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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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博士学位論文要約  
Summary 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 two immiscible liquids in which one liquid distributes as submicron-sized 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 bioaccessibility. One of the challenging problems in nanoemulsions study is the detection of nanoemulsions instability which the difficulty comes from their relatively small droplet size. We investigated the effect of mechanical stresses on nanoemulsions coalescence stability by fluorescence microscopy image analysis.

The concept of study was detecting of mixing of fluorescent dyes that were dispersed inside the oil droplets. 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 cellulose nanocrystal solutions. Three types of surfactants were compared the stability including phosphatidylcholine (PC), sodium oleate (Na-oleate), and Tween60. Consequently, two type of cellulose nanocrystals were also compared the stability including sulfated type cellulose nanocrystals (sul-CNCs), and desulfated type cellulose nanocrystals (desul-CNCs). The mixture of nDiO and DiI nanoemulsions at ratio 1:1 were treated with mechanical stresses, (i) gentle mixing with a spoon, (ii) mild mixing with a stirrer, (iii) intermediate mixing with a vortex, (iv) moderate mixing with a rotor-stator, and (v) slashing mixing with a microfluidizer. The mixtures were determined droplet size distribution by light scattering and their mixing behaviors using fluorescence microscope and the data were analyzed using a 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 refer to isolated green and red droplets, respectively which are resulting from non-coalescence droplets and  $R_{mix}$  around 0.5 refer to mixed droplets which are resulting from coalescence droplets.

All surfactants-stabilized nanoemulsion mixtures subjected 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 the multimodal distribution and cloudy appearance. The PC, Na-oleate, and Tween60 nanoemulsions exhibited 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 were close in proximity, which was attributed 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, whereas the moderate and slashing mixings could raise droplet aggregation and coalescence depending on the interfacial layer integrity.

The fluorescence microscopy image analysis was performed to study the instability of CNC-stabilized nanoemulsions against mechanical stresses. The sulfated and desulfated CNCs were used to prepare nanoemulsions referring to high charge and low charge particles. The desulfated CNCs exhibited better performance in forming the droplets. However, the sulfated CNC particles stabilized 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  $R_{\text{mix}} = 0$  and 1, except for the slashing mixing by microfluidizer gave high frequencies at  $R_{\text{mix}}$  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 the impingement area.

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