ナノエマルジョンの合体安定性の新規研究手法の開発

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博士学位論文内容要旨
Abstract of Dissertation

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論文題目 Title	Development of new methodology to investigate coalescence stability in nanoemulsions		

Nanoemulsions are colloidal dispersions of immiscible liquids where one liquid distributes as submicron-size droplets in another liquid. Nanoemulsions have been widely used in food application for encapsulation bioactive compounds, nutrients, vitamins, colors, flavors, and preservatives because of their optical property, high surface area, long-term stability, and high bioaccesscibility. The most commonly used emulsifier to fabricate nanoemulsions is surfactant which are amphiphilic surface-active molecules that can reduce surface tensions of oil and water and facilitate droplet formation. Phosphatidylcholine (PC) is regarded as a zwitterionic surfactant because it contains both positive and negative charges in head group which stabilizes droplet through electrostatic stabilization. Sodium oleate (Na-oleate) is a synthetic amphiphilic molecule that contain positive charge in head group giving electrostatic stabilization. Tween60 is a nonionic surfactant which stabilizes droplet through steric stabilization. Tween60 provides a good stability in mild acids, alkalis, and electrolytes. In recent years, solid particles have been used to stabilize emulsions which are called Pickering emulsions. The particles have no surface-active ability but they can be wetted by oil and water. Unlike surfactants, adsorption of the particles at the oil-water interface is irreversible. Cellulose nanocrystals (CNC) are rod-shape particles obtained from cellulose, they can be used to stabilize nanoemulsions because they have nano-scale dimension.

One of the challenging problems in nanoemulsions study is the detection of nanoemulsions instability which the difficulty comes from their small droplet size. We investigated the effect of mechanical stresses on nanoemulsions coalescence stability by fluorescence microscopy image analysis. Two fluorescent dyes, nDiO (green fluorescence) and DiI (red fluorescence), were dissolved in squalane as oil phase (5%w/w) of nanoemulsions. The aqueous phase contains surfactant solutions or CNC solutions. The mixture of nDiO and DiI nanoemulsions at ratio 1:1 were treated with mechanical stresses, (i) a gentle mixing by a spoon, (ii) a mild mixing by a stirrer, (iii) an intermediate mixing by vortex, (iv) a moderate mixing by rotor-stator, and (v) a slashing mixing by microfluidizer. The mixtures were determined droplet size distribution by light scattering and their mixing behaviors using fluorescence microscope and the data were analyzed using the lab-made

program of image analysis. The image analysis program was developed for calculation of the mixing ratio (R_{mix}) between red and green dye intensities in the droplets where $R_{mix} = 0$ and 1 are referred to isolated green and red droplets, respectively and R_{mix} around 0.5 are referred to mixed droplets.

The first experiment, all nanoemulsions mixtures after subjecting to the mechanical stresses did not demonstrate the change in their droplet size distribution except Tween60 nanoemulsions treated by moderate mixing. It showed droplet aggregation by observing multimodal distribution and cloudy appearance. The PC, Na-oleate, and Tween60 nanoemulsions showed mixed droplets when they were treated with mechanical stresses. These mixed droplets referred two possible cases (i) droplet coalescence and (ii) droplet aggregation because the fluorescence microscopy could not discern the droplet that are close in proximity attribute to its spatial resolution. The gentle, mild, and intermediate mixings could promote droplet encounter leading to aggregation but these mixings were not strong enough to promote droplet coalescence, while the moderate and slashing mixings could raise droplet aggregation and coalescence depending on the interfacial layer integrity. Moreover, PC nanoemulsions exhibited better stability against mechanical stresses comparing with Na-oleate and Tween60, it is corresponding to high electrostatic repulsion of PC on the droplet surface that prevents droplets aggregation.

The second experiment, the fluorescence microscopy image analysis was applied to study instability of CNC nanoemulsions against the mechanical stresses. The sulfated and desulfated CNC were used to prepare nanoemulsions referring to high charge and low charge particles. The desulfated CNC exhibited better performance to form the droplets. However, the sulfated CNC particles could stabilize the droplet when their charge was partially screened by a counter ion. The sulfated CNC nanoemulsions were stable against the mechanical stresses by observing high frequencies at $R_{mix} = 0$ and 1, except the slashing mixing by microfluidizer gave high frequencies at R_{mix} around 0.5. On the other hand, the desulfated CNC nanoemulsions were prone to aggregate under gentle to moderate mixings because of low repulsion between the droplets and slashing mixing caused coalescence by intensive shearing and high pressure at impingement area.

In conclusion, the fluorescence microscopy with lab-made image analysis program provided an evidence of nanoemulsions instability in microscopic level which could not detect using the light scattering technique. However, it could not recognize adjacent droplets that has distance less than 700 nm due to the limitation of its spatial resolution. This developed methodology is applicable for studying instability of nanoemulsions and macroemulsions without altering their microstructure.