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Method for the Preparation of Secondary Emulsions of Fish Oil in Water

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Abstract

The method for the preparation of stable secondary emulsion has been detailed and discussed in terms of polymer capping and emulsion ratio. The dispersion of omega-3 oil in water by emulsion stabilized with casein was further coated with a polycationic polyelectrolyte, Poly (dially dimethyl ammonium chloride). The stability of the emulsion was studied by turbidity measurements using a UV-visible spectrophotometer. The particles size of the primary and secondary emulsion was investigated by light scattering and zeta potential measurements. Results shown in this article provide some details about the mechanism of the formation of secondary emulsion, mainly regarding the polymer/emulsion-mixing ratio.

Key words : Emulsion, Secondary emulsion, Polyelectrolyte

Introduction

Omega-3 fatty acids are a family of polyunsaturated fatty acids that human being cannot synthesize, but which can be found in nut, fruits, or in fish. Oil containing the precious omega-3 can be used from the waste of the fish industry in the form of fish oil such as tuna oil. These long-chain omega-3 fatty acids are considered beneficial for growth and development throughout the life cycle. However, omega-3 fatty acids are highly unsaturated and therefore are highly susceptible to oxidation.(1) Oxidation of lipid is a major cause of deterioration in the quality of products that affects many characteristics such as flavor, color and nutritive value. Lipid oxidation can occur under free radical mechanism.

Emulsions can be seen as a dispersion of two immiscible phases usually described as oil-in-water (O/W) or water in oil (W/O). The microcapsules have a size which can range from a few nanometers, in the case of liposome or micro-emulsion, to several hundred nanometers as found in traditional emulsions.(2) In order to stabilize the microcapsule, an emulsifier is needed to reduce the surface tension between the two phases and prevent coalescence of the droplets. Factors such as type of emulsifiers and the ratio of emulsifier and oil type control the size of emulsion droplets. Oil-in-water emulsions are prepared by homogenizing oil and aqueous phase in the presence of an emulsifier. Proteins such as whey protein, casein or albumin have been used as emulsifiers forming, interfacial membrane around the emulsion.(3) This interfacial membrane can be used to inhibit lipid oxidation by decreasing the interactions between the omega-3 and transition metals, such as Fe and Cu, in the aqueous phase. Recently, the layer-by-layer assembly technique was used to prepare multilayer interfacial membrane coated emulsion droplet and further prevent the oxidation of oil.(4,5) In the layer-by-layer technique, a polyelectrolyte layer is formed on a charged surface due to strong electrostatic attraction between the surface and oppositely charged polyelectrolyte molecules in solution. The basic method of producing multilayer oil-in-water emulsions can be summarized as follows: The primary emulsion is prepared by homogenizing oil and water phases in the presence of a negatively charged emulsifier. The 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 of oppositely charged polyelectrolytes to form the tertiary emulsion.(6-7) This layer-by-layer technique has been reported and extensively studied by Decker and coworker in a series of reports which demonstrated the anti-oxidative or

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higher mechanical resistance of the final multilayer emulsion. Yet, although their work used multilayer stabilized emulsion, no report has been made about the method to prepare the secondary emulsion and more particularly what polymer / emulsion ratio should be used.

In the present article we have refined and detailed the method applied in the preparation of secondary emulsions. UV-Visible spectroscopy was used to monitor the solution turbidity as an evidence of the dispersion of the emulsion or its aggregation as a function of the primary emulsion / capping ratio. Zeta potential measurements and light scattering were also used to monitor the formation of the secondary emulsion.

Materials and Experimental Procedures

Chemicals

Poly (dially dimethyl ammonium chloride) (PDADMAC) and Casein sodium salt(sodium caseinate) used in the preparation of the secondary emulsion were purchased from Sigma Aldrich (USA). Tuna oil was kindly provided by T.C. Union Global Company (Thailand). All pH adjustments were made with mixing the appropriate amount of Acetic acid glacial and Sodium acetate purchased from Labscan (USA). Double distilled water produced in our laboratory was used in all experiments.

Emulsion Preparation

Primary emulsions were prepared by homogenizing a mixture of 5% tuna oil in 95% water solution containing 1.5%w casein, with a homogenizer (Polytron PT3100, USA) at 20,000 rpm. 5 successive homogenization cycles of 2 min were used for the preparation of each types of emulsion solution (either primary or secondary). Secondary emulsions were prepared by adding the primary anionic emulsions into a cationic polyelectrolyte 10 mM PDADMAC solution at pH6. The PDADMAC acted here as a capping agent. The volume ratio between primary emulsions and polyelectrolyte (PDADMMAC) was varied from values equal to 0, 0.1, 0.3, 1 and 2. The mixed solution was then characterized by measuring the % transmission using a UV-Visible spectrophotometer (Analyticjena, S100, Germany) at 550nm, which correspond to a turbidity measurement. The particle size diameter and zeta potential of secondary emulsions were characterized by using the Zeta-seizer nano series (USA).

Results and Discussion

In order to prepare a suitable primary emulsion, the mixture of oil, casein and water needs to be homogenized to induce the formation of the microscopic droplets. The energy provided to the system by mechanicals means using the homogenizer is necessary for the formation of the oil/water interface, which is energetically unfavorable and not spontaneous. When the homogenization steps are increased the solution becomes more turbid until the transmission remains stable. Five homogenization steps were found to be sufficient to achieve constant turbidity, suggests minimal particle size. The pH of the solution was fixed to pH 6 in order to provide a negative charge to the surface of the emulsion droplets. The pH value equal to 6 is sufficiently far from the iso-electric point of the casein, which has a value of 4.5, to insure complete ionization of the protein to inhibit droplet coalescence and flocculation.

Figure 1 shows a diagram depicting the preparation of the secondary emulsion. The negatively charged droplets found in the primary emulsion are mixed with a polycationic polyelectrolyte (PDADMAC). This polyelectrolyte was conveniently picked due to its pH stability since the presence of quaternary amine induces total ionization at all pH ranges from 1 to 14. The mixing order of the two constituents, namely the primary emulsion and the PDADMAC, is of great importance (data not shown), and successful mixing is only possible if the emulsion is dropped into the PDADMAC solution. If opposite steps are taken and the PDADMAC is poured into the emulsion solution, a white precipitate is rapidly formed. This precipitate is due to the high molecular weight of the PDADMAC, which will induce rapid flocculation of the droplets by bridging. The bridging of droplets by high molecular electrolytes can be avoided by reversing the order of mixing, which means adding the emulsion to the PDADMAC solution.

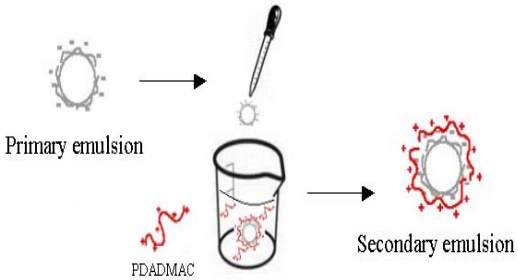

Figure 1. Schematic depiction of the formation of secondary emulsion.

Beside the order of mixing, it is necessary for the preparation of secondary emulsions to find the ratio amounts of primary emulsion and PDADMAC solution that are to be mixed. The need for the proper ratio is due to the fact that if the PDADMAC concentration used is too high, excess PDADMAC will remain in solution and could prevent the formation of tertiary emulsion through complexation if one desired to prepare such emulsion. If on the other hand, not enough PDADMAC is used, primary and secondary emulsion will coexist in the solution leading to the aggregation and flocculation of the oppositely charged emulsion. Figure 2 shows the effect on % transmission of the molar ratio between volume of primary emulsion and a 10mM PDADMAC solution. It can be seen that the graph is separated into 3 sections. In the first section (0 to 8ml of added emulsion), the % transmission decreased gradually when increasing the volume of emulsion, which corresponds to the formation of the secondary emulsion. For the second range (8ml to 10ml), the % transmission sharply increases which signifies that the solution becomes clear as a result of the precipitation of the emulsion droplets. This suggests that a complex occurred between the already formed cationic secondary emulsion and the further added anionic primary emulsion. For the last range (12ml-onward), the % transmission was found to decrease due to the addition of primary emulsion making the solution more turbid. This experiment, which can be seen as an optical transmission titration of the emulsion, is very useful to understand the complexation mechanism between the PDADMAC, the primary emulsion and the secondary emulsion.

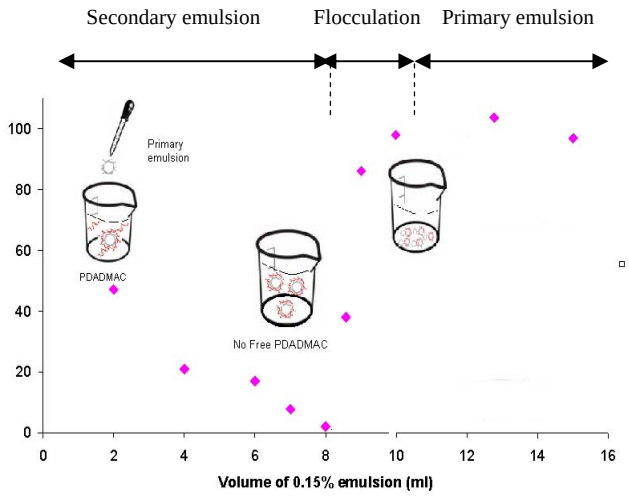

Figure 2. Effect of ratio between the volume of primary emulsion and PDADMAC on %transmission

To further understand the dynamics of secondary emulsion formation, particle size and zeta potential of primary and secondary emulsions were investigated. The volume ratio between volume of primary emulsion (1.5% casein) and the PDADMAC (10mM) solution was varied from 0, 0.1, 0.3, 1 and 2. Figure 3 depicts a graph of the measured particle size of emulsion droplet as a function of the ratio between volume of primary emulsion and 10mM PDADMAC. It can be seen that the initial particle sizes of the primary emulsion is 281nm, which is typical for emulsion prepared by homogenization. The particle size measured for the two mixing ratios of 0.1 and 0.3, found in zone 1, have a particle size of 755 and 706, respectively, which corresponds to the secondary emulsion. As expected upon mixing with PDADMAC, a secondary polyelectrolyte is added to the droplets making them larger. Interestingly, when the volume-mixing ratio of the emulsion with PDADMAC was increased to 1 and 2, the particle size was found to decrease back to its initial value around 250nm. This, in fact, does not represent the desorption of the secondary layer but is the primary emulsion since the secondary has already precipitated at the bottom of the test tube.

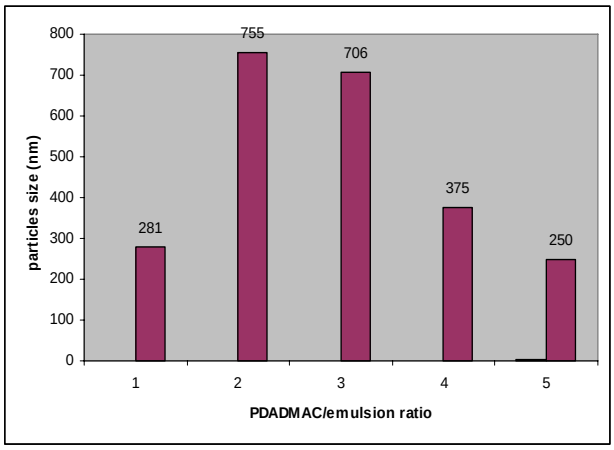

Figure 3. Effect of ratio between the volume of primary emulsion and PDADMAC on particle size

In order to further confirm that the emulsion droplets in zone 3 are of primary nature, zeta potential measurements were executed to investigate the surface charge of the emulsion droplet under various mixing ratios. Figure 4 exhibits the zeta potential of the mulsion droplet as a function of the ratio between volume of primary emulsion and 10mM PDADMAC. It was found that zeta potential initially had a negative value (-16mV), which corresponds to the anionic casein

found in the primary emulsion at pH6. The zeta potential of the emulsion droplets used in the 0.1 to 0.3 mixing ratio was found to be largely positive (+31mV) as a result of the adsorption of the polycationic PDADAMAC. These values further confirm the formation of the secondary emulsion of casein-PDAD. Thus, secondary emulsion completely occurred at volume ratio 0.1 and 0.3. At volume ratio equal to 1 the zeta potential has a value nearly equal to 0 mM, which suggests the presence of neutral particles in the solution. While solution mixed with this mass ratio were found in zone two, which represent the sharp increase in % transmission, it can be understood that they represent the neutralized emulsion droplets between the secondary and primary emulsion. When excess polyelectrolyte is added, the seta potential reverses to –17mM, which corresponds to the value of the zeta potential found for the primary emulsion. This confirms that the particles found in zone three are in fact primary emulsion.

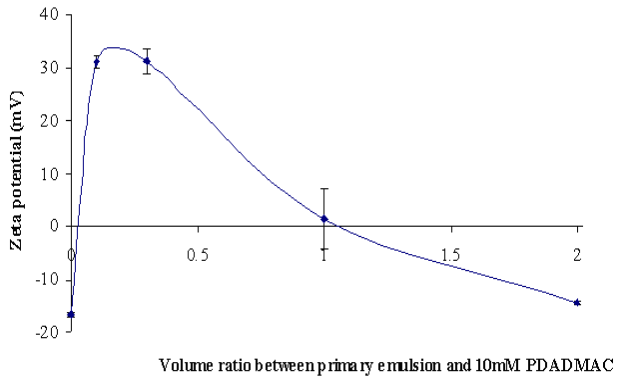

Figure 4. Effect of ratio between the volume of primary emulsion and PDADMAC on zeta potential

Conclusion

The mechanism of the formation of secondary emulsion has been investigated. The molar ratio between the primary emulsion and the secondary polyelectrolyte was found to play a major role in the stability of the solutions, their sizes and surface charges. Turbidity, particle size and zeta potential measurements confirmed that the optimum component ratio is found right before the solution is destabilized and flocculates and equal to 0.3. Maximum zeta potential and maximum particle size suggest the formation of a robust and stable secondary emulsion. These results should allow for the preparation of emulsion coated with a larger number of layers.

Acknowledgements

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