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Study on rheological property of mixed aqueous solutions of Phosphatidylcholine and Lysophosphatidylcholine

Master's Thesis

STUDY ON RHEOLOGICAL PROPERTY OF MIXED AQUEOUS SOLUTIONS OF PHOSPHATIDYLCHOLINE AND LYSOPHOSPHATIDYLCHOLINE

September 2016

Graduate School of Marine Science and Technology

Tokyo University of Marine Science and Technology

Master's Course of Food Science and Technology

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Content

Chapter 1: General Introduction

Chapter 2: Rheological properties of the lecithin mixtures between Phosphatidylcholine (PC) and Lysophosphatidylcholine (LPC) with different molar ratio at intermediate and low temperature

Chapter 3: Effect of temperature on Reorganization of wormlike structure based on Phosphatidylcholine (PC) and Lysophosphatidylcholine (LPC) with different ratio

Chapter4: Conclusions

Acknowledgements

List of figures

List of Tables

修士学位論文内容要旨
Abstract

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論文題目 Title	Study on rheological property of mixed aqueous solutions of Phosphatidylcholine and Lysophosphatidylcholine.		

Lecithin are phospholipid substances which their structures consist of phosphoric acid with choline, glycerol and fatty acids. The main source of lecithin is egg yolk, milk, marine source, sunflowers or soybeans. They are useful material which is applied in several fields. For example, in food industry, lecithin are used for food textures, antioxidant, emulsifier or surfactant. Lecithin are used as zwitterionic surfactants, they have ability to self-assembly into a several of micellar structure in aqueous solution or organic solvent, depending on the chemical structure of lecithin, temperature, pH, light; for instance, spherical micelles, vesicle micelles, bilayer micelles or wormlike micelles.

Phosphatidylcholine (PC) is a major phospholipid component in lecithin. PC is commonly studied as surfactant or emulsifier because of PC structure consist of hydrophilic part (head group) and hydrophobic part (tail group) which can reduce surface tension. The hydrophobic head group of PC is choline, while the hydrophobic tail groups is fatty acid chains. Furthermore, PC can be used to produce Lysophosphatidylcholine (LPC) by the hydrolysis reaction with phospholipase A_2. From this reaction, the one mole of fatty acid of PC is removed, causing LPC has more hydrophobicity than PC.

From recent study, the researcher investigated the formation of wormlike micelle structure of lecithin mixtures which consist of PC and LPC in aqueous solution. They found that pure PC normally formed planar structure, while pure LPC will aggregate to form spherical micelle. However, the lecithin mixtures at total concentration about 56 mM or above could formed the wormlike structure at temperature more than 45 ˚C. Because the

alkyl chain of PC and LPC were melted at high temperature, resulting in high flexibility and molecular mobility. And further, PC and LPC would be bind each other and aggregated to form a new structure which show a high viscosity and transparent relate to a characteristic of wormlike structure. Moreover, they also found that after formation on heating, the wormlike micelles remained stable on cooling.

To our knowledge, this is the first report on the formation of phospholipid wormlike micelles without the addition of salts in aqueous solutions. Since, normally, lecithin will form reverse wormlike micelle with addition of salt in organic solvent. And there are only a few researches on zwitterionic worms. To get more information about this system, in present study, the rheological properties of mixed LPC and PC with different molar ratio in aqueous solution at varied temperature was determined. In addition, the reorganization of wormlike micelle based on LPC and PC was also investigated.

Firstly, LPC and PC system with different molar ratio was measured the rheological properties by falling ball method and oscillatory measurement. It is found by falling ball test that the mixture with high concentration of PC showed a high viscosity at low temperature and exhibited gel-like behavior. However, the viscosity decreased with increasing temperature since a reduction of micelle size and diminution of micellar network. On the other hand, the mixtures contained high concentration of LPC show the opposite tendency which showed a low in viscosity and transparent at low temperature and switched to a high in viscosity with increasing temperature. However, from oscillation frequency sweep test showed that an increase in viscosity with transparent correlated with a formation of wormlike structure. In the other word, only the sample with LPC: PC is 40:24 and 44:20 had ability to form a wormlike structure. For LPC: PC 40:24 system, the sample could form wormlike micelle at wide range of temperature (5-30 ˚C.), while LPC: PC =44:20 system could form only at 30 ˚C.

Second, the reorganization of wormlike micelle based on LPC and PC was measured. In this experiment, only the mixtures of LPC and PC with molar ratio were 40 to 24 and 44 to

20 which had ability to form a wormlike structure was investigated. The samples include LPC: PC = 40: 24 at 10-30 ˚C and LPC: PC = 44:20 at 30 ˚C. To prepare, the mixture was heated followed by cooled down and kept at specific temperature for 24 hours for equilibration of system. Further, the mixture was cut the length of wormlike structure by Silverson machine before rheological measurement. The viscosity of sample was determined as a function of time. From our result showed that both of samples at 30 ˚C presented a high in viscosity depend on time, indicating they had an ability to recover their structure. While the sample at 10-20 ˚C could not recover its structure, resulting in a low viscosity with time. In addition, steady-shear measurement also supported our result which showed a shear-thinning behavior at 30 ˚C which is a typically wormlike characteristic. On the other hand, the sample at 10-20 ˚C presented a Newtonian fluid behavior, indicating the formation of rod-like micelle.

Chapter 1

General Introduction

1.1   Surfactant

Surfactant is an amphiphilic molecule which can reduce the interfacial tension between two phases such as the system of oil and water phase or the system of air and water because their structure consist of two parts; hydrophobic and hydrophilic part (Myers, 2006). Generally, the hydrophobic part is a non-polar or no charge such as hydrocarbon chain, while the hydrophilic part is a polar or charge compound such as sulfate or quaternary amine group (Soderman *et al,* 2004)

According to the hydrophilic head group of surfactant, the surfactant can be classified into 4 classes

1. Cationic surfactants are substances with their head group containing positively-charge ion, for instance, Erucyl bis(hydroxyethyl) methylammonium chloride (EHAC) and Erucyl trimethylammonium chloride (ETAC) (Raghavan and Kaler, 2000)

2. Anionic surfactants are substances with carrying the negative charge ion in head group. Normally anionic corresponds to sulfate compound such as alkyl sulfate, Sodium 4-(8-methacryloyloxyoctyl)oxybenzene sulfonate (MOBS) (Zhu *et al.,* 2006)

3. Zwitterionic surfactants or Amphoteric surfactants are amphiphilic substances with their hydrophilic part contain both of negative and positive charge, for example, Erucyl dimethyl amidopropyl betaine (EDAB) (Kumur *et al.* 2007) and dioctanoylphosphatidylcholine (diC$_8$PC) (Martinez-Landeira *et al.,* 2002)

4. <u>Non-ionic surfactants</u> are surfactants with a non-charge in their hydrophilic group. The head group of this kind of surfactants are alcohol, phenol, ether, ester or amide. The example of nonionic surfactant is Pentaetylene glycol monododecyl ether (Bernheim- Groswasser *et al.,* 2000)

Surfactants can self-assembly form micelle in polar and non-polar solution, and they have the ability aggregate in various micellar characteristics, for example spherical micelle, vesicle micelle, cylindrical micelle, bilayer micelle or planar micelle (Myers, 2006). However, the geometry of the surfactant can be predicted depend on critical packing parameter (CPP*; p*). The critical packing parameter can calculate by the concept of Israelachivili (2011) as followed: $p = v/a_0 l$, where l is the effective maximum of length of the hydrophobic tail, v is the volume of hydrophobic tail and a_0 is the effective surfactant area of hydrophilic head group. The amphiphilic compounds expected to aggregate into spherical micelle when CPP less than 1/3 (about 0.33), wormlike micelle, cylindrical micelle or rod-shaped micelle when CPP is more than 0.33 but less than 0.5, vesicle micelle when CPP is between 0.5 and 1, the surfactant formed planar structure when CPP about 1 and aggregate to form reversed or inverted micelle when CPP more than 1 (Soderman *et al,* 2004; Chu *et al,* 2013; Myers, 2006)

Table1: Expected Aggregate Characteristic with prediction by Critical Packing Parameter.

$v \, / \, a_0 l$

Critical Packing Parameter	General Surfactant Type	Expected Aggregate Structure
< 0.33	Single tailed surfactants and large head groups	Spherical or ellipsoidal micelles
0.33-0.5	Single surfactants with small head groups, or ionic surfactants	Cylindrical or rod-shaped micelle
0.5-1.0	Two hydrophobic tail surfactants with large head groups and flexible chains	Vesicle and flexible bilayer structures
1	Two hydrophobic tail surfactants with small head groups and inflexible chains	Planar bilayer structures
> 1	Two hydrophobic tail surfactants with small head groups. Very large hydrophobic groups	Reversed or inverted micelles

Source: Myers, D., Surfactant Science and Technology, Third Edition, 2006, 126

1.2   Application of surfactant

Surfactants are used in many applications. For example; surfactants are applied in recovery and processing of petroleum industry (Schramm L. L. *et al.,* 2003). When petroleum are pumped from an oil stock, some of them have been left. However, surfactants can reduce these problems by controlling mobility of the drive of oil form reservoir. (Nelson R.C., 1982)

In the personal care products, surfactants are used as foaming agents like soap, toothpaste and shampoo. Sodium dodecyl sulfate (SDS) is anionic surfactant which is extensively used in cleaning and hygiene products. Besides, some of surfactants were also used like detergents such as laundry detergents and domestic cleaning products because of their cleaning properties. (Falbe J., 1987). The surfactants which their structure consist of alkylbenzene sulfonates, alcohol ethoxylates or sulfates are mainly used in laundry detergents and personal care products. (Schramm L. L. *et al.,* 2003). The surfactants have ability to remove dirt form dishes and clothes by diminution of interfacial tension between two Interfaces and force away the dirt form the surface of clothes and dishes.

Surfactants are also used in pharmaceutical application. For instance; they are used in mouth washed, respiratory distress therapy, suppository bases, enhancers for percutaneous absorption. (Mishra M. *et al.,* 2009).

Furthermore, surfactants are important role in food industry. They acted as emulsifier in food emulsion system such as oil in water emulsion or water in oil emulsions. In both system, oil and water will separate into two phase when they are mixed. However, when the surfactant are added into these system, the surfactants will adsorb at interface between oil and water by reaching out of hydrophobic group into oil phase and persisting of hydrophilic group in water phase, resulting in reduction of surface tension between oil and water phase. Therefore, the oil in water or water in oil system is more stable. Oil in water products are mayonnaise, salad dressing, milk, cream and soft drink. While water in oil products

are butter, cheese and margarine.(Guzey D. and McClements J., 2006)

1.3   Wormlike micelle

Surfactant will aggregate to form rod-like micelle when the length of micelle is shorter than its persistence length which the length in direction with no tangent. However, they will transfer to form wormlike micelle if the whole length is much greater than its persistence length. Wormlike micelle or a flexible cylindrical micelle has been reported by aggregation of several kind of surfactant; non-ionic, cationic, anionic and zwitterionic surfactant. For instance, non-ionic wormlike micelle formed by polyoxyethylene phytosteryl ether ($PhyEO_n$) and polyoxyethylene alkyl ether (C_nEO_m),
cationic wormlike micelles from erucyl bis-(hydroxyethyl)methylammonium cholide (EHAC) with adding organic compound. (Sharma S.C. *et al.*, 2009; Kalur G.C. *et al.* 2005; Walker L. M. 2001)

Wormlike micelle show a similar behavior to polymer which they can entangle into viscoelastic networks. However, the micellar chains have ability to break and recombine because their chain are held by only weak physical bonds, while polymers are formed by covalent bonds.

The rheological behavior of wormlike micelle solutions can be classified into two classes.

1. <u>Linear rheological behavior</u> wormlike micelles become entangled with increasing of contour length. The rheological properties will be present both of viscous and elastic behavior with a single relaxation time, thereby showed the Maxwell behavior when the breaking time is much less than reputation time. In this case, the important parameters consist of a plateau modulus (G_0) refers to the entanglement density of net and a relaxation time (t_R) relates to the reptation and breaking/reforming times of the micelle.

2. <u>Non-Linear rheological behavior</u> the non-linear response will be observed when a

shear rate is high enough to annoy the wormlike structure. In this situation, wormlike systems demonstrate many distinct phenomena. For example, in cationic micelle system show a shear thickening in steady-shear measurement. Shear induces the growth of micelle and a changing of rod-like micelles to wormlike micelles. (Chu. Z. *et al.,* 2013; Zonglin C. *et al.,* 2013; Tung S. H. *et al.,* 2007; Walker L. M. 2001)

However, the characteristic of wormlike micelle can change depend on the changing of external stimulator or change in environment such as pH, temperature, UV/Visible light, salt, CO_2, counterion and electrical. (Zonglin C. *et al.,* 2013). Davies T.S. *et al.* (2006) investigated on the cationic surfactant, cetyltrimethylammonium bromide (CTAB) with adding 5-methyl salicylic acid (5mS).The mixtures containing 12.5 mM CTAB with 5 mS at concentration between 5 and 15 mM showed a clear, viscoelastic and exhibited shear-thinning fluids as evidence by rheometer. This result suggested that salt have influence on the formation of wormlike micelle. Moreover, they also studied on the effect of temperature on CTAB/5mS system. They found that temperature induce the transition from vesicle to wormlike micelle.

Moreover, cetyltrimethylammonium bromide (CTAB) is pH sensitive, thereby this surfactants were studied with potassium phthalic acid (PPA) to determine the pH effect on viscoelastic wormlike micelle by Lin Y. (2009). In this report shown a switch between wormlike micelle and short cylindrical micelles as evidence by NMR, rheometer, UV-Vis spectrometer and dynamic light scattering technique.

For the work of Kumar R. *et al.* 2010 studied on reverse wormlike micelle from the combination of lecithin and the organic derivative, trans- para-coumaric acid (trans-PCA). As evidence from rheological measurement, Small Angle Neutron Scattering (SANS) shown the reduction of length of wormlike micelle upon UV irradiation since trans-PCA is tuned into cis - form which less ability of H-bond with lecithin, resulting in, the smaller sizes of micelles.

1.3.1   Application of wormlike micelles

Wormlike micelles are very useful materials. Generally, they are used in many technological and industrial fields, for instance, they are applied in the oil industry, biomedicine, drag reduction, and clean processes. ((Zonglin C. *et al.,* 2013)

In oil fields, viscoelastic wormlike cationic surfactants are used as fracturing fluids and these wormlike micelles are combined with hydrophobically modified polymer for controlling the water operation. And cationic surfactant also used as drag reduction agents for district heating and cooling fluids. Besides, wormlike micelles also found in some personal care products such as shampoo and body wash. (Yang J., 2002)

1.4   Lecithin

Lecithin is phospholipid compound which its structure consist of phosphoric acid with choline, glycerol, glycolipids, triglycerides, phospholipids and fatty acids. It is generally found in egg yolk, milk, marine source, sunflowers or soybeans. Lecithin is necessary for cell membrane. It controls the cholesterol level and protected gallstones in our bodies. Moreover, it is applied in several fields. For example, in food industry, lecithin is used for smoothing food textures, antioxidant, emulsifier, and surfactants. When lecithin is used as surfactants, it can form various characteristics in aqueous solution or organic solvent depend on the type of lecithin, temperature, such as, spherical micelles, vesicle micelles, bilayer micelles or wormlike micelles. There are many important substances in lecithin, that is, phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, phosphatidylserine and phosphatidic acid. (Smith J. and Hong L., 2003; Szuha B.F., 1989)

1.4.1   Phosphatidylcholine (PC)

Phosphatidylcholine (PC) is a major compounds in lecithin and it is found in almost all cell components such as brain, heart, liver and kidney but the largest

7

concentration in the membrane. In addition, PC also abundant in dietary source for example, egg yolk, soybean, milk, beef, fish and sunflower. The structure of PC consist of hydrophobic part, two fatty acid chains and hydrophilic part, choline group which is a precursor of acetylcholine and a compound for brain activity about learning, memory and motor control. (Kane E. and Kane P., 2005; Christie W. W., 2011)

1.4.2 Lysophosphatidylcholine (LPC)

Lysophosphatidylcholine; LPC is a phospholipid compound which can be obtained from the hydrolysis reaction of phosphatidylcholines; PC with phospholipase A_2. From this enzymatic action, one of the fatty acid groups of PC is removed.

LPC can be found in some parts of our body such as cell membrane and blood plasma. In plasma, the fatty acid chain of LPC is palmitic acid (16:0) or stearic acid (18:0) (Munder P.G. *et al.,* 1979). Moreover, LPC is abundant in various foods. Bemiller J. and Whistler R. reported that LPC were discovered in the lipids composition of oat starch about 70%. Eskin N.A.M. and Shahidi F. represented acyltransferase produced LPC from phospholipids in egg yolk, vegetable oils, processed meat, cake products and milk products such as cheese.

In the bioavailability of dietary, LPC is useful to enhance the cellular absorption of carotenoids such as β-carotene and lutein and cholesterol absorption (Sugawara T. *et al*., 2010; Nakano T. *et al.,* 2009).

1.5 Rheological measurement

Rheology is study about changing in shape for liquid and solid material by their flowing after applied some stress. Each material will show unique behavior. There are many parameters for identify the fluid behavior of these materials such as viscosity which is a resistance of liquid flow. This parameter is dependent with many factors, for example, molecular weight, shape and structure of material, temperature and pressure.

The flowing behavior can be classified into two groups, that is; Newtonian systems and Non-Newtonian system.

Newtonian system

$F/A = \eta \, (dv/dr)$

Where, F/A = shear stress; F = force, A = area (Unit is N/m^2)

dv/dr = shear rate or velocity gradient

η = viscosity

The relationship between shear stress and shear rate called flow curve is linear line. Even though the temperature of Newtonian materials increases, but their viscosity remains constant.
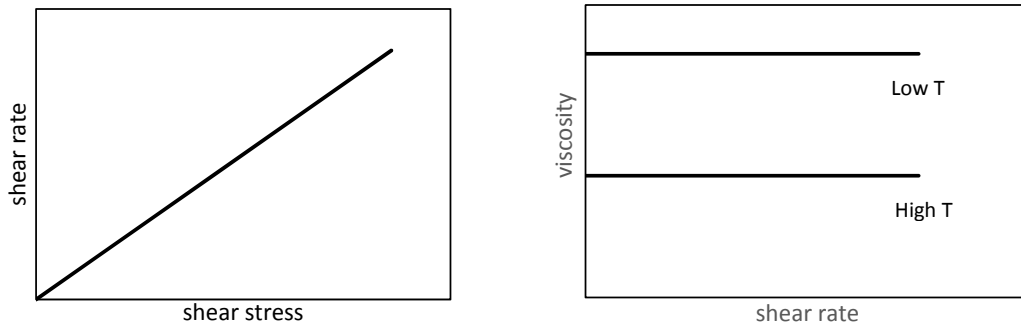
Fig.1 Flow curve of Newtonian system

Unit of viscosity is $dyne.sec.cm^{-2}$ or $gram.cm^{-1}.sec^{-1}$ called poise. Another unit of viscosity is Pascal-second (Pa.s). One Pa.s is equal 10 poise.

The liquid which showed Newtonian behavior is water, alcohol, glycerin, milk and vegetable oil.

Non-Newtonian-time independent

A non-Newtonian fluid is a material which its relationship between shear rate and shear stress are not constant. Non-Newtonian materials consist of colloidal solution, emulsion, liquid suspension. There are several types of these materials depend on their flow behavior as following.

1. Plastic flow and Bingham Flow

For Bingham behavior, fluid materials can be flowed when the obtained force equal to yield value (yield stress). These materials behave like solid when applied stress less than yield value and they will show Newtonian fluid when force exceeding yield value. In Figure 2 showed the flow curve of Bingham flow. Slope of this graph is mobility. However, for Plastic flow, materials also start to flow after input stress equal to yield stress. But after obtained yield stress, some materials shows shear-thinning behavior which their viscosity decreases followed by plateau for example tomato plate, lipsticks and toothpastes.
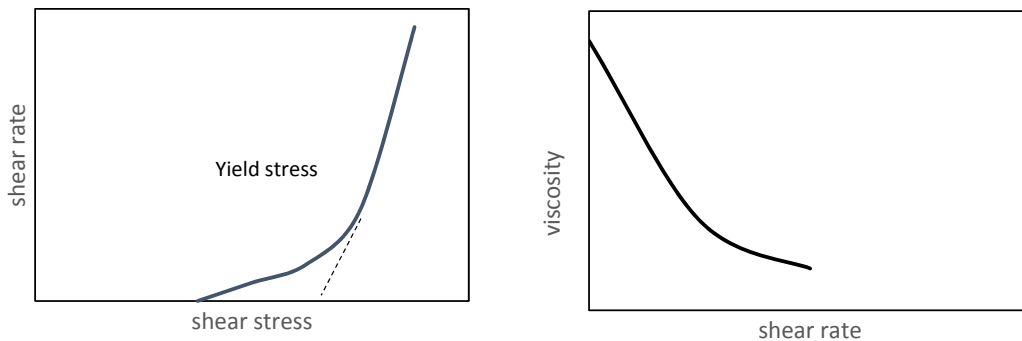
Fig.2 Flow curve of Bingham system

2. Pseudoplastic flow (shear thinning system)

In this case, there is no yield stress. The solution can be moved after obtained some force but the velocity rate of movement is not directly proportion with this force. Shear-thinning materials will show a decreasing in viscosity with shear rate. The sample which present this behavior conclude emulsions, suspending agent and polymer.
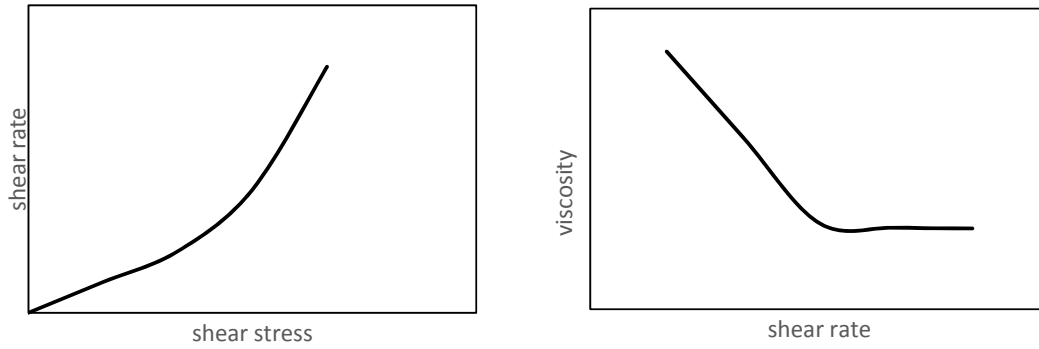
Fig.3 Flow curve of Pseudoplastic system

3. Dilatant flow (shear thickening system)

Some fluid materials have a high resistance of flowing when force increase. Therefore their viscosity will increase upon shearing. This phenomenon can be found in some pharmaceutical products such as a suspension of penicillin or in food products for example, corn starch solution and some type of honey.
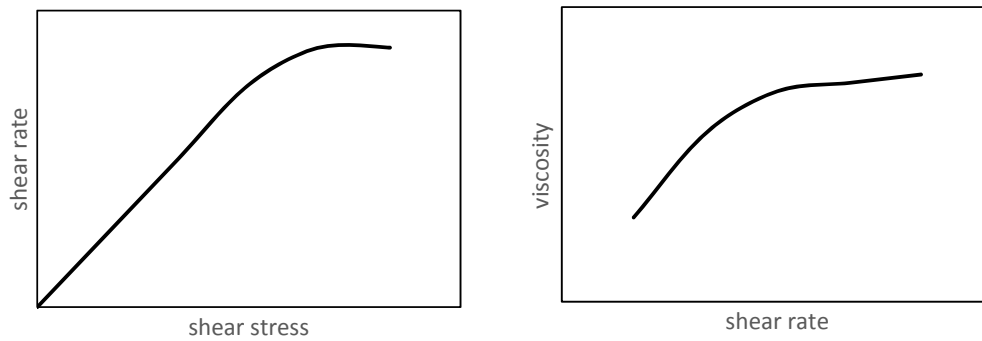
Fig.4 Flow curve of Dilatant system

<u>Non-Newtonian-time dependent</u>

Some material shows a change in viscosity with time at constant shear rate.

1. Thixotropic

For these materials, their viscosity decrease with increasing time because the structure of these materials are destroyed. However, when these materials are kept for a long time, their viscosity will increase because they can restore their structure. For instant, Some particle can form three dimensional network and form gel at force = 0. However, these sample will change form gel to solution when they are obtained some force such as shaking. And they will form gel again after stop shaking, the particle will be moved by random Brownian movement to restore their structure.
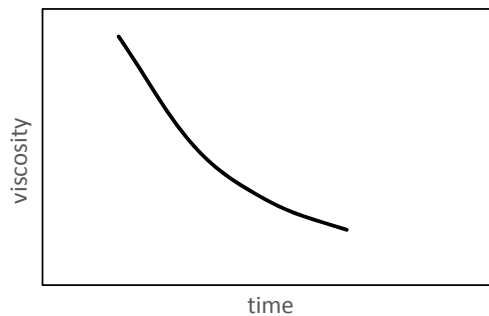
Fig.5 Flow curve of Thixotropic system

2. Rheopectic

These materials will show opposite behavior with Thixotropic materials which their viscosity increase with time.

12
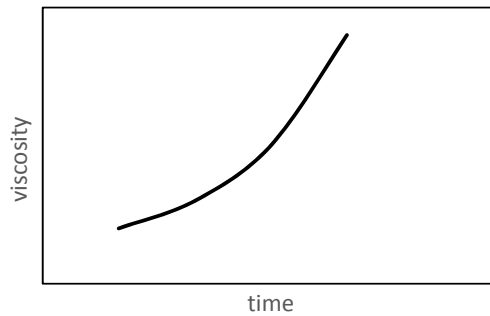
Fig.6 Flow curve of Rheopectic system.

Equipment for viscosity measurement

1. One –point instrument for Newtonian material such as capillary viscometer and falling sphere viscometer

2. Multipoint instrument for Newtonian and Non-Newtonian material for example, Cup & bob viscometer, Cone &plate viscometer and Brookfield synchrolectic viscometer.

(Daubert C. R. *et al.,* 2010; Faith A. M., 2001; Martin A. *et al.* 1993; Rao M. A., 1999)

1.5.1 Oscillatory experiment

Some of materials such as polymer systems emulsion or dispersions are difficult to investigate their rheological properties because their response intermediate between solid and liquids which called viscoelastic behavior. However, studying of rheological behavior of these materials is important to find a suitable condition for application in several fields. In this case, Oscillatory rheology is always used to analyze the rheological properties of these samples. Since oscillatory measurement can be used to quantify both viscous response and elastic response of each material at different time scales. Oscillation measurement can be classified into four tests, the Stress sweep, the Frequency Sweep, the Time Sweep and the Temperature Sweep.

The Oscillation stress sweep test is a first step to consider the linear viscoelastic range which loss modulus/ viscous modulus (G''), storage modulus/ elastic modulus (G') are

13

independent with stress or strain. This test is necessary for all of oscillation test.

Oscillation frequency sweep test is a measurement which present the relationship of G'', G' and frequency (ω). G' is a measure of energy stored and released per cycle of deformation per unit volume which refers to elastic nature of each material. G'' is a measure of the dissipated energy as heat per cycle of deformation per unit volume which relates to viscous nature of materials. To prove the viscoelastic behavior of materials by the frequency dependent with G' and G'', an important thing is applied strain deformations are small so as not to destroy the material properties. The area which viscous modulus and elastic modulus are independent with strains called linear viscoelastic region. In this region, if the materials which exhibit G'' are larger than G' at (ω), that mean this material behaves like liquids. In contrast, materials will present G' is greater than G'' when this materials response as solid behavior. For the region which G'' and G' are depending on strain, called nonlinear viscoelastic region. The large strain is applied in this area resulting changing in G'' and G' with strain. This measurement is used to study about the dynamic properties of some materials such as soft glassy materials. In addition, relaxation time can also be investigated at a crossover between G' and G''. Relaxation time relates to a change in structure with time. For wormlike structure, if the relaxation time is long, indicating long wormlike structure.

Oscillatory time sweep is usually used to investigate a rearrangement of materials with time. This rearrangement directly affects the rheological behavior of each material. This test provides information about how materials change with time, for instance, using oscillatory time test to determine polymer degradation, dispersion settling.

Oscillatory temperature sweep is determined the changing of material structure with temperature. (Wyss H.M. *et al.*, 2007; Gunasekaran S. *et al.* 2000)

1.6   Previous report

Recently, the mixtures based on phosphatidylcholine (PC) and lysophosphatidylcholine (LPC) in aqueous solution were studied by Natdanai *et al.* (2014). From this study, they found that at temperature below 45 ˚C, the mixtures present a spherical micelle for LPC in any temperature while PC formed planar structure.

However, they observed that the lecithin mixtures at total concentrations more than or equal to 56 mM have highly viscous and more transparent when the mixtures were heated up more than 45˚C. Therefore, they assumed that this phenomenon caused by the self-assembly wormlike structure of these phosphatidylcholine mixtures. This occurrence found only the sample contained LPC fraction range 0.5-0.7 that meant a minimum concentration of both LPC and PC were prerequisite for formation of wormlike micelles in aqueous solutions. (Natdanai *et al.*, 2014)

In many reports also showed the same appearance that the surfactants will present a clear solution with high viscosity when the surfactant aggregated to form wormlike micelle. Davies *et al.* (2006) studied on self-assembly of cationic surfactant, cetyltrimethylammonium bromide (CTAB) with adding 5-methyl salicylic acid (5mS) transition from vesicle to viscoelastic wormlike micelle upon heating. In this report, they found that when the CTAB/5mS system transformed into wormlike structures, the sample showed a colorless solution with a dramatic increase in viscosity.

In addition, Natdanai *et al.* (2014) studied more detail about the phosphatidylcholine mixture in aqueous solution by measuring the diffusion coefficient with nuclear magnetic resonance (NMR) technique and investigated the endothermic peak with differential scanning calorimeter (DSC) technique.

From their NMR result presented a decrease in diffusion coefficient of LPC and PC mixture at temperature more than 55˚C that related to a formation of wormlike micelle. And

DSC suggested that this wormlike structure can be formed after the melting of crystalline like structure in both LPC and PC at higher 40˚C. Furthermore, they investigated the effect of temperature on the viscosity of lecithin mixture. To investigate, the 48 and 64 mM lecithin mixture with different fraction of LPC were heated at temperature form 25 to 75 ˚C followed by cooling process. In case of 48 mM mixtures, all of samples had a low in viscosity less than 10 Pa.s throughout the heating process which indicate the formation of short wormlike micelles. However, on cooling process, the viscosity of mixtures with LPC fraction more than 0.5 increase. This increasing occurred presumably an increase in the length of the wormlike micelle. Turn to 64 mM mixtures, this samples also shown the same tendency which the viscosity increased on heating above 50˚C and viscosity also increased on cooling process below 60˚C. The viscosity on cooling process remained close its values at high temperature that meant the wormlike micelle is the thermodynamically stable state after forming. (Nadanai *et al.* 2014). Therefore, from this result suggested that these samples can maintained wormlike structure at low temperature. We would like to continue this research. Therefore, in this report, the objective is to determine a rheological properties of these lecithin mixtures based on LPC and PC at intermediate and low temperature. In addition, for the lecithin mixtures which showed a typical wormlike structure, their reorganization which is a specific characteristic of wormlike micelle will be investigated.

(a)   Before heating   (b) After heating
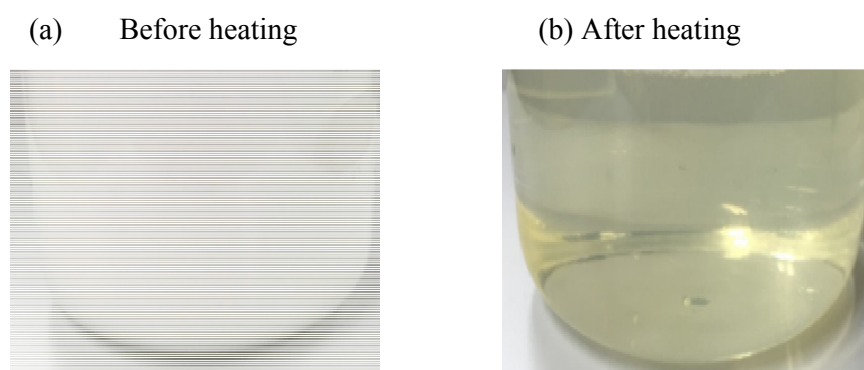
Fig.7 A 64 mM lecithin mixture ratio LPC: PC 40: 24; (a) before and (b) after heat process at 80 ˚C for 30 minutes. Before heating, the sample is low viscosity and high turbidity. After heating for 30 minutes, the sample become more transparent and high viscous.

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Chapter 2

Rheological properties of the lecithin mixtures between Phosphatidylcholine (PC) and Lysophosphatidylcholine (LPC) with different molar ratio at intermediate and low temperature

Abstract

The system of mixed solution between Lysophosphatidylcholine (LPC) and Phosphatidylcholine (PC) with different molar ratio was investigated the rheological properties by falling ball method and oscillatory measurement. The samples with LPC molar fraction 0.5-0.6 (LPC:PC 32:32 and LPC:PC 36:28) showed a high viscosity with turbidity at low temperature (5-10 ˚C) and exhibited gel-like behavior. On the other hand, the mixtures with LPC molar fraction > 0.6 exhibited an opposite tendency which showed a low in viscosity at low temperature and switch to a high viscosity with transparent when temperature increase. We assumed that the high viscosity with clear solution was a typically characteristic of wormlike micelle. To identify the wormlike characteristic, the oscillation frequency sweep test was determined. From dynamic result suggested that the system of LPC: PC = 32:32, 36:28 systems at 5-10 ˚C had no a single relaxation time, G' and G" were independent with all range of frequency, suggesting both of samples were not form wormlike structure. However, at 20 ˚C a crossover of G' and G" was observed in LPC: PC = 32: 32 and 36: 28. It is possible in according with the formation of wormlike micelle, nevertheless, our experimental data were not fitted with the Maxwell model with a single relaxation time. That meant both of samples did not consist with wormlike structure. In case of LPC: PC = 40:24 system, we found that this system had an ability to form wormlike structure at all range of temperature but the length of wormlike micelle depend on temperature. Lastly the LPC: PC = 44:20 system formed rod-like micelle at 5-20 ˚C and converted to wormlike structure at 30˚C.

2.1 Introduction

Phosphatidylcholine (PC) and lysophosphatidylcholine (LPC) are phospholipid compounds called lecithin. In nature, they were found in egg-yolk or soybean. Lecithin is used as emulsifier and surfactant in cosmetic, pharmaceutical or food industries (Palacios L. E. and Wang T., 2005). LPC and PC are zwitterionic surfactants which can aggregate to form micelle. Generally, LPC form spherical micelle because their molecular shape contained a large head group with single tail. On the other hand, the chemical structure of PC consist of small head group with two hydrophobic tail, indicating the formation of planar structure. (Natdanai F *et al.* 2014; Attwood D. *et al.* 1983; Kang K. C. *et al.* 2004)

Oscillatory rheology is a useful tool to determine viscoelastic materials at different time scale. This tool provides storage modulus (G') and loss modulus (G'') properties which refer to the structural and dynamic properties of these materials. If the storage modulus of viscoelastic materials is larger than loss modulus, these sample will show solid-like behavior. In contrast, the materials will response viscous behavior when the loss modulus dominates storage modulus. Furthermore, frequency sweep measurement is a valuable method to investigate the characteristic of materials. The relationship between G', G'' and frequency will be probed. Each material will show the different behavior in this measurement, for example, the materials which form wormlike structure will present storage modulus (G') is greater than the loss modulus (G'') at high frequency and they are independent of frequency over a wide range with a single relaxation time, resulting in Maxwell behavior. However, Oscillation stress sweep measurement is necessary to do first for determining a material's linear viscoelastic range before measuring the frequency sweep test. (Wyss H. M. *et al.*, 2007; Gunasekaran S. *et al.* 2000)

From previous report, Natdanai *et al.* (2014) found that the mixtures of lecithin between phosphatidylcholine (PC) and lysophosphatidylcholine (LPC) with total concentration above 56 mM can be formed wormlike structure after heating at temperature above 45˚C. In addition, this wormlike structure is the thermodynamically stable state after

forming. That means wormlike micelle can from after heating and also maintains at low temperature. To our knowledge, there are no reports about the formation of normal wormlike structure based on PC and LPC mixture. Normally, lecithin form reverse wormlike micelle in non-polar solvent .(Tung S. H. *et al.*, 2006; Imai M. *et al.*, 2013; Njauw C. W. *et al.*, 2013) In addition, there are only few reports about wormlike micelle by zwiiterionic surfactant with addition of salt. (Kumar *et al.,* 2007). Therefore, in this research, to get more details about the mixed lecithin systems based on PC and LPC. The rheological properties of these mixtures with different molar ratio will be investigated at low and intermediate temperature.

2.2 Materials and Method

2.2.1 Materials

Phosphatidylcholine with a purity 98% and Lysophosphatidylcholine which extracted form egg yolk were obtained from Kewpie Corporation (Tokyo, Japan). Their chemical structures are shown below. Deionized water was provided form a Water Purifier (Autostill WA500, Yamato Scientific Co., LTD., Tokyo, Japan)
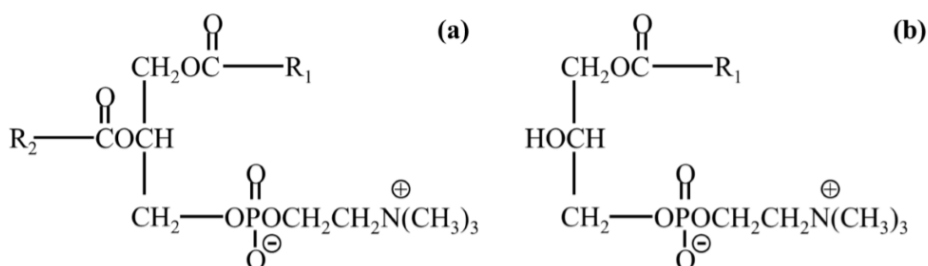

Fig.8   The chemical structure of PC (a) and LPC (b)

Source: Natdanai, F. *et al.,* Formation of wormlike micelle structure in Phosphatidylcholine aqueous mixture, 2014, 32

24

2.2.2 Preparation

       LPC and PC were mixed in deionized water by using a Silverson Machines Limited L5M-A (HP5 1pQ, Waterside, Chesham, Bucks, England) for 10 minutes at 40˚C. Further, the lecithin mixtures were heated at 80˚C for 30 minutes in water bath to induce the formation of wormlike structure. In our experiment, we investigated 64 mM lecithin mixtures at various molar ratio of LPC and PC ,that is, 32:32, 36:28, 40:24 and 44:20. In figure 9 showed a changes of viscosity and absorbance of the lecithin mixtures which LPC and PC ratio 40 to 24 depend on temperature. From this data showed that this samples exhibited a high in viscosity and transparent at 80 ˚C, indicating the characteristic of wormlike structure. Therefore, 80˚C is a suitable condition to provide a wormlike micelle. Notable, there is no any precipitate, that is, both of lecithin are completely dispersed in aqueous solution.
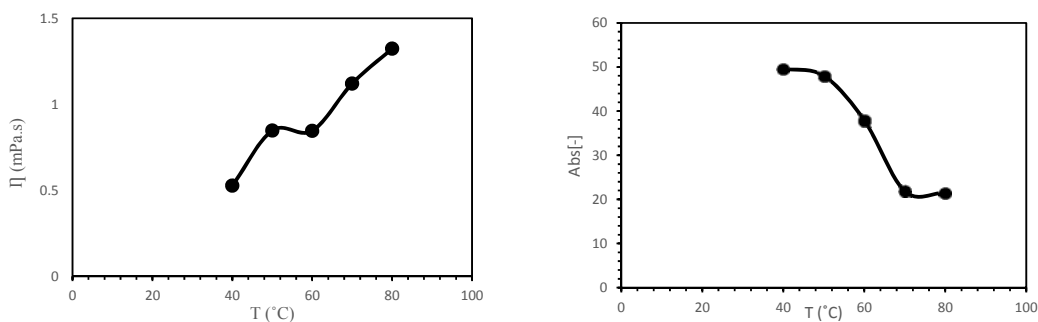

Fig.9   Illustration of viscosity (left) and absorbance at 500 nm (right) as a function of temperature of 64 mM lecithin mixture with LPC and PC molar ratio 40 to 24. Absorbance were measured by UV spectrophotometer (U-3300 spectrophotometer, Hitachi, Japan).

2.2.3 Falling ball measurement

After heat process, all of samples with different molar ratio lecithin mixtures were investigated by falling ball measurement (Natdanai *et al.,* 2014; Brizard *et al.,* 2005). The samples were transferred in glass tubes (diameter of tube is 1.1 cm and height is 8 cm) and kept in a cooler incubator at least 8 hours for equilibration at specified temperature. Further, the falling time were measured by investigation of the movement of steel ball (diameter about 0.6 cm) from the top to the bottom of sample tubes. The viscosity of these samples were calculated by the equation as followed,

$$\eta = \eta_0 \, (t \, / \, t_0) \qquad\qquad (1)$$

where is η is the viscosity of sample, η_0 is the viscosity of reference and t_0 is the falling time of reference. In this experiment, the reference is deionized water.

2.2.4 Rheological measurement

All of samples were kept in rheometer at specified temperature (5- 30˚C) for at least 10 minutes in order to certifying equilibration previous to determining of frequency sweep measurement. Frequency spectra were recorded in the linear viscoelastic regime of the samples as determined by dynamic strain sweep measurement.

Rheological of lecithin mixtures were performed with a Haake (MARS II) rheometer. A cup with inner diameter 27.208 mm and bob (diameter =25.080 mm.) were used. The volume of each sample for measurement was about 16 ml. A sample cover was used to reduce a change in the samples by evaporation during measurement. The temperature was controlled at 5, 10, 20 or 30 ˚C by a temperature controller (Haake UTM controller).

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2.3   Results and Discussion

2.3.1 Falling ball result
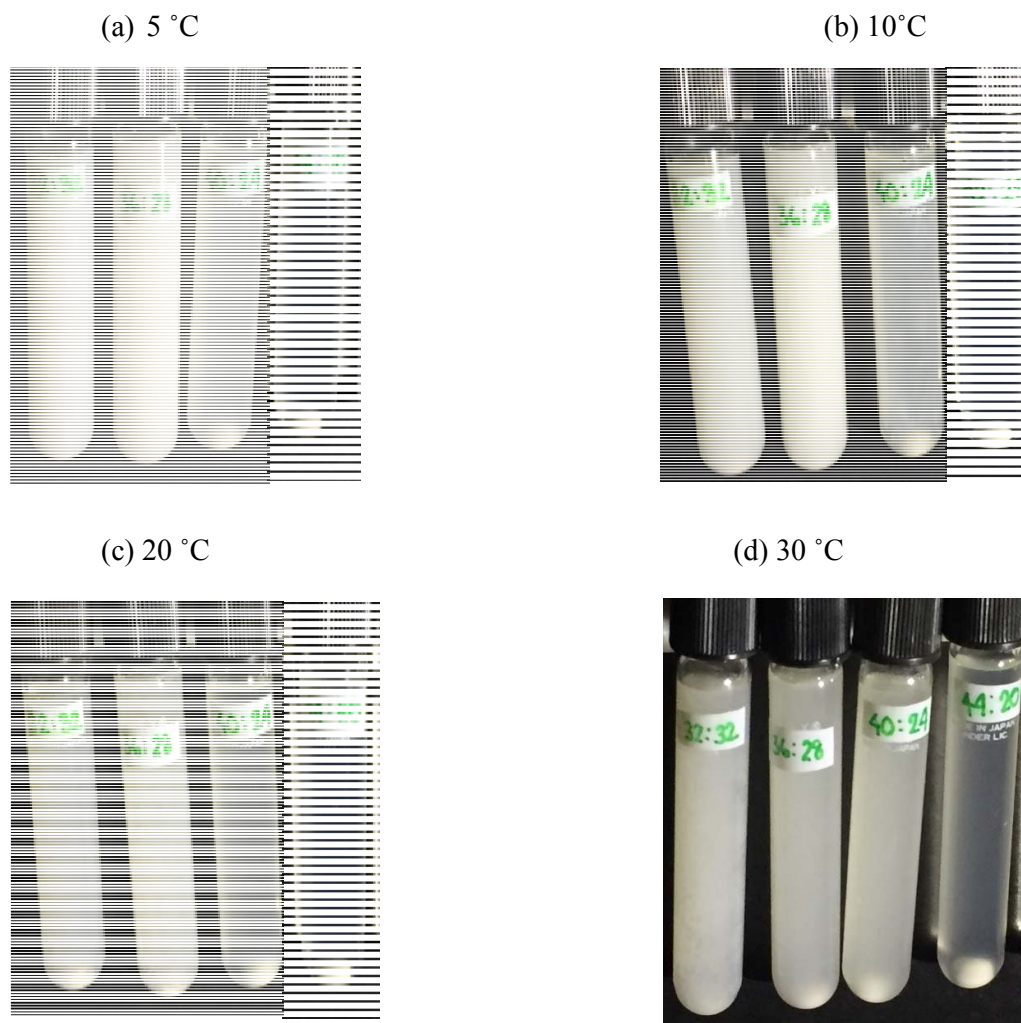
(a) 5 ˚C

(b) 10˚C

(c) 20 ˚C

(d) 30 ˚C

Fig.10 Photograph showing the appearance of 64 mM lecithin mixtures between LPC and
PC with different molar ratio in aqueous solutions. The samples include LPC: PC
mixtures = 32:32, 36:28, 40:24 and 44:20 are kept in cooler incubator at 5˚C
(a), 10 ˚C (b), 20˚C(c) and 30 ˚C(d).

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Figure 10 a-d presented a photograph of the appearance of aqueous solutions containing mixtures of LPC and PC with molar ratio of LPC: PC = 32:32, 36:28, 40:24 and 44:20 at 5, 10, 20 and 30°C, respectively. At low temperature range between 5-10°C, Mixtures containing a large amount of PC (LPC: PC 32:32 and 36:28) shows a turbidity and both of samples showed a slightly transparent with increasing temperature. On the other hand, the samples which LPC molar ratio is higher than PC molar exhibited more transparent at any temperature.
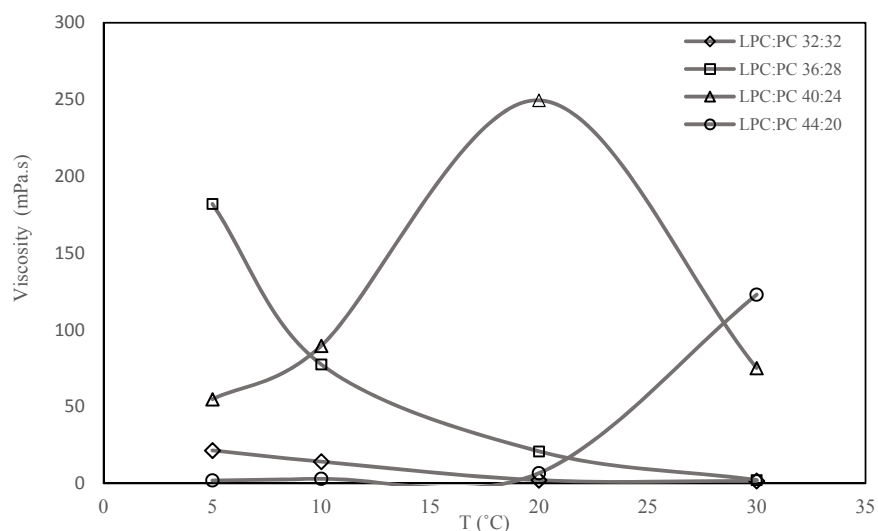

Fig.11 Viscosity versus temperature of lecithin mixture with different molar ratio (LPC: PC = 32:32, 36:28, 40:24 and 44:20). All of samples were hold in cooler incubator at least 8 hours before viscosity measurement by falling ball method.

Changing of the viscosity for the mixed solution based on LPC and PC system with different molar ratio at varied temperature as shown in Figure 11. At low temperature (5-10°C), the viscosity of lecithin mixtures containing high concentration of LPC (LPC: PC = 44:20) exhibited a low in viscosity but showed a clear solutions form visual observation. This phenomena presumably indicated that PC were melt by heat process and further formed a short rod-like structure with LPC. However, the viscosity was a dramatic increase with increasing temperature, causing network structure or formation of wormlike structure. Comparison with other samples, a clearness of both of samples are highest. The reason is a

low hydrophobicity of LPC because their structure is consist of only one fatty acid chain, resulting in a high ability to dissolve in water and formed spherical micelle.

On the other hand, for the mixtures with high concentration of PC (LPC: PC =32:32 and 36:28) presented an opposite behavior. The viscosity of these samples at low temperature displayed a high in viscosity followed by a dramatic decrease with increasing temperature. We have two hypotheses from this situation. First hypothesis is most of PC in this system formed a large planar structure, resulting a high turbidity and high viscosity at low temperature. However, the samples switched to lower viscosity and slightly clearness with increasing temperature, suggesting some of PC surfactants formed other type micellar structure such as wormlike or rod like structure with LPC. While, the residue PC still aggregated to form planar structure. The second hypothesis is both of samples had not ability to form a wormlike structure micelle. LPC formed a spherical micelle while PC formed a large planar structure at low temperature, indicating a high opacity and high viscosity. And the samples became more transparent and dropped in viscosity because of the size of spherical micelle and planar structure decreased.

In case of lecithin mixture with LPC: PC = 40:24 in aqueous solution demonstrated different tendency with the other samples. Upon increasing the temperature from 5 to 20 ˚C, the viscosity raised by more than two order of magnitude. However, upon a further increase in temperature, the viscosity dropped monotonically. We assumed that the sample formed rod-like or short wormlike structure at low temperature and transferred to from a wormlike structure at 20 ˚C , indicating a dramatic increase in viscosity with clearness. However, at 30˚C, the viscosity dropped and the value is same at 10 ˚C. That meant the sample reverted from wormlike micelle to rod-like structure or the samples transferred from long wormlike micelle to short wormlike structure.

To get more details about mixed lecithin systems, the oscillation frequency sweep measurement was used in order to study the dynamic and rheological properties of the solution in the mixed LPC and PC.
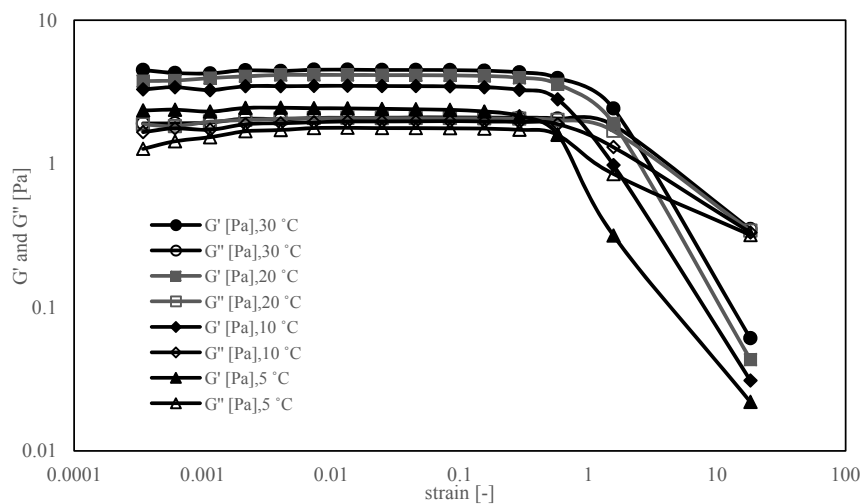

Fig.12   Dynamic strain sweep of LPC: PC system = 40:24 at different temperature.

.   A dynamic strain sweep measurement were used to determine the linear viscoelastic region for dynamic frequency sweep test. As shown in Figure 12, both of the storage modulus (G') and the loss modulus (G") of the mixed solution of LPC and PC molar ratio is 40 to24 were independent of strain from 0.001 to 1 Pa at any temperature, indicating the structure of samples were not destroyed in this region. And G' dominating G'', referring the samples is highly structured. However, at above 1% of strain, G' and G" value decrease, meaning the network structures were disturbed by strain sweep. In our experiment, a strain value for oscillation frequency test is 0.02 %. The others samples showed a linear viscoelastic regime at the same range of strain (data not shown).

(a) LPC:PC 32:32
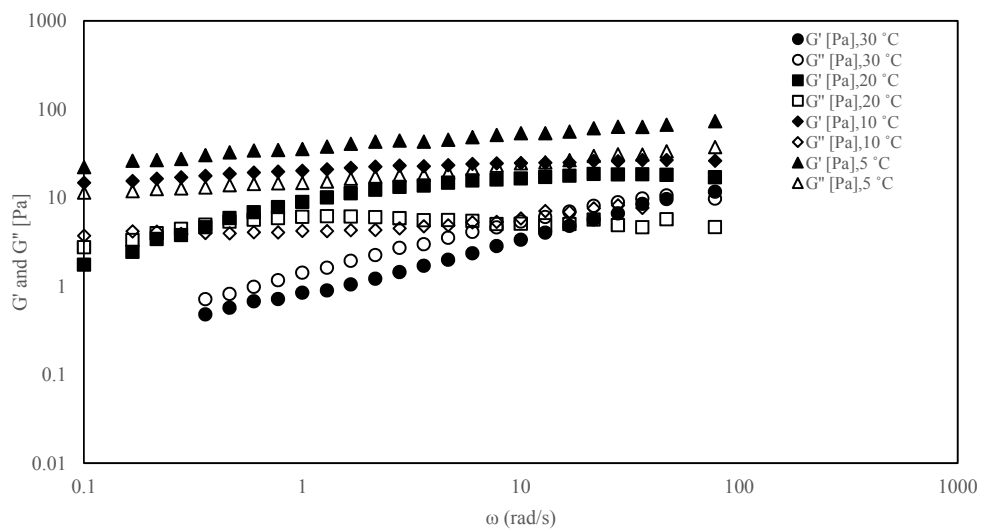
(b) LPC : PC 36:28
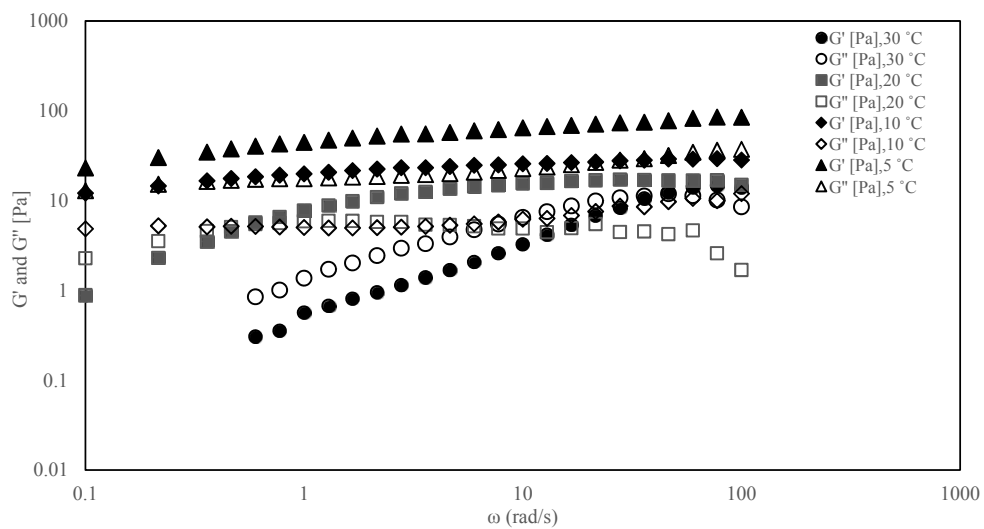
(c) LPC: PC 40:24

(d) LPC: PC 44:20

Fig.13 Storage modulus (G', filled symbol) and loss modulus (G", open symbol) varying as a function of angular frequency (ω) for a mixed leci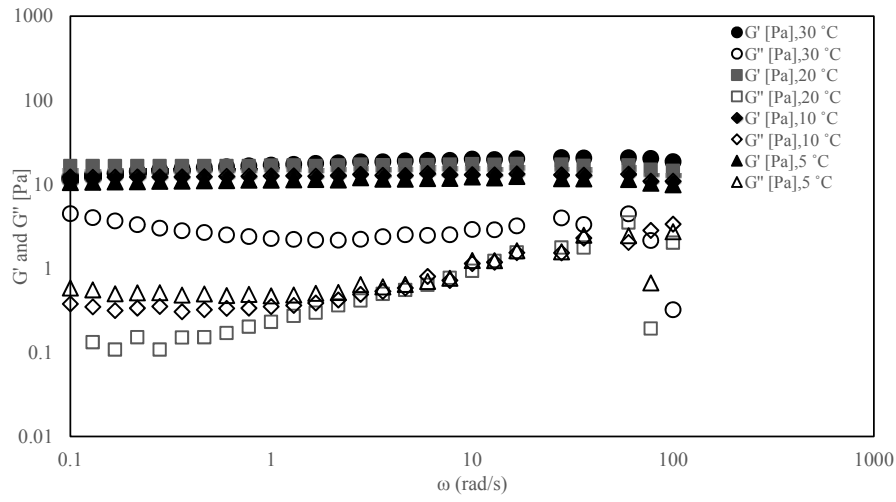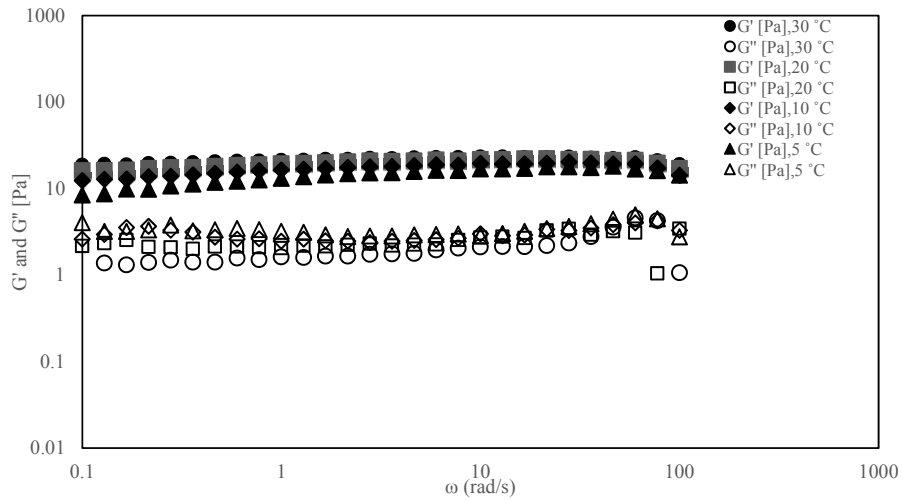thin solution with the composition between LPC and PC is 32:32 (a), 36:28 (b), 40:24 (c) and 44:20 (d) at various temperature.

A variation in G' and G'' versus an angular frequency for the LPC: PC system is 32:32, 36:28, 40:24 and 44:20 were shown in Fig 13a-d, respectively. The G' value represented elasticity, while the G'' value replaced viscous of samples. For LPC: PC = 32: 32 and 36: 28 systems, at low temperature range from 5 to 10˚C, both of G' and G'' values were independent as a function of ω and no relaxation time. Besides, G' also exceeded G'' over range of ω, indicating the gel-like behavior with infinite relaxation time. On the other hand, at intermediate temperature between 20 and 30 ˚C, the mixtures became more fluid response. At 20˚C, both of samples exhibited a viscoelastic behavior. Both of G' and G'' value raised with the increasing of ω, and G'' was slightly greater than G'. However, at high frequency, G' exceeded G'' with a plateau region. Moreover, the samples also showed a crossover point between G' and G''. This phenomenon is presumably consisted with Maxwell model, which used to explain the dynamic behavior of wormlike structure. Generally, the rheological behavior of wormlike micelle is considered by the balance between breaking and reptation of the micelles. If the breaking time is sufficiently shorter than reptation time, the sample responses follow a single Maxwell model. The Maxwell equation is described as bellows. (Hashizaki K. *et al.*, 2009; Shchipunov Y. A., 2001; Yan H. *et al.*, 2014)

$$G' = (\omega^2 t_R^2 G_0) / (1 + \omega^2 t_R^2) \qquad (2)$$

$$G'' = (\omega t_R G_0) / (1 + \omega^2 t_R^2) \qquad (3)$$

where, G_0 is the plateau modulus at high frequency, t_R is the relaxation time at the G' = G''. The Maxwell curve (solid line) was shown in Fig 14a-b. The experimental data of both samples were not fitted well with the Maxwellian curve based on equations 2 and 3, indicating all of samples did not form a wormlike structure. The frequency sweep data also ensured by the Cole-Cole plots, our result supported that the samples were not consisted with the wormlike micelle. The experimental data did not fit with Maxwell curve (data not shown).

At 30 ˚C, both of samples exhibited more fluid like with G'' exceeding G' at low and intermediate frequency. And both of values increased with frequency.

Based on the above falling ball and dynamic frequency result, it could conclude that the systems of LPC and PC with molar ratio were 32:32 and 36:28 were not a suitable molar ratio to formation of wormlike micelle. The high viscosity with turbidity reasonable occurred because of a large planar structure at low temperature. In contrast, a low viscosity with a slightly transparent at intermediate temperature presumably caused a reduction of the planar micellar size and/or a transition to form other shape. (Lee H. Y. *et al.,* 2010)
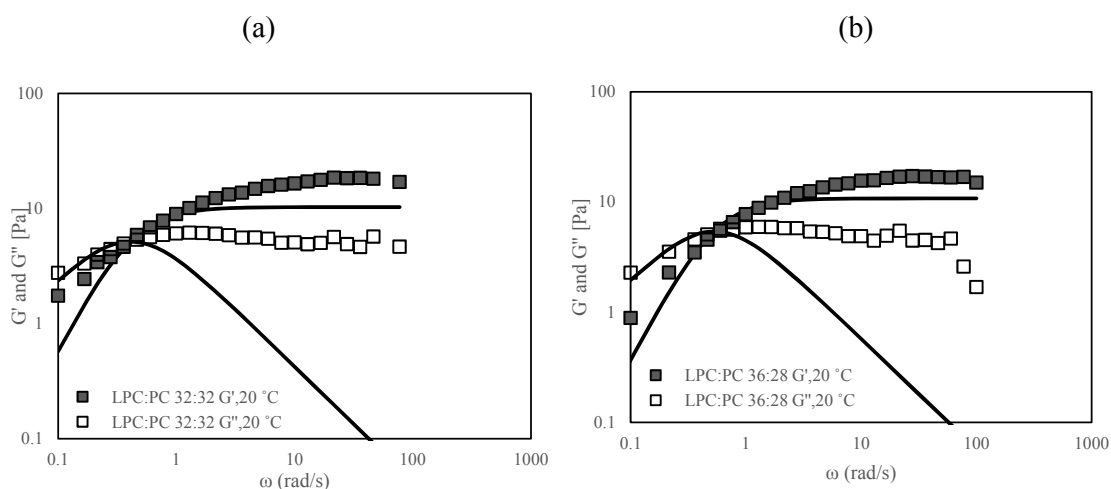
Fig.14 Rheological data for LPC: PC = 32:32 (a) and 36:28 (b) at 20˚C: frequency sweep rheology (a) G' (filled symbols) and G" (open symbols) as a function of frequency. The solids curve is the best fit of calculation using Maxwell model.

For LPC: PC 40: 24 solutions, G' and G" modulus exhibited a weak depending on the over range of frequency with G' exceeding G" modulus at any temperature. At temperature range from 5 to 10 ˚C, the gap between the G' and G" modulus at low frequency quite maintained, but no intersection between G' and G". This implied that the relaxation time of this sample was very high and fell outside the experimental window. However, we found that the gap was broader at 20 ˚C and became narrow at 30 ˚C, suggesting the relaxation time at 20 ˚C was longer than at temperature range 5-10 ˚C and relaxation time became shorter at 30 ˚C.

34

Therefore, we could summarized from falling ball measurement and frequency sweep test that a flexible wormlike micelle possible formed in LPC: PC =40:24 system at temperature range from 5 to 10 ˚C, reflecting a high viscosity and clear solution. However, the sample show higher in viscosity with transparent at 20 ˚C., indicating the expanding of wormlike structure. And a dropping in viscosity at 30 ˚C was possible occurred because of a reduction of entanglement of network or diminution of the length of wormlike structure.

For LPC: PC = 44:20 system, G' and G" were very weak dependent with frequency at all range of temperature. G' exceeded G" and no crossover of G' and G" was observed over range of frequency at any temperature, suggesting the sample responded   gel-like behavior. However, G' decreased with a reduction of temperature, thereby, sample became liquid-like at low temperature, causing a drop in viscosity. From above result and falling ball test revealed that at temperature 5-20 ˚C, a low in viscosity with transparent suggest the rod-like structure and converted to wormlike structure at 30 ˚C. Therefore, the sample at 30 ˚C, presented a very high viscosity with transparent and the relaxation time is very long than the accessible experimental window.

2.4   Conclusion

We studied the system of mixed lecithin based on LPC and PC in aqueous solution with molar ratio is 32:32, 36:28, 40:24 and 44:20 at varied temperature. Our result suggested that the LPC: PC system is 32:32, 36:28 could not form wormlike structure at any temperature, while the LPC: PC is 40:24 and 44:20 solution could aggregate to form wormlike micelle at specific temperature. From our report suggested that the optimize temperature in order to form a wormlike structure for LPC: PC = 40: 24 is temperature range from 5 to 30 ˚C, while LPC: PC = 44:20 had an ability to form wormlike micelle only at 30 ˚C. We hoped that our report is useful to better understanding about this system and possible apply in several fields such as oil field, cosmetics, health product care.

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Chapter3

Effect of temperature on Reorganization of wormlike structure based on Phosphatidylcholine (PC) and Lysophosphatidylcholine (LPC) with different ratio

Abstract

The reorganization of wormlike structure formed by mixing Lysophosphatidylcholine (LPC) solution with Phosphatidylcholine (PC) dispersion at 80 ˚C for 30 minutes followed by cooling for 24 hours in order to form a long wormlike structure at low temperature was determined. The samples were cut by silversons machine before rheological measurement. Form previous reports, we found that only LPC: PC = 40:24 at 5-30 ˚C and 44: 20 solutions at 30 ˚C could form wormlike structure. Therefore, in this study, only these samples were investigated the recovery properties. Form our result showed that the viscosity of samples only at 30 ˚C increased as a function of time, that is, the length of wormlike structure expanded with increasing time. In the other word, the sample displayed a reformation of wormlike micelle at specific temperature. Furthermore, the steady-shear measurement also confirmed our result which the samples exhibited a shear-thinning behavior, corresponding to the characteristic of wormlike structure.

3.1 Introduction

Lecithin are fatty substances which can used zwitterionic surfactants. Therefore, at critical micelle concentration, lecithin have ability to self-assembly into a several of micellar structure depending on the chemical structure of each lecithin such as vesicle, spherical, planar or wormlike structure. For our experiment, we focus on lecithin called Phosphatidylcholine (PC) and Lysophosphatidylcholine (LPC) which has an ability to form wormlike micelle.

Wormlike micelles are a long flexible cylindrical micellar structure. Wormlike micelles can entangled into transient network same as polymer, causing viscoelastic properties to the solution. The network of these micelles is reversible, that is, constantly break down and reform spontaneously in a time scale. These micelles have been observed and explained in a wide variety of surfactant types. However, there are only a few reports studied on wormlike micelle formed by zwitterionic surfactants so far. Therefore, better understanding about the formation of wormlike micelle based on zwitterionic surfactants is advantage for application in several fields such as oil recovery, drag reduction and health care products. (Chu Z. *et al.,* 2013; Raghavan S. R. *et al.,* 2002)

From previous report, we found that some of mixed lecithin systems could form wormlike structure at specific temperature, which is LPC: PC = 40:24 system at 10-30 ˚C and LPC: PC 44:20 at 30 ˚C. Therefore, in this chapter, we will investigate the restructure of wormlike micelle which is a specific characteristic of wormlike micelle. The viscosity as a function of time for this sample was determined after holding at specific temperature for at least 24 hours followed by blending. The steady- shear measurement was also used to confirm the characteristic of wormlike.

3.2 Material & Method

3.2.1 Material

Egg yolk phosphatidylcholine (PC) and lysophosphatidylcholine (LPC) were obtained from Kewpie Corporation(Tokyo, Japan). Distilled water was provided from Water Purifier (Autostill WA500, Yamato Scientific Co., LTD., Tokyo, Japan)

For preparation, PC was mixed with LPC in aqueous solutions by using a Silverson Machines Limited L5M-A (HP5 1pQ, Waterside, Chesham, Bucks, England) for 10 minutes at 40˚C. Further, these mixtures were heated up at 80˚C for 30 minutes followed by cooled down at specific temperature for at least 24 hours for ensuring equilibration before investigation of rheological properties. After storage, the samples were blended with Silverson machine before considering their viscosity by Haake (MARS II) rheometer.

3.3.2 Rheological measurement

Rheological properties were performed on a Haake (MARS II) rheometer. A coquette with a cup of 27.208 mm diameter and a bob of 25.080 mm diameter were used. A cover was used to prevent the evaporation of the samples during measurement. The lecithin mixtures which showed a characteristic of wormlike structure from chapter two were investigated at specific temperature. Before investigation of rheological properties by Haake (MARS II) rheometer, the samples were blended for 2 minutes with Silverson Machines at specific temperature for cutting the long length wormlike structure to short wormlike. A high in viscosity with transparent indicating a long wormlike structure.

3.3 Result and Discussion

(a)
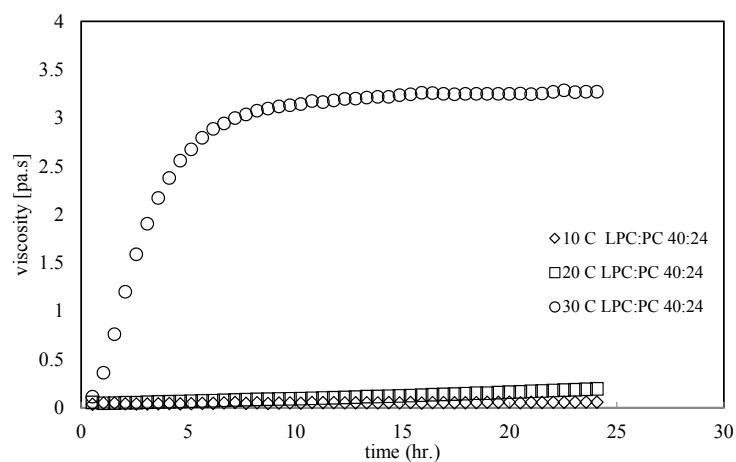
(b)

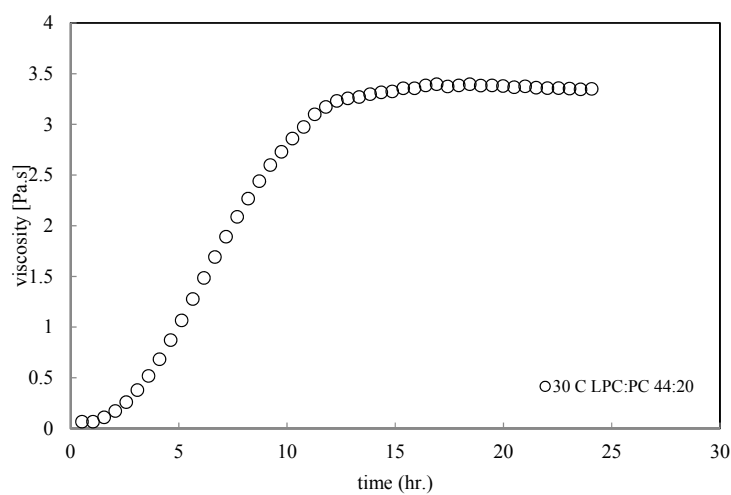Fig.15 Viscosity versus time for 64 mM lecithin mixtures of LPC: PC = 40:24 (a) and 44:20
(b) in aqueous solutions. The sample was kept in cooler incubator at specific
temperature for 24 hours for equilibration. Sample was blended before rheological
measurement. The diamond symbols (◊), square symbols (□) and circle symbols (○)
for the sample was determined at 10 ˚C, 20 ˚C and 30 ˚C, respectively.

Generally, LPC were formed a spherical micelle because of their critical packing parameter (CPP) is less than 1/3. While PC with CPP is between ½ and 1 were aggregated to form a large structure such as planar, vesicle or bilayer structure (Gennis R. b., 1989)

However, form our previous research, by heating process lecithin mixtures at 80˚C, the samples transition from turbidity with low viscosity to a clearness with slightly viscous. Since at high temperature, the samples had ability to overcome the kinetic barrier and the alkyl chain of samples were also melted, resulting in a higher molecular mobility and flexibility of samples. This increased mobility caused a formation of wormlike structure (Natdanai *et al.,* 2014). In this experiment, the samples were kept at specific temperature for 24 hours after heating. The samples were observed still colorless while the viscosity became higher that mean the samples formed longer wormlike structure. And the viscosity of samples were lower after blending by Silverson machine because of a reduction in length of wormlike micelle.

Changing of viscosity with time of LPC: PC = 40: 24 and 44:20 solutions at different temperature were shown in Figure 15. For LPC: PC = 40: 24 systems, the viscosity was quite low and slightly increased with increasing time at 10 and 20 ˚C, suggesting the sample could not reform a wormlike structure at this range of temperature. It possible caused a slow movement of micelle at low temperature and it required longer time to reformation. In contrast, this sample demonstrated a dramatic increase in viscosity with time at 30 ˚C, causing the rearrangement of wormlike structure from short worms to long wormlike micelles. Further, the viscosity demonstrated a steady value after holding at least 10 hours. This indicated that this sample formed a stable structure. The LPC: PC =44:20 also presented a similar tendency with LPC: PC = 40:24 at 30 ˚C which showing the increase in viscosity followed by a maintain value. In other word, this sample also had reformation ability same as LPC: PC = 40:24 system at 30 ˚C. However, this sample could reform long wormlike micelle after 15 hours, that mean, LPC: PC = 40:24 had a recovery ability greater than this system.
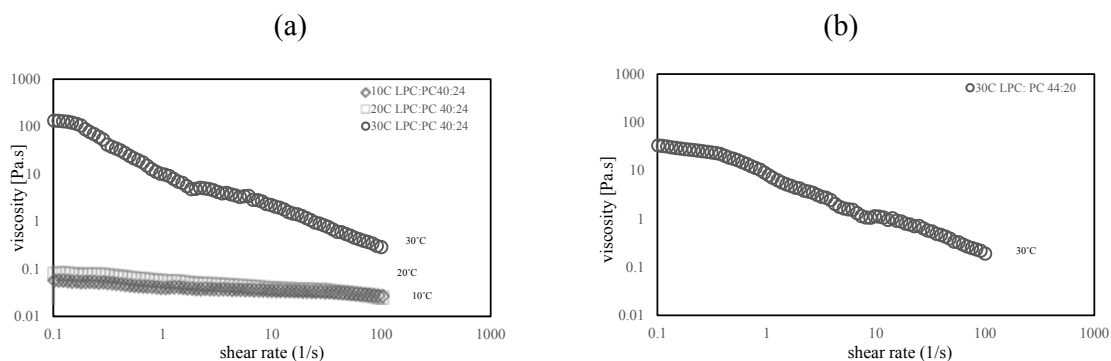
Fig.16 Viscosity plotted as a function of shear rate for LPC: PC molar ratio = 40:24 at 10-30˚C(a) and 44:20 at 30 ˚C. The diamond symbols (◊), square symbols (□) and circle symbols (○) for the sample was determined at 10 ˚C, 20 ˚C and 30 ˚C, respectively.

To ensure an increase in viscosity is corresponding to the restructure of wormlike micelle. The sample was investigated the steady shear measurement after the sample was cut and kept for 24 hours.

Figure 16 demonstrated the variation of viscosity as a function of shear rate for mixed lecithin solutions at specific temperature. In case of LPC: PC = 40:24 system, the sample at temperature range between 10 and 20 ˚C showed a Newtonian fluid like behavior, expecting a rod-like behavior. However, the sample at 30 ˚C exhibited a Newtonian flow at low shear rate further followed by a shear thinning behavior which a viscosity decrease with shear rate at high shear rate. This behavior corresponded to the presence of typical of wormlike structure. The long wormlike micelle will align in same direction at high shear rate, resulting in a drop in viscosity. (Chu z. *et al.,* 2010; Raghavan S. R. *et al.,* 2002; Fan H. *et al.,* 2010; Yan H. *et al.,* 2014). A zero shear viscosity (plateau viscosities) of sample was around 100 Pa.s.. LPC: PC = 44:20 system at 30 ˚C also demonstrated a shear-thinning behavior as evidence for the formation of wormlike structure. Comparison both of samples at 30 ˚C, a zero shear viscosity of LPC:PC 40:24 solution was greater than LPC: PC = 44: 20 solution, indicating a longer length of wormlike micelle.

3.4 Conclusion

We investigated the reorganization of wormlike structure in LPC: PC = 40:24 and 44:20 systems. The sample was kept at specific temperature and cut before determined its reformation. Our results showed that the viscosity of the both samples at 30 ˚C increased with time, indicating all of them had an ability to restructure wormlike micelle. However, LPC: PC = 40: 24 system formed only short rod-like at 10-20 ˚C. It is reasonable that the samples require a long period of time in order to reformation. Moreover, the steady shear measurement also supported an increasing of viscosity consist with the wormlike structure. The samples exhibited a shear thinning behavior, which is a typically wormlike characteristic.

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Chapter4: General conclusions

Phosphatidylcholine (PC) and Lysophosphatidylcholine (LPC) are zwittrionic surfactants which their structure consist of positive and negative charge, thereby they have an ability to form several types of micelle depending on external environments such as temperature, pH, salt and solvent. In this research, we focus on the wormlike structure formed by these surfactants. There are a few reports has been studied on a wormlike micellar structure of these surfactants. In general, PC formed a reverse wormlike micelle in non-polar solvent or water with addition of salt. Recent report, however, found that wormlike structure could formed by mixing both of surfactants at appropriate total concentration and temperature. That is the wormlike structure formed with addition of LPC and PC with total concentration above 56 mM at temperature more than 45˚C. In this work, we interested in the stability of wormlike micelle based on LPC and PC after heating followed by cooling at specific temperature. For preparation, LPC and PC with different molar ratio concentration were mixed and heated up at 80˚C to induce the onset of formation of wormlike structure. Further, the samples were hold at temperature range from 5 to 30 ˚C for a long time.

Falling ball measurement is an effective technique to observe an appearance and determine the flowing of materials. From our falling ball result, a transparent with low viscosity at temperature range between 5-10 ˚C was observed in LPC: PC = 44: 20 system. The sample showed a dramatic increase in viscosity with increasing temperature but the transparent still maintained. For LPC: PC =32:32 and 36:28 exhibited a similar response, that is, the samples had a high viscosity, turbidity at low temperature and switch to low viscosity with slightly transparent at intermediate temperature. However, the LPC: PC = 40:24 systems presented a viscosity increased with temperature until a maximum followed by a decreased in viscosity.

The oscillation frequency sweep is a valuable tool for investigation the dynamic properties of materials. This revealed that the wormlike structure was only observed in LPC: PC = 40:24 at temperature range 10 to 30˚C and LPC: PC = 44:20 at 30 ˚C.

Furthermore, the reformation of wormlike micelle of both systems at specific temperature also investigated. Our result showed that both of them had ability to restructure at 30 ˚C and the reorganization for LPC: PC = 40:24 system was faster than LPC:PC = 44:20 system. After cutting, the sample at 10 and 20 ˚C could not reform wormlike structure. It formed only rod-like micelle. The reason is probable the micelle move quite slow at low temperature, thereby, it require long period of time to recombine the wormlike structure.

However, our research studied on only macrostructure for mixed lecithin of LPC and PC solutions. To get more understanding about this system, the microstructure should be proved by specific techniques such as small-angle neutron scattering (SANS) and cryo – transmission electron microscopy (cryo-TEM) in order to apply in several fields such as food industry, oil industry, drug delivery, personal care products or future developments.

Acknowledgements

First, I would like to express my gratitude to my supervisor, Professor Dr. Matsukawa Shingo, for accepting me as a student in his laboratory and for his support, valuable advice and guidance throughout my thesis. I would like to express my grateful thank to my committee members, Assistant Professor Dr. Tashiro Yuri and Professor Dr. Suzuki Toru for their comments and suggestion on my thesis. My thanks also extended to all laboratory members and staff for their help during my research.

My sincere thanks to Ms. Paphawee Nantarajit, Ms. Yada Sangpring, Dr. Natdanai Fafaungwithayakul, Thai friends and Japanese friends for any helps during my stay in Japan.

Special Thank to my family and my friends in Thailand, who always support and motivation.

Finally, I am grateful to thank the Ministry of Education, Culture, Sports, Science and Technology (Monbukagakusho: MEXT) and Tokyo University of Marine Science and Technology for scholarship and opportunity to study in Japan.