thickness of this multilayer coating is in the nanometer range. Therefore, we could not see any change in the mean diameter when number of coating layer increased.

The particle size distribution of tertiary emulsion coated with casein-PDADMAC-alginate exhibited the narrower size distribution as compared to tertiary emulsion coated with casein-chitosan-alginate. Furthermore, the mean diameter of the first peak was smaller diameter size than the tertiary emulsion containing casein-chitosan-alginate.

However, the tertiary emulsion containing casein-chitosan-alginate membrane had larger particle size than secondary emulsion coated with casein-chitosan (diameter around 912 nm).

The zeta potential of this tertiary emulsion was negative, which exhibited the coating of alginate polyelectrolyte onto the cationic secondary emulsions.

The tertiary emulsion coated with casein-PDADMAC-alginate at pH 6 containing 1.25 %w tuna oil, 0.38 %w casein, 7.5 mM PDADMAC and 0.5 %w/v alginate was prepared from the mixing of secondary emulsion contained 0.75 %w casein and 15 mM PDADMAC with 1 %w/v alginate at volume ratio of 1. For the tertiary emulsion coated with casein-chitosan-alginate containing 0.24 %w tuna oil, 0.071 %w casein 0.024 %w/v chitosan and 0.071 %w/v alginate was prepared from the mixing of secondary emulsion contained 0.25 %w casein with 0.083 %w/v chitosan with 0.1 %w/v alginate at volume ratio of 0.40 for quaternary emulsion preparation in the later study.

4.2.3 Investigate the optimum volume ratio between tertiary emulsion and cationic polyelectrolyte for quaternary emulsion preparation

Similarly, quaternary emulsion can be prepared by mixing tertiary emulsion which exhibited anionic charged droplets with cationic polyelctrolyte. The tertiary emulsion containing casein-chitosan-alginate could not be used to prepare quaternary emulsion as we observed the precipitating complex between tertiary emulsions and chitosan. This might be the result of the complex size, which was too large to stay in the emulsion form. On the other hand, the quaternary emulsion coated casein-PDADMAC-alginate-PDADMAC membrane could be prepared.

In Figure 4.27, the titration curve between tertiary emulsion coated with casein-PDADMAC-alginate and 40 mM PDADMAC on %transmission was shown. The volume ratio of 1.0 showed the lowest %transmission indicating the equivalent point of the titration between tertiary emulsion and PDADMAC solution. The mass

ratio between tertiary emulsion and PDADMAC at this equivalent point was 0.77. After this point, the precipitation of complex between cationic PDADMAC and anionic tertiary emulsion was observed.

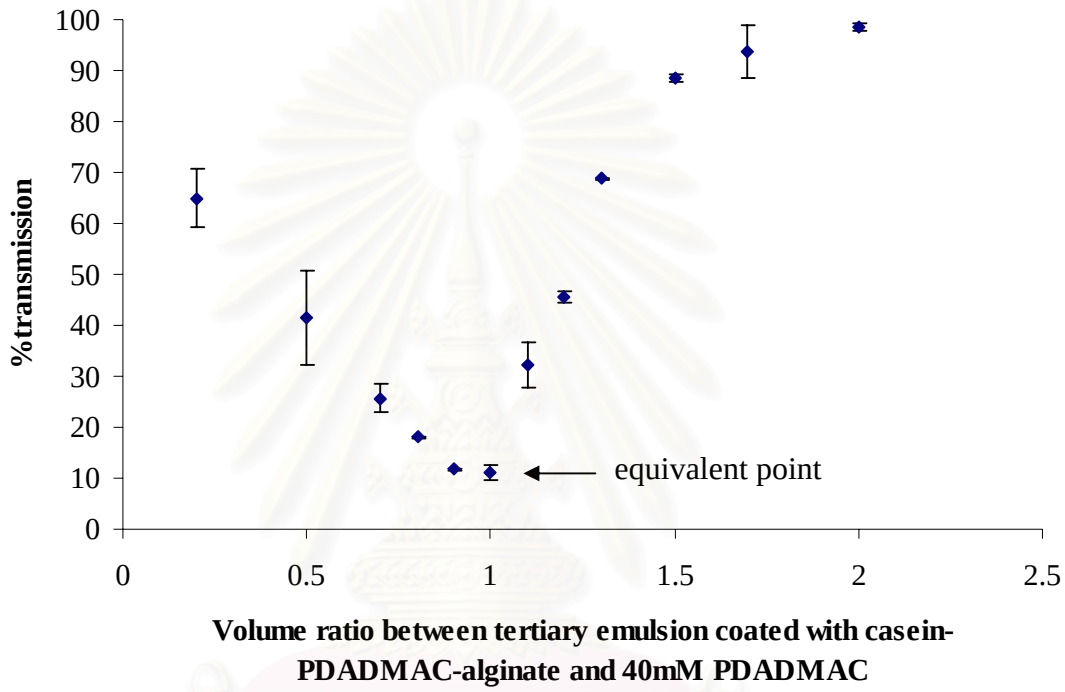


Figure 4.27 The titration curve between tertiary emulsion coated with casein-PDADMAC-alginate and 40 mM PDADMAC at pH 6.

The particle size distribution curve of quaternary emulsion coated with casein-PDADMAC-alginate-PDADMAC was shown in Figure 4.28. The particle size diameter and zeta potential of this quaternary emulsion was shown in Table 4.2. The mean diameter of quaternary emulsion coated with casein-PDADMAC-alginate-PDADMAC of the first peak, which contained 90% of droplet population, was very close to the mean diameter sizes of the tertiary emulsion containing casein-PDADMAC-alginate. The zeta potential of the quaternary emulsion was positive

(around 30 mV), which exhibited the coating of PDADMAC polyelectrolyte onto the anionic tertiary emulsion coated with casein-PDADMAC-alginate membrane.

The obtained quaternary emulsion coated with casein-PDADMAC-alginate-PDADMAC membrane at pH 6 containing 0.63 %w tuna oil, 0.19 %w casein and 20 mM PDADMAC was prepared from the mixing of tertiary emulsion coated with casein-PDADMAC-alginate contained 1.25 %w tuna oil, 0.38 %w casein, 7.5 mM PDADMAC and 0.5 %w/v alginate at volume ratio of 1 for measuring creaming and oxidative stability in the later study.

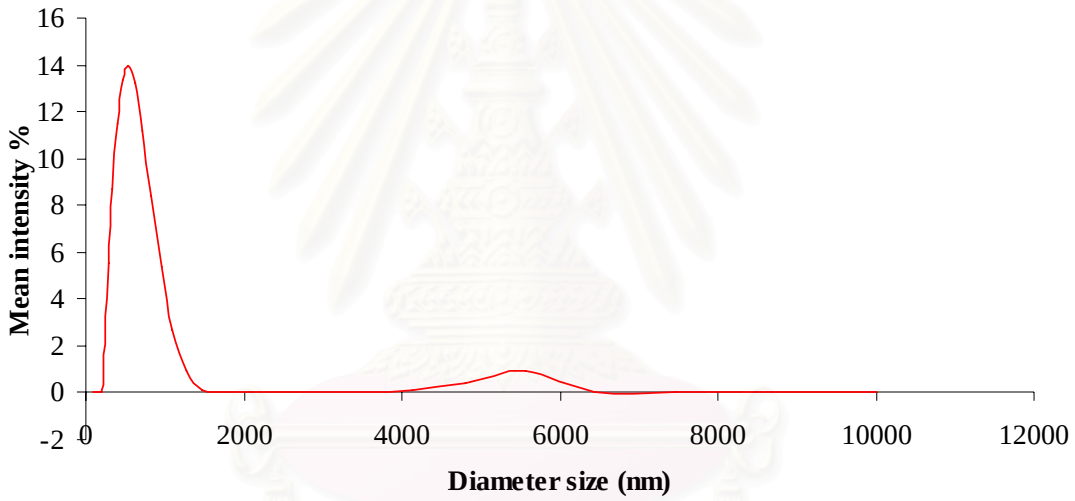


Figure 4.28 The particle size distribution of quaternary emulsion coated with casein-PDADMAC-alginate-PDADMAC at pH 6. The mixture solution was measured after 24 hours of storage then diluted 50 times for the measurement.

Table 4.2 The summary of particle size mean diameter and the intensity of the first peak and zeta potential of primary, secondary, tertiary and quaternary emulsion. The mixture solution was measured after 24 hours of storage then diluted 50 times for the measurement.

Emulsion	Concentration of polyelectrolyte	Volume ratio	Mean diameter (nm)	Intensity (%)	Zeta potential (mV)
Primary emulsion	-	-	422.4 (±48.8)	92.6 (±1.3)	-13.0 (±0.5)
Secondary emulsion coated with PDADMAC	30mM	1	565.6 (±30.6)	91.8 (±1.9)	30.3 (±2.2)
Tertiary emulsion coated with PDADMAC-alginate	1.0%w/v	1	589.5 (±74.1)	98.7 (±1.1)	-24.2 (±1.3)
Quaternary emulsion with PDADMAC-alginate-PDADMAC	40mM	1	569.0 (±32.7)	95.3 (±2.1)	30.8 (±1.6)

4.3 Creaming stability measurement

%Transmission of diluted emulsions at 550 nm, which can imply to the stability of emulsion was measured using an UV-Visible spectrophotometer according to the creaming stability measurement in the previous studies [23, 33, 35].

From Figure 4.29, at the first day, freshly prepared primary emulsion exhibited very similar stability to multilayer emulsions. After the first day of storage, the %transmission clearly increased more than another indicating that the primary emulsion was less stable to creaming compare to multilayer emulsions for all cases.

For multilayer emulsion, we observed similar stability among freshly prepared multilayer emulsions. However, after three storage day, we could see clearly trends that the creaming stability seemed better as the increasing in the number of layer around droplets when compared at the same day, especially on the sixth and seventh day where the quarternary emulsion showed very good stability compared to the rest and tertirary emulsion showed better stability compared to secondary emulsion. At seven day storage, the %transmission values, of primary emulsion was 46.91, while the %transmission of secondary, tertiary and quaternary emulsions were 32.94, 25.47 and 16.24, respectively. As compared to primary emulsion, the secondary, tertiary and quaternary emulsions could, therefore, enhance about 30, 46 and 65% of the stability, respectively. This is because the relatively strong electrostatic and steric repulsion associated with the relatively thick and electrically charged multilayer interfacial membrane [30]. Moreover, the creaming stability may have been improved because the increasing of the number of layers increased the overall density of the droplets, therefore reducing the density difference between the dispersed and continuous phases and decreasing the driving force for the gravitational separation [31].

For all cases, the stability of emulsion declined with increasing the storage time. However, the quarternary emulsion showed the smallest changes with time (least slope).

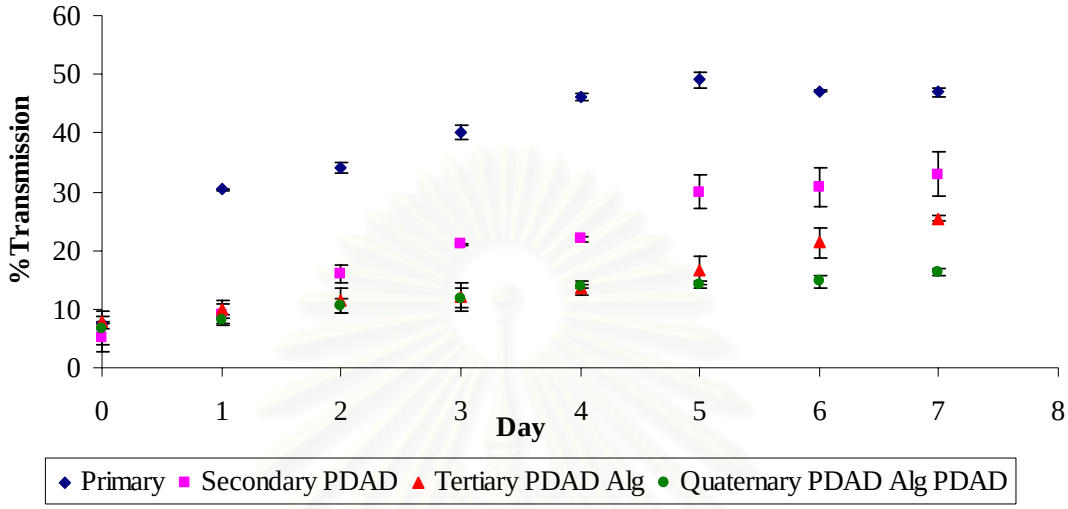


Figure 4.29 The creaming stability of primary emulsion (stabilized by 0.19 %w casein), secondary emulsion (stabilized by 0.19 %w casein and 3.8 mM PDADMAC), tertiary emulsion (stabilized by 0.19 %w casein, 3.8 mM PDADMAC and 0.25 %w/v alginate) and quaternary emulsion (stabilized by 0.19 %w casein, 3.8 mM PDADMAC, 0.25 %w/v alginate and 20 mM PDADMAC) at pH 6.

For casein-chitosan-alginate system (Figure 4.30), similar results were observed that at the first day, all freshly prepared emulsions showed similar stability. After the storage, we observed that creaming stability of primary and secondary emulsions became worse and their trends were more dramatic compared to tertiary emulsion, especially from the forth to seventh day. It is confirmed that more layer coating on the emulsion droplet can help enhance the emulsion stability. The %transmission value at day 7 of tertiary emulsion coated with casein-chitosan-alginate was 31.82, which could increase about 32% of the stability as compared to primary emulsion.

Comparison between the secondary emulsions coated with casein and chitosan and casein-PDADMAC at pH 6, we could see that the secondary emulsion

coated casein and chitosan was less stable to creaming because the lower charge density of chitosan at pH 6 as compared to PDADMAC. This can reduced the electrostatic repulsions between the droplets.

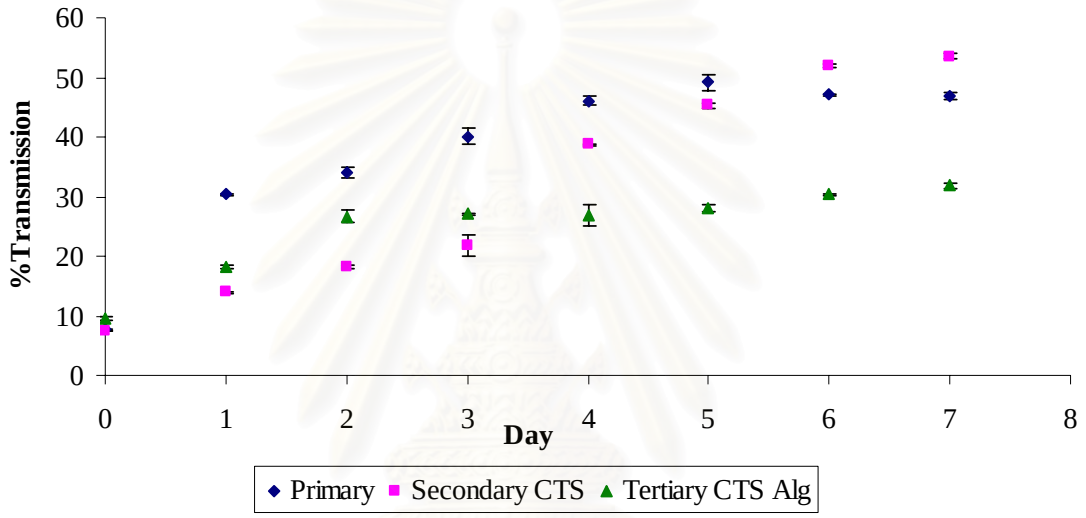


Figure 4.30 The creaming stability of primary emulsion (stabilized by 0.071 %w casein), secondary emulsion (stabilized by 0.071 %w casein and 0.024 %w/v chitosan) and tertiary emulsion (stabilized by 0.071 %w casein, 0.024 %w/v chitosan and 0.071 %w/v alginate) at pH 6.

4.4 Lipid Oxidation

Lipid hydroperoxide is formed in the oxidation of oil. Thus amount of lipid hydroperoxide can be used as the indiciator for lipid oxidation of oil [11]. Ferric thiocyanate can be used to monitor the lipid hydroperoxide amount. The ferric thiocyanate method is based on the oxidation of ferrous ion (Fe^{2+}) to ferric ion (Fe^{3+}), which are determined as ferric thiocyanate $[FeSCN]^{2+}$. The absorbance of

ferric thiocyanate solution was measured at 510 nm. Hydroperoxide concentrations were determined using a standard curve made from cumene hydroperoxide [33]. Lipid hydroperoxide produced from each emulsion solution was measured as a function of time (0-7 days) to monitor the differences in kinetic of lipid oxidation of emulsions which varied the number of layer.

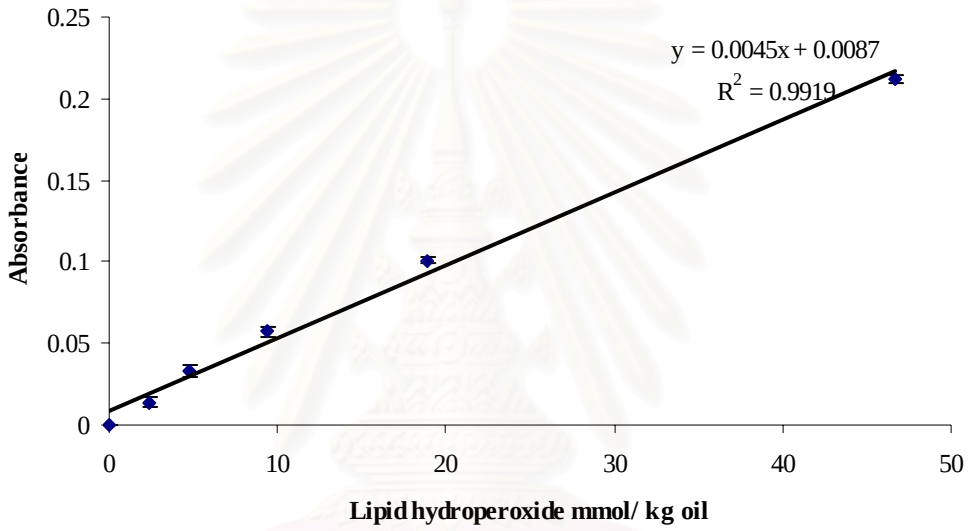


Figure 4.31 The calibration curve of hydroperoxide by using cumene hydroperoxide as standard.

The calibration curve of hydroperoxide was constructed. The standard curve of cumene hydroperoxide showed the linear relationship between the cumene hydroperoxide concentration and the absorbance with linear regression of 0.9919.

Figure 4.32 showed a comparison of oxidation rates in tuna oil-in-water emulsions stabilized by casein alone (primary emulsion), casein plus PDADMAC (secondary emulsion), casein plus PDADMAC plus alginate (tertiary emulsion) and casein plus PDADMAC plus alginate plus PDADMAC (quaternary emulsion).

On the first three storage days, there were no any change on the oil oxidation rate occurred in all emulsions. In primary emulsion, the oil oxidation rate reached the maximum on the forth day and then the declination was observed because the lipid hydroperoxide decomposed to aldehydes. This observation has been reported in the previous studies [8, 35, 37] The lipid hydroperoxide concentration of primary emulsion increased to 35.84 mmol/kg of oil on day 4, respectively.

For secondary and tertiary emulsions, the maximum value of hydroperoxide was seen on the forth day, which were around 22.20 mmol/kg of oil. The oxidative stability of tertiary emulsion which top layer was alginate exhibited not significantly different as compared to secondary emulsion coated with casein-PDADMAC. This observation might be the result of the different thickness, where the tertiary emulsion had the thicker layer than the secondary emulsion even it posse anionic droplet.

For the quaternary emulsion, the maximum of lipid hydroperoxide observed on the 5^{th} day, which its value was 22.26 mmol/kg of oil. Thus the maximum amount of lipid hydroperoxide of multilayer emulsion was lower around 38% than primary emulsion. This implied that the multilayer coating can help retard the oil oxidation. Furthermore, the quaternary emulsion might be able to retard the oxidation rate slightly more than the secondary and tertiary emulsions as we could see from it took longer time to reach the maximum value of lipid hydroperoxide.

Comparison between primary and multilayer emulsions on the maximum values of the observed lipid hydroperoxide and the time to reach the maximum values, the oxidative stability of multilayer emulsion was higher than primary emulsion.

In our study, the performance of multilayer emulsions on the oxidative stability were not significantly enhanced as compared to the previous studies [8, 35]. It may be due to the using of casein as the emulsifier. Casein has been reported that casein could inhibit the lipid oxidation better than the other protein (WPI and SPI) [27]. Thus, the multilayer emulsion contained casein as the emulsifier exhibited the smaller different of the oxidative stability.

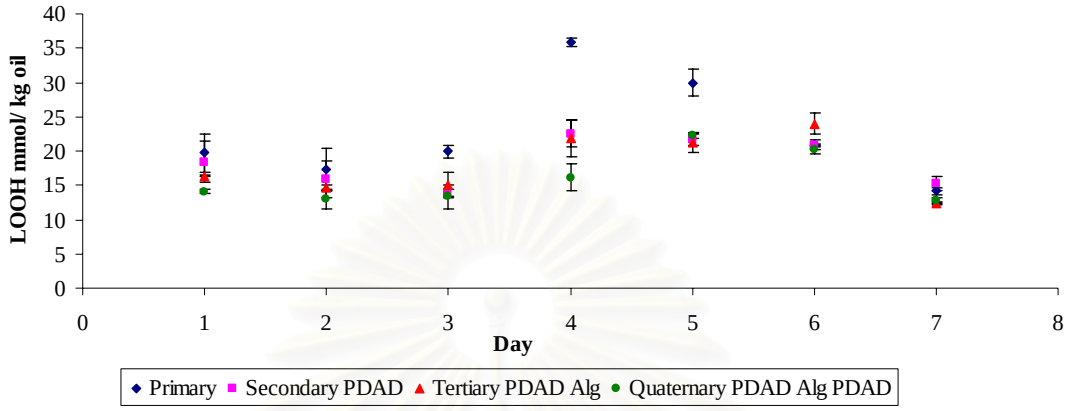


Figure 4.32 Plot of hydroperoxide as a function of observation days of primary emulsion (stabilized by 0.19 %w casein), secondary emulsion (stabilized by 0.19 %w casein and 3.8 mM PDADMAC), tertiary emulsion (stabilized by 0.19 %w casein, 3.8 mM PDADMAC and 0.25 %w/v alginate) and quaternary emulsion (stabilized by 0.19 %w casein, 3.8 mM PDADMAC, 0.25 %w/v alginate and 20 mM PDADMAC) at pH 6.

From Figure 4.33, it can be seen that the lipid oxidation for various emulsions namely primary emulsion, secondary emulsion coated with chitosan, tertiary emulsion containing casein-chitosan-alginate coated droplets displayed different profiles.

Similarly, the changing of the oil oxidation pattern in primary emulsion was observed that the oxidation of oil increased from day one to day four (the maximum values). Then, the oil oxidation declined because of the decomposition of lipid hydroperoxide. The lipid hydroperoxide concentration of primary emulsion at day 4 increased to 36 mmol/kg of oil.

However, the amount of lipid hydroperoxide of secondary and tertiary emulsion seemed no different after 7 days of storage. If we considered the amount of hydroperoxide obtained from the multilayer emulsions on the forth day, we observed

that the lipid hydroperoxide of secondary and tertiary emulsions were around 20 mmol/kg of oil. Thus the amount of lipid hydroperoxide of multilayer emulsions at day 4 was 36.17% lower than primary emulsion.

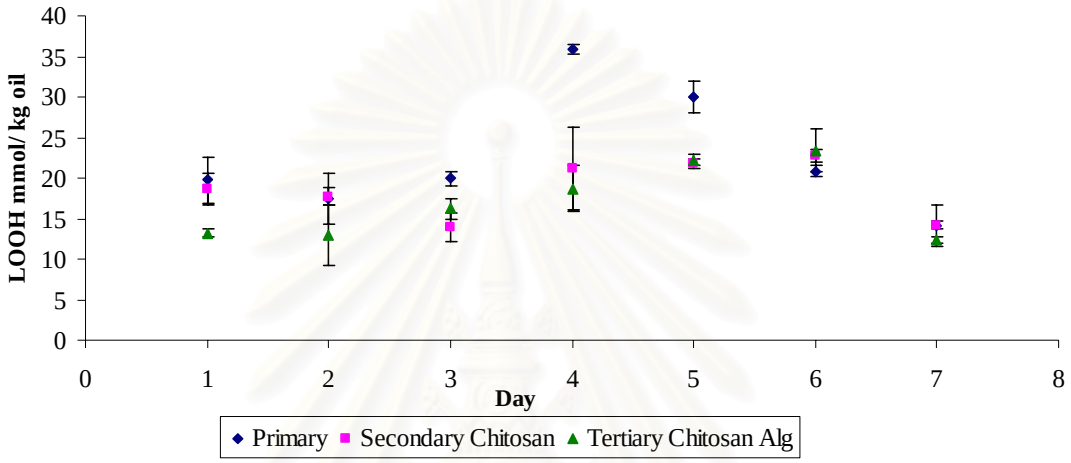


Figure 4.33 Plot of hydroperoxide as a function of observation days of primary emulsion (stabilized by 0.071 %w casein), secondary emulsion (stabilized by 0.071 %w casein and 0.024 %w/v chitosan) and tertiary emulsion (stabilized by 0.071 %w casein, 0.024 %w/v chitosan and 0.071 %w/v alginate) at pH 6.

Multilayer emulsions could decrease lipid oxidation rate because the thicker layer of interfacial membrane as we have reported. This thicker layer help decreasing the interactions between omega-3 in the droplets and oxidant species in the aqueous phase. The increasing of number of layer coated around droplets of emlsion provided the thicker layer around the droplets which could increase the oxidative stability of emulsion as compared to primary emulsion [8].

CHAPTER V

CONCLUSION

This research has shown that stable monolayer and multilayer emulsions could be prepared by using simple and effective method. The parameters controlling formation of stable primary emulsions such as number of homogenization cycle (1-10), pH of emulsion (3-7), emulsifier concentrations (0.5-2.5 %w of casein) were investigated. Primary emulsion was containing small droplets (diameter around 400nm) was prepared by homogenizing for 2 minutes at 20,000 rpm and 5 homogenization cycles. The obtained primary emulsion exhibited polydispersed size distribution which had bimodal size distribution. The mean diameter of the first peak which exhibited the higher population (more than 90%) was used to represent the droplet size in the prepared emulsions. Primary emulsion contained 1.5 %w casein concentration exhibited the narrowest particle size distribution and also showed good stability as compared to the others. Primary emulsion prepared at pH 6 showed the most stability as compared to the other pH. The prepared primary emulsion by homogenizing 5 %w tuna oil and 95 %w aqueous emulsifier solution, which contained 1.5 %w of casein concentration at pH 6 exhibited anionic charged droplets (zeta potential around -13 mV) and its diameter was around 400 nm.

Therefore, the primary emulsion contained 5 %w tuna oil and 95 %w aqueous emulsifier solution, which contained 1.5 %w of casein concentration at pH 6 will be used for the multilayer emulsion.

To prepare the stable multilayer emulsions, the maximum amount of polyelectrolyte completely coating around the oppositely charged emulsion droplets, the equivalent point, were studied by measuring %transmission of mixing solution at each volume ratio between emulsion and polyelectrolyte. The equivalent point was the volume ratio which showed the lowest of %transmission. First, the preparation of secondary emulsions coated with casein-PDADMAC and casein-chitosan were

studied. Then, the tertiary emulsions preparation coated with casein-PDADMAC-alginate and casein-chitosan-alginate were studied. Last the quaternary emulsion preparation coated with casein-PDADMAC-alginate-PDADMAC was also investigated.

The secondary emulsion coated with PDADMAC as the outer layer could be prepared by mixing primary emulsion contained 1.5 %w casein with 30 mM PDADMAC at volume ratio of 1. In this study, the average mass ratio of casein to PDADMAC was 3.2. Secondary emulsion could increase the creaming stability after 7 day of storage 30% as compared to primary emulsion.

Therefore, the secondary emulsion containing 2.5 %w tuna oil, 0.75 %w casein and 15 mM PDADMAC with the zeta potential values around +30 mV could be prepared in this study.

The secondary emulsion coated with casein-chitosan at pH 6 containing 0.83 %w tuna oil, 0.25 %w casein and 0.083 %w/v chitosan was produced from the mixing of primary emulsion contained 1.5 %w casein with 0.1 %w/v chitosan at volume ratio of 0.2 (mass ratio of 3). Prepared secondary emulsion at pH 6 obtained the zeta potential values around +20 mV which exhibited the cationic charge of the emulsion droplet. The effect of pH of chitosan solution from pH 5.5, 6.0 and 6.5 was also studied. Chitosan at pH 6 could prepare stable secondary emulsion as compared to the other pH. The creaming stability of this secondary emulsion was lower than primary emulsion due to the low charge density of chitosan at pH 6.0 which observed from the higher %transmission than primary emulsion at day 6 and 7 of storage.

Tertiary emulsion at pH 6 containing anionic droplets coated with casein-PDADMAC-alginate (zeta potential around -25 mV) containing 1.25 %w tuna oil, 0.38 %w casein, 7.5 mM PDADMAC and 0.5 %w/v alginate was prepared from the mixing of secondary emulsion contained 0.75 %w casein and 15mM PDADMAC with 1 %w/v alginate at volume ratio of 1 (mass ratio of 0.24). The tertiary emulsion coated with casein-chitosan-alginate (zeta potential around -4 mV) containing 0.24

%w tuna oil, 0.071 %w casein 0.024 %w/v chitosan and 0.071 %w/v alginate was prepared from the mixing of secondary emulsion contained 0.25 %w casein with 0.083 %w/v chitosan with 0.1 %w/v alginate at volume ratio of 0.4 (mass ratio of 0.33). These tertiary emulsions could increase the stability to creaming more than primary emulsion. The increasing in creaming stability of tertiary emulsions coated with casein-PDADMAC-alginate and casein-chitosan-alginate at day 7 were 45.70 and 32.17% as compared to primary emulsion, respectively.

Finally, quaternary emulsion coated with casein-PDADMAC-alginate-PDADMAC membrane (zeta potential around +30 mV) at pH 6 containing 0.63 %w tuna oil, 0.19 %w casein, 0.25 %w/v alginate and 20 mM PDADMAC was prepared from the mixing of tertiary emulsion coated with casein-PDADMAC-alginate contained 1.25 %w tuna oil, 0.38 %w casein, 7.5 mM PDADMAC and 0.5 %w/v alginate with 40 mM PDADMAC at volume ratio of 1 (mass ratio of 0.77). Quaternary emulsion could increase 66% of the stability to creaming at day 7 as compared to the primary emulsion. Thus, multilayer emulsions exhibited the increasing in the creaming stability as compared to primary emulsion which quaternary emulsion coated with casein-PDADMAC-alginate-PDADMAC exhibited the most stability as compared to the others.

The oxidative stability of these emulsions was also compared using UV-Vis spectrophotometry. The lipid hydroperoxide primary product of the oil oxidation was determined by using the ferric thiocyanate method. All multilayer emulsions showed the increase in the oxidative stability as compared to primary emulsion. The oxidative stability of multilayer emulsion could enhance significantly for 4 and 5 days of storage. However, the performance of mulatilayer emulsions on the oxidative stability was insignificantly different. It may be due to the using of casein as the emulsifier which had been reported that casein could inhibit the lipid oxidation better than the other protein (WPI and SPI) [27]. Thus, the multilayer emulsion contained casein as the emulsifier exhibited the insignificantly different of the oxidative

stability. The average increasing in oxidative stability of all multilayer emulsion at day 4 and 5 was around 36-38% as compared to primary emulsion.

The improved creaming and oxidative stability of multilayer emulsion may be due to the ability of multilayered interfacial membranes to either increase the repulsive colloidal interactions between the droplets such as electrostatic and steric repulsive force, or to block the cations interact with the oil droplets.

Hence, multilayer emulsion may have a number of potential applications in the food industry for example protection of lipid oxidation, improving the stability of emulsion to environmental stresses and also for controlled flavor release application.

Suggestion for future research

The increasing of oxidative stability of tuna oil-in-water-emulsion, which contained omega-3 is important for the usage in food application. We can enhance the oxidative stability of oil-in-water emulsions by increasing the number of layer and adding antioxidant as mentioned in the chapter 2. Many antioxidants can be used in the food industry such as capsaicin, β-carotene, carotenoid, curcumin, etc. The amount of antioxidants can be varied to exhibit the effect of antioxidant quantity on the lipid oxidation rate.

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

REFERENCES

[1] Kubow, S. Lipid oxidation products in food and atherogenesis. Nutrition Reviews 51 (1993): 33-40.

[2] Park, Y.; Kelleher, S. D.; McClements, D. J.; and Decker, E. A. Incorporation and stabilization of omega-3 fatty acids in surimi made from cod, *Gadus morhua.* Journal of Agricultural and Food Chemistry 52 (2004): 597-601.

[3] Heinzelmann, K.; Franke, K.; Velasco, J.; and Marquez-Ruiz, G. Microencapsulation of fish oil by freeze-drying techniques and influence of process parameters on oxidative stability during storage. European Food Research and Technology 211 (2000): 234-239.

[4] Dickinson, E. Properties of emulsions stabilized with milk proteins: overview of some recent developments. Journal of Dairy Science 80 (1997): 2607-2619.

[5] Mei, L.; Decker, E. A.; and McClements, D. J. Evidence of iron association with emulsion droplets and its impact of lipid oxidation. Journal of Agricultural and Food Chemistry 46 (1998): 5072-5077.

[6] Kellerby, S. S.; McClements, D. J.; and Decker, E. A. Role of proteins in oil-in-water emulsions on the stability of lipid hydroperoxides. Journal of Agricultural and Food Chemistry 54 (2006): 7879-7884.

[7] Ogawa, S.; Decker, E. A.; and McClements, D. J. Production and characterization of o/w emulsions containing cationic droplets stabilized by lecithin-chitosan membranes. Journal of Agricultural and Food Chemistry 51 (2003): 2806-2812.

[8] Klinkesorn, U.; Sophanodora, P.; Chinachoti, P.; McClements, D. J.; and Decker, E. A. Increasing the oxidative stability of liquid and dried tuna oil-in-water emulsions with electrostatic layer-by-layer deposition technology. Journal of Agricultural and Food Chemistry 53 (2005): 4561-4566.

[9] Uauy, R.; and Valenzuela, A. Marine oils: the health benefits of n-3 fatty acids. Nutrition 16 (2000): 680-684.

[10] Akoh, C. C.; and Lai, O. M., eds. Healthful lipids. Champaign: AOCS Press, 2005.

[11] Frankel, E. N. Lipid oxidation. 2nd ed. Bridgwater: Oily Press, 2005.

[12] Schramm, L. L. Emulsions, foams, and suspensions: fundamentals and applications. Weinheim: Wiley-VCH, 2005.

[13] Grove, R. Notes on making cola [Online]. 2005. Available from: http://sparror.cubecinema.com/cube/cola/chemistry/cola.pdf [2009, March 21]

[14] Sjoblom, J. Emulsions and emulsion stability. 2nd ed. Boca Raton: Taylor & Francis, 2006.

[15] Surh, J.; Gu, Y. S.; Decker, E.A.; and McClements, D. J. Influence of environmental stressed on stability of o/w emulsions containing cationic droplets stabilized by SDS-fish gelatin membranes. Journal of Agricultural and Food Chemistry 53 (2005): 4236-4244.

[16] Gibbs, R. J. An Introduction to zeta potential and its measurement [Online]. 2005. Available from: http://www.silver-colloids.com/Tutorials/Intro/pcs1.html [2009, January 25]

[17] Dautzenberg, H.; and Jaeger, W.; Kotz, J. Polyelectrolytes: formation, characterization and application. New York: HANSER, 1994.

[18] Decher, G.; and Schlenoff, J. B. Multilayer Thin Films. New York: Wiley-VCH Verlag Gmbh&Co, 2002.

[19] Guzey, D.; and McClements, D. J. Formation, stability and properties of multilayer emulsions for application in the food industry. Advances in Colloid and Interface Science 128-130 (2006): 227-248.

[20] Gu, Y. S.; Decker, E.A.; and McClements, D. J. Influence of pH and carrageenan type on properties of beta-lactoglobulin stabilized oil-in-water emulsions. Food Hydrocolloid 19 (2005): 83-91.

[21] Ogawa, S.; Decker, E.A.; and McClements, D. J. Influence of environmental conditions on the stability of oil in water emulsions containing droplets stabilized by lecithin-chitosan membranes. Journal of Agricultural and Food Chemistry 51 (2003): 5522-5527.

[22] Gu, Y. S.; Regnier, L.; and McClements, D. J. Influence of environmental stresses on stability of oil-in-water emulsions containing droplets stabilized by beta-lactoglobulin–iota-carrageenan membranes. Journal of Colloid and Interface Science. 286 (2005): 551–8.

[23] Aoki, T.; Decker, E. A.; and McClements, D. J. Influence of environmental stresses on stability of o/w emulsions containing droplets stabilized by multilayered membranes produced by a layer-by-layer electrostatic deposition technique. Food Hydrocolloids 19 (2005): 209-220.

[24] Caruso, F.; Trau, D.; Mohwald, H.; and Renneberg R. Enzyme encapsulation in layer-by-layer engineered polymer multilayer capsules. Langmuir 16 (2000): 1485-1488.

[25] Schuler, C.; and Caruso, F. Decomposable hollow biopolymer-based capsules. Biomacromolecules 2 (2001): 921–926.

[26] Hu, M.; McClements, D. J.; and Decker, E. A. Lipid Oxidation in corn oil-in-water emulsions stabilized by casein, whey protein isolate and soy protein isolate. Journal of Agricultural and Food Chemistry 51 (2003): 1696-1700.

[27] Hu, M.; McClements, D. J.; and Decker, E. A. Impact of whey protein emulsifiers on the oxidative stability of salmon oil-in-water emulsions. Journal of Agricultural and Food Chemistry 51 (2003): 1435-1439.

[28] Guzey, D.; and McClements, D. J. Impact of electrostatic interactions on formation and stability of emulsions containing oil droplets coated by β-lactoglobulin-pectin complexes. Journal of Agricultural and Food Chemistry 55 (2007): 475-485.

[29] Shaw, L. A.; McClements, D. J.; and Decker, E. A. Spray-dried multilayered emulsions as a delivery method for ω-3 fatty acids into food systems. Journal of Agricultural and Food Chemistry 55 (2007): 3112-3119.

[30] Gu, Y. S.; Decker, E.A.; and McClements, D. J. Production and characterization of oil-in-water emulsions containing droplets stabilized by multilayer membranes consisting of β-lactoglobulin, *i*-carrageenan and gelatin. Langmuir 21 (2005): 5752-5760.

[31] Guzey, D.; and McClements, D. J. Influence of environmental stresses on o/w emulsions stabilized by β-lactoglobulin-pectin-chitosan membranes produced by electrostatic layer-by-layer deposition technique, FOBI 1 (2006): 30-40.

[32] Mancuso, R. J.; McClements, D. J.; and Decker, E. A. Iron-accelerated cumene hydroperoxide decomposition in hexadecane and trilaurin emulsions. Journal of Agricultural and Food Chemistry 48 (2000): 213-219.

[33] Ogawa, S.; Decker, E. A.; and McClements, D. J. Production and characterization of o/w emulsions containing cationic droplets stabilized by lecithin-chitosan-pectin multilayerd membranes. Journal of Agricultural and Food Chemistry 52 (2004): 3595-3600.

[34] Gray, J. I. Measurement of lipid oxidation: a review. Journal of The American Oil Chemists' Society 55 (1978): 539-546.

[35] Klinkesorn, U.; Sophanodora, P.; Chinachoti, P.; McClements, D. J.; and Decker, E. A. Stability of spray-dried tuna oil emulsions encapsulated with two-layered interfacial membranes. Journal of Agricultural and Food Chemistry 53 (2005): 8365-8371.

[36] Kellerby, S. S.; Gu, S. Y.; McClements, D. J.; and Decker, E. A. Lipid oxidation in a menhaden oil-in-water emulsion stabilized by sodium caseinate cross-linked with transglutaminase. Journal of Agricultural and Food Chemistry 54 (2006): 10222-10227.

[37] Silvestre, M. P. C.; Chaiyasit, W.; Brannan, R. G.; McClements, D. J.; and Decker, E. A. Ability of surfactant headgroup size to alter lipid and

antioxidant oxidation in oil-in-water emulsions. Journal of Agricultural and Food Chemistry 48 (2000); 2057-2061.

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

APPENDICES

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

APPENDIX A

Table 1 The average %transmission @550 nm of primary emulsion at each casein concentration which varied number of homogenization cycle

Number of homogenization cycle	Casein concentration				
	0.5 %w	1.0 %w	1.5 %w	2.0 %w	2.5 %w
1	60.50	45.57	27.37	22.87	20.97
2	49.37	33.17	20.00	17.97	14.83
3	47.10	32.37	19.23	15.90	12.77
4	48.10	31.33	18.30	13.67	11.67
5	46.90	30.4	16.33	12.27	10.53
6	46.60	30.46	14.97	12.13	9.77
7	42.27	30.3	15.27	12.60	9.67
8	43.07	27.4	13.73	11.90	8.50
9	43.70	27.03	15.17	11.07	8.70
10	42.00	26.96	14.70	10.77	8.03

Table 2 The average %transmission @550 nm of primary emulsion which varied pH on the 1 week storage

pH	Day							
	0	1	2	3	4	5	6	7
3	4.40	32.04	41.39	45.71	46.69	50.19	55.29	58.39
4	13.86	39.33	66.20	70.95	77.33	78.13	79.21	82.32
5	80.53	82.65	86.16	88.70	88.51	85.95	86.38	90.37
6	3.84	30.37	36.28	40.04	40.94	41.78	41.17	37.64
7	7.47	37.07	43.24	48.35	45.48	46.27	52.70	59.11

Table 3 The average mean diameter at each peak of primary emulsion which varied casein concentration

Casein concentration (%w)	Mean diameter (nm)	
	Peak 1	Peak 2
0.5	473.8 ± 56.0	3420.2 ± 1101.7
1.0	457.0 ± 72.2	3436.98 ± 1368.6
1.5	417.0 ± 35.8	3334.81 ± 1024.6
2.0	435.3 ± 38.0	3184.06 ±1406.6
2.5	422.0 ± 32.0	3208.43±750.3

Table 4 The average zeta potential value of primary emulsion which varied casein concentration

Casein concentration (%w)	Zeta potential (mV)
0.5	-13.156 ± 0.54
1.0	-11.29 ± 0.62
1.5	-13.12 ± 1.73
2.0	-13.58 ± 0.57
2.5	-11.97 ± 1.27

Table 5 The average %transmission @550 nm of primary emulsion which varied casein concentration on the 1 week storage

Casein concentration (%w)	Day			
	1	3	5	7
0.5	43.07	55.99	63.33	64.13
1.0	30.80	46.57	49.67	50.27
1.5	27.47	39.78	41.17	41.98
2.0	2.57	35.04	37.67	40.04
2.5	23.33	30.37	33.84	36.28

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

APPENDIX B

Table 6 The average %transmission @550 nm of secondary emulsion preparation which varied the volume ratio between primary emulsion and 10 mM PDADMAC

The volume ratio between primary emulsion and 10 mM PDADMAC	%transmission
0.050	53.55
0.10	31.75
0.15	16.06
0.20	13.23
0.25	16.30
0.30	18.77
0.35	41.98
0.40	92.88
0.45	93.50
0.50	99.66
0.55	97.55
0.60	94.72

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

Table 7 The average zeta potential value of secondary emulsion preparation which varied the volume ratio between primary emulsion and 10 mM PDADMAC

The volume ratio between primary emulsion and 10 mM PDADMAC	Zeta potential (mV)
0	-13.16 ± 0.54
0.10	31.05 ± 1.11
0.30	31.27 ± 2.22
1.0	1.41 ± 5.51
2.0	-14.53 ± 0.19

Table 8 The average mean diameter at each peak of secondary emulsion preparation which varied the volume ratio between primary emulsion and 10 mM PDADMAC

The volume ratio between primary emulsion and 10mM PDADMAC	Mean diameter (nm)	
	Peak 1	Peak 2
0	422.4 ± 48.8	4953.7 ± 301.2
0.10	503.4 ± 18.5	3474.9 ± 326.5
0.30	565.7 ± 30.6	4582.7 ± 219.2
1.0	412.0 ± 22.3	5124.3 ± 17.9
2.0	376.0 ± 12.3	3372.9 ± 114.4

Table 9 The average %transmission @550 nm of secondary emulsion preparation which varied the volume ratio between primary emulsion and 0.1 %w/v chitosan

The volume ratio between primary emulsion and 0.1 %w/v chitosan	%transmission
0.10	16.79
0.20	4.89
0.30	101.97
0.40	92.94
0.50	81.05
0.60	77.47

Table 10 The average zeta potential value of secondary emulsion preparation which varied the volume ratio between primary emulsion and 10 mM PDADMAC

The volume ratio between primary emulsion and 10 mM PDADMAC	Zeta potential (mV)
0	-16.75 ± 0.35
0.10	15.63 ± 0.28
0.20	18.55 ± 0.49
0.50	-1.59 ± 4.36

Table 11 The average mean diameter at each peak of secondary emulsion preparation which varied the volume ratio between primary emulsion and 0.1 %w/v chitosan

The volume ratio between primary emulsion and 0.1 %w/v chitosan	Mean diameter (nm)	
	Peak 1	Peak 2
0	422.4 ± 48.8	4953.7 ± 301.2
0.10	981.9 ± 84.5	4495.4 ± 1271.5
0.20	912.9 ± 65.6	3967.0 ± 1056.5
0.50	531.2 ± 87.5	4071.0 ± 1677.7

Table 12 The average %transmission @550 nm of tertiary emulsion coated with casein-PDADMAC-alginate preparation which varied the volume ratio between secondary emulsion coated with casein-PDADMAC and 1 %w/v alginate

The volume ratio between secondary emulsion and 1 %w/v alginate	%transmission
0.20	41.43
0.40	44.23
0.60	59.06
0.70	39.20
0.80	33.82
0.90	27.08
1.0	26.76
1.1	31.38
1.2	39.36
1.3	66.44
1.4	66.68
1.6	79.12

Table 13 The average %transmission @550 nm of tertiary emulsion coated with casein-chitosan-alginate preparation which varied the volume ratio between secondary emulsion coated with casein-chitosan and 0.1 %w/v alginate

The volume ratio between secondary emulsion and 0.1 %w/v alginate	%transmission
0.10	30.76
0.20	24.60
0.30	19.74
0.40	8.96
0.45	20.69
0.50	26.19
0.55	36.98
0.60	37.08
0.70	41.12
0.80	48.52
0.90	55.36
1.0	52.26

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

Table 14 The average %transmission @550 nm of quaternary emulsion coated with casein-PDADMAC-alginate-PDADMAC preparation which varied the volume ratio between tertiary emulsion coated with casein-PDADMAC-alginate and 40 mM PDADMAC

The volume ratio between tertiary emulsion and 40 mM PDADMAC	%transmission
0.20	65.33
0.50	41.39
0.70	25.65
0.80	18.12
0.90	11.73
1.0	11.05
1.1	32.21
1.2	45.47
1.3	68.75
1.5	88.56
1.7	93.77
2.0	98.49

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

APPENDIX C

Table 15 The average %transmission @550 nm of emulsion which varied the number of layer coated on droplet of emulsion on the 1 week storage

Emulsion	Day							
	0	1	2	3	4	5	6	7
Primary emulsion (coated with casein)	7.65	30.33	33.99	40.13	46.13	49.11	47.15	46.91
Secondary emulsion (coated with casein-chitosan)	7.44	13.97	18.28	21.91	38.74	45.24	51.94	53.55
Tertiary emulsion (coated with casein-chitosan-alginate)	9.60	18.17	26.59	27.12	26.76	28.01	30.37	31.82

Table 16 The average %transmission @550 nm of emulsion which varied the number of layer coated on droplet of emulsion on the 1 week storage

Emulsion	Day							
	0	1	2	3	4	5	6	7
Primary emulsion (coated with casein)	7.65	30.33	33.99	40.13	46.13	49.11	47.15	46.91
Secondary emulsion (coated with casein-PDADMAC)	5.23	9.19	15.89	20.99	21.91	30.11	30.65	32.94
Tertiary emulsion (coated with casein-PDADMAC-alginate)	7.73	10.07	11.38	12.02	13.53	16.55	21.38	25.47
Quaternary emulsion (coated with casein-PDADMAC-alginate-PDADMAC)	6.67	8.11	10.54	11.87	13.83	14.15	14.75	16.24

APPENDIX D

Table 17 Absorbance @ 510 nm of standard solution of cumene hydroperoxide

Cumene hydroperoxide concentration (mmol/kg oil)	Absorbance
2.35	0.01409
4.71	0.03340
9.42	0.05730
18.8	0.10081
46.6	0.21200

Table 18 Absorbance @ 510 nm of lipid hydroperoxide of monolayer and multilayer emulsions on the 1 week storage

Emulsion	Day						
	1	2	3	4	5	6	7
Primary emulsion (coated with casein)	0.09764	0.08701	0.09843	0.16998	0.14331	0.10255	0.07236
Secondary emulsion (coated with casein-chitosan)	0.09265	0.08844	0.07126	0.10404	0.10678	0.11101	0.07236
Tertiary emulsion (coated with casein-chitosan-alginate)	0.06819	0.06707	0.08150	0.09287	0.10882	0.11410	0.06412

Table 19 Absorbance @ 510 nm of lipid hydroperoxide of monolayer and multilayer emulsions on the 1 week storage

Emulsion	Day						
	1	2	3	4	5	6	7
Primary emulsion (coated with casein)	0.09764	0.08701	0.09843	0.16998	0.14331	0.10255	0.07236
Secondary emulsion (coated with casein-PDADMAC)	0.09147	0.08006	0.07126	0.11019	0.10624	0.10282	0.07745
Tertiary emulsion (coated with casein-PDADMAC-alginate)	0.08212	0.07458	0.076359	0.10703	0.10437	0.11668	0.06462
Quaternary emulsion (coated with casein-PDADMAC-alginate-PDADMAC)	0.07203	0.06706	0.06866	0.08141	0.10888	0.09922	0.06639

VITA

Name: Miss SOMPRASONG SIRAMARD

Date of Birth: May 23th, 1985

Place of Birth: Saraburi, Thailand

Address: 27 Moo 8 Bhuddhamonthon Sai 1 Road, Bangramard, Talingchan, Bangkok 10170, Thailand

Telephone: 081-3989770

E-mail address: ssd_ying@hotmail.com

Educations:

2006-2008	M.Sc. (Petrochemistry and Polymer Science), Chulalongkorn University, Thailand
2002-2005	B.Sc. (Chemistry), Kasetsart University, Thailand
1999-2001	Photisarnpittayakorn School, Bangkok, Thailand
1990-1998	Piyamitr School, Bangkok, Thailand

Scholarship:

2002-2005 The Thailand Science Scholarship Program student

Presentation:

Somprasong Siramard, Luxsana Dubas and Stephan T. Dubas, Zn and Fe alginate complex blend omega-3 microemulsion for food application (Poster Session): 33rd Congress of Science and Technology 0f Thailand, 18-20 October 2007. Walailak University, Nakhonsithammarat, Thailand

Somprasong Siramard, Luxsana Dubas and Stephan T. Dubas, Oxidative stability of omega-3 emulsions coated with polyelectrolyte multilayer (Poster Session): Pure and Applied Chemistry International Conference 2009, 14-16 January 2009. Naresuan University, Phitsanulok, Thailand