APPLICATION NOTE

Differential Scanning Calorimetry

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Investigating the Destabilization of Solid Emulsions Using Differential Scanning Calorimetry (DSC)

Introduction

Emulsions are systems that consist of two or more liquid phases that are partially or completely immiscible with one liquid being dispersed in the other in the form of droplets. Emulsions constitute an important product class in various industries including the food, chemical and pharmaceutical industries. Emulsions are inherently unstable since the two immiscible liquids have a tendency to phase separate over time in order to minimize the thermodynamically unfavorable interaction between the two or more molecular species. As such, the kinetic stability of emulsions that is their ability to resist phase separation due to destabilization processes such as flocculation, coalescence, or Ostwald ripening is of key importance to manufacturers.

There are a number of methods available to determine the stability of emulsions, with each method having certain advantages and disadvantages. Light scattering is a very common method to determine the stability of emulsions, because it can be used to assess changes in the droplet size distribution of the emulsion. Unfortunately, light scattering does not yield information about differences in composition of individual particles in the emulsions, and therefore, it cannot give specific information on emulsion

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stability if the sample contains chemically diverse structures aside from the emulsion droplets such as, polymer aggregates. Furthermore, light scattering does not yield information about whether the breakdown of an emulsion is due to flocculation (i.e. the aggregation of several droplets to form large flocs) or coalescence (i.e. the merging of several droplets into a larger one). Optical microscopy can in some cases be used to distinguish between flocculation and coalescence, but it is limited to the observation of larger particles (> 500 nm). Moreover, under the microscope and in the absence of specialized staining techniques, air bubbles may appear similar to coalesced oil droplets; and thus, give the researcher false information on the stability of the sample. In contrast, differential scanning calorimetry (DSC) can be used to detect the presence of coalesced emulsion particles, because of a shift in crystallization temperatures. Moreover, DSC is sensitive to the chemical nature of the material and thus the stability of emulsion droplets may be assessed in samples that contain other colloidal particles. Finally, the presence of air droplets will not affect the measurement unless it causes thermal conductivity to be significantly reduced.

In order to understand why DSC is able to assess emulsion stability, it is necessary to understand why alterations in crystallization temperature of emulsions occur when the size of emulsion droplets changes, a phenomena that is related to the degree of supercooling required to induce solidification. Supercooling describes the phenomena of cooling a material below its crystallization point without it becoming solid (Sato and Garti 1988). Supercooling occurs since substantial energy expenditure or activation energy is required to form the initial solid crystal seeds in the liquid. If the magnitude of the activation energy, required for crystal formation, is sufficiently high compared to the thermal energy of the system, crystallization will not occur, even though the transition is thermodynamically favorable. The existence of an energy barrier is also due to the fact that very small crystals are thermodynamically unstable and re-dissolve. Crystallization can therefore only occur if crystals have grown large enough to be stable; otherwise the material will remain in a supercooled state (Sato and Garti 1988). In a supercooled state, the material, which in the case of an emulsion is generally a lipid, may form crystals under two conditions. Firstly, if the temperatures are substantially lower than the crystallization temperature of the bulk lipid, the energy barrier may be overcome allowing the lipid to crystallize. Secondly, the lipid may come in contact with solid impurities in the liquid that may serve as a crystallization template; in which case less activation energy is required for crystallization.

All naturally occurring lipids contain some amount of impurities that can function as a crystal template, and only a small amount of impurities are needed to initiate crystal formation in large volumes of samples. For example, in 1 ml of lipid, numerous impurities can be found that are able to act as nuclei. However, if that 1 ml of lipid is emulsified into 1,000,000 small droplets then only a handful of droplets will contain impurities, while the other droplets are void of them (Figure 1). Thus, if a lipid is emulsified into smaller and smaller emulsion droplets, then less and less volume of the lipid will be in contact with nuclei that are able to lower the required activation energy. As a result, smaller droplets will increasingly crystallize at lower temperatures because majority of the lipid droplets will not contain any nuclei. Conversely, if the oil droplets coalesce and form larger droplets, then increasing volume of lipid will be in contact with nuclei and will; therefore, crystallize at higher temperatures. In other words, an emulsion containing droplets with small droplet sizes crystallize at low temperatures while emulsions containing highly coalesced droplets crystallize at higher temperature (McClements 1999; Helgason, Awad et al. 2008).

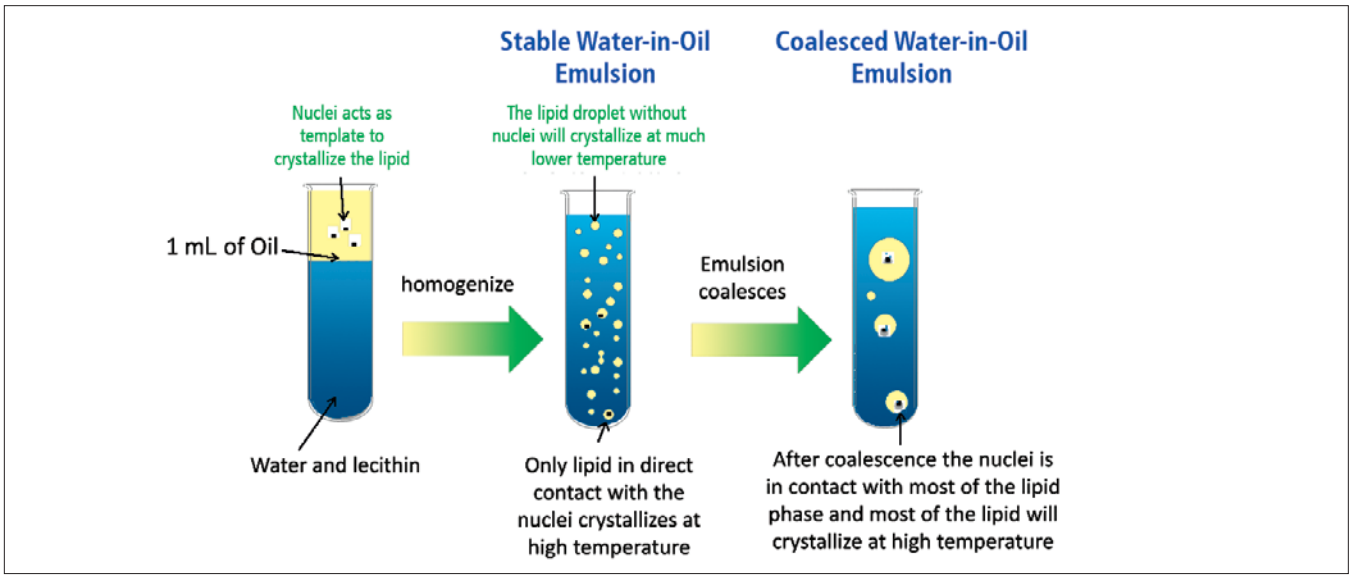

Figure 1. Schematic drawing of the nucleation mechanism in oil-in-water emulsions, and the impact of coalescence.

The temperature at which the crystallization occurs can thus be used to follow the coalescence of emulsified lipid. Most lipids will crystallize above -50 °C. Therefore, a DSC with a two-stage cooling unit is well suited to accurately follow this process. In this application note, we present an example of thermal analytical measurements that may be used by manufacturers and researchers to assess stability of emulsions, particularly those that contain solidified droplets that are obtained by cooling a hot emulsion below the crystallization temperature of the lipid. Many manufacturers have encountered stability issues when attempting to manufacture emulsions that contain solid lipid droplets.

Materials and Methods

A lipid phase [10% (w/w) octadecane] was fully melted by heating to 40-45 °C. The lipid phase was then mixed with a 3% lecithin solution [Alcolec® PC 75, obtained as a gift from Lipoid LLC (Newark, NJ, USA)] held at 40-45 °C in 10 mM citrate buffer. An emulsion premix was made using a hand-held high-speed blender at 100% power for 1 min (Homogenizer Standard Unit, Labworld-Online.com, Straufen, Germany). The hot emulsion premix was homogenized with a thermostated Microfluidizer® 3 times at 10,000 psi (Microfluidics, Newton, MA, USA). This process yielded droplets with z-average sizes of 170.9 ±0.8 nm and polydispersity index of 0.13 ±0.02. Finally, the solution was placed in a refrigerator set at 5 °C to initiate crystallization of the lipid.

Acetic acid was used to titrate the samples to pH 2.8, 3.1, 3.7, 4, and 5. Between 8-10 mg of emulsion was placed in aluminum pans and hermetically sealed. Empty pans were used as a reference for the emulsion samples. Each sample was first heated to 40 °C at 10 °C/min to melt the lipid, and then cooled to 1 °C at 10 °C/min in a differential scanning calorimeter (DSC 8000, PerkinElmer, Shelton, CT, USA). All samples were measured 1 day after pH had been adjusted. Nitrogen 20 mL/min was used as purge gas.

Results and Discussion

At high pH, the surfactant lecithin stabilizes the emulsion droplet because of its high electronegative surface charge. When two droplets come into close proximity, the negative charges on the interface of the droplets will cause an electrostatically driven repulsion causing the droplets to remain separated. When the pH is decreased, the lecithin increasingly loses its electronegativity, which leads to a destabilization of the emulsions since the electrostatic repulsion is no longer sufficient to prevent droplets from coming into contact due to Brownian motion. The pH-induced reduction of surface charges was therefore used to demonstrate how a DSC could be used to investigate stability of emulsions, particularly those that undergo a liquid-solid transition.

An emulsion that contains solidified instead of liquid droplets is often prone to destabilization. At a low pH, the solid particles have a tendency to quickly aggregate, since there are firstly insufficient repulsive forces present between the individual droplets allowing the solid lipid droplets to touch each other. Secondly, the transition from liquid to solid is often accompanied by increases in surface area (since imperfect crystals are formed) causing the surfaces to be insufficiently covered by protective surfactant. Thus, the lipid phases of two solid lipid droplets may be in direct contact, causing them to merge together when the emulsion is heated. As a consequence, many solid lipid emulsions coalesce upon melting. In turn, the coalesced droplets upon cooling crystallize at higher temperature than the uncoalesced particles, a fact that is visible in DSC experiments.

The crystallization onset temperature of octadecane in a bulk phase is 28 °C; therefore, if there is any coalescence, the particles will crystallize close to or slightly below 28 °C. Emulsified droplets on the other hand will crystallize at temperatures far below the crystallization temperature of an octadecane bulk phase. This is demonstrated in Figure 1, where the emulsion was first heated, and then upon cooling at 10 °C/min, crystallization temperatures of 11.37 ±0.18 and 11.32 ±0.23 °C at pH 5 and 4 respectively, are observed. The low crystallization temperatures indicate that the oil remained dispersed in small droplets despite the heating/cooling cycle as long as the pH was sufficiently high (> pH 4). Conversely, at pH 3.7 two crystallization peaks, can be observed; the first one at 25.43 ±0.01 °C and the second at 11.86 ±0.08 °C. The presence of two distinct crystallization peaks indicates that some of the emulsion droplets had begun to coalesce when the emulsion was heated. However, not all the droplets crystallized at the higher temperature indicating that some of the droplets did not coalesce and remained stable. At pH 3.1 and 2.8 only one crystallization peak at 25.22 ±0.01 and 25.24 ±0.06 °C, respectively, were visible. This indicates that at pH 3.1 and 2.8, the emulsion had undergone extensive coalescence due to the fact that the droplets' lipid surfaces had been able to come into direct contact with each other causing a merging upon heating.

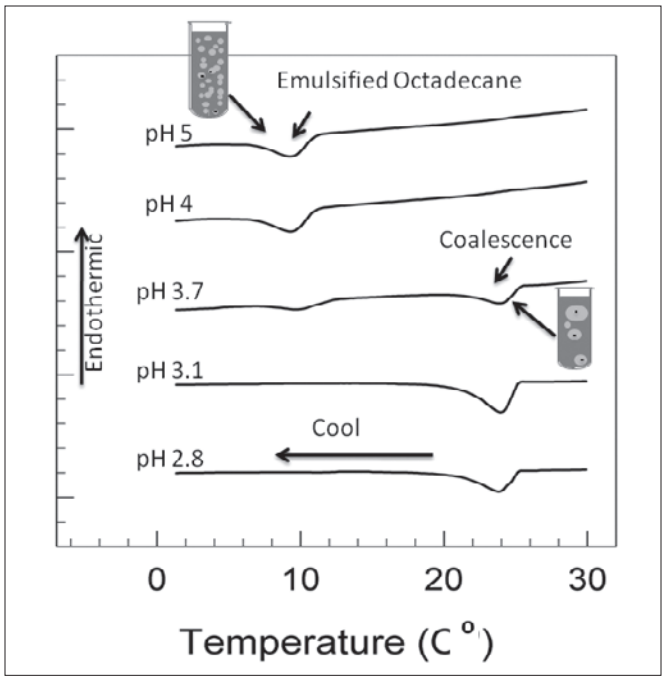

Figure 2. DSC thermographs during cooling of octadecane emulsions, stabilized with lecithin at various pH values.

Conclusion

The brief results shown demonstrate that DSC is an extremely useful method to assess the degree of coalescence in samples, without having to conduct light scattering or microscopic experiments. In combination with microscope or light scattering (dynamic or static) methods, a detailed understanding of ongoing processes responsible for a deterioration of an emulsion's quality can be gained. It should be noted that lipids with relatively sharp crystallization behavior needs to be used, since otherwise the crystallization becomes too complex and it will not be possible to distinguish between coalesced crystallization and crystallization of emulsified lipids. Moreover, high heating and cooling rates can amplify signals obtained from low volume or dilute samples. This method can also be used to investigate emulsions with water as a dispersed phase and oil as the continuous phase (water-in-oil emulsions). In that case, water would crystallize at a different temperature depending on how small the water droplets are. Moreover in multiple emulsions such as water-in-oil-in-water, bulk water and internal phase water could be distinguished.

References

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