Journal of Food Process Engineering ISSN 1745-4530

A NEW METHOD FOR DETERMINING THE EMULSION STABILITY INDEX BY BACKSCATTERING LIGHT DETECTION

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Received for Publication November 27, 2013 Accepted for Publication January 17, 2014

doi:10.1111/jfpe.12078

ABSTRACT

In the present study, the Δ backscattering (Δ BS) method was developed as a simple and sensitive tool for determining the emulsion stability index (ESI) by backscattering light detection. The backscattering light detection method uses emulsion-scanning technology to monitor physical behavior in an emulsion as a function of storage time. Emulsions were prepared by the homogenization (8,000 rpm for 2 min at 25C) of distilled water and perilla oil containing Tween 60, and the sample was quiescently incubated in a vial at 25C for 11 h. To confirm this new methodology, the sum of ΔBS at the clarified layer, obtained from the backscattering flux profiles over the storage period, was compared with the ESI determined by the volumetric method. Our backscattering light detection method was compared with the volumetric method based on the emulsifier content. The sum of ΔBS at the clarified layer decreased as the ESI decreased, with a correlation of 0.999, 0.993, 0.998 and 0.998 for emulsions stabilized with 200, 300, 400 and 500 μ mol of emulsifier, respectively. Regardless of the emulsifier content in the emulsion, the R^2 values for the fit curve, calculated by plotting the sum of ΔBS at the clarified layer against the ESI determined by the volumetric method, revealed strong linear relationship ($R^2 = 0.953$). Our results suggest that the backscattering light detection method is a new tool for the quantitative determination of the ESI in an emulsion system.

PRACTICAL APPLICATIONS

The acceptable way for the emulsion characterization is the determination of emulsion stability by measuring creaming rate and/or oil droplet diameter. Although the determination of oil droplet diameter provides the various information on the emulsion stability, it needs the expensive instruments based on dynamic light scattering or laser diffraction analysis. On the other hand, creaming is monitoring the change in the height of the boundary between the creamed and serum layers with naked eyes. In many cases, however, the condition of emulsions (such as their color, viscosity, and oil content, *etc*) prevents the clear reading of the boundary. The backscattering light detection described in this study could be a promising method which enables the accurate analysis of emulsion stability regardless of the limitations mentioned above.

INTRODUCTION

An emulsion consists of two or more immiscible liquids, such as oil and water, with one of the liquids dispersed in the other phase in the form of droplets. In most emulsion foods, the diameter of the dispersed droplets ranges from 0.1 to 100 μ m (Dickinson and Stainsby 1982; Dickinson 1992; Friberg *et al.* 2004). An oil-in-water (o/w) emulsion

consists of oil droplets dispersed in the aqueous phase (e.g., milk, cream, dressing, mayonnaise, a beverage, soups and sauces), whereas a water-in-oil emulsion consists of water droplets dispersed in an oil phase (e.g., margarine and butter).

An emulsion may become unstable due to various physical and chemical processes. Common physical mechanisms that cause destabilization over time include creaming, sedimentation, flocculation, coalescence and Ostwald ripening (McClements 2005). Gravitational separation generally causes creaming and sedimentation, whereas droplet aggregation results in flocculation and coalescence. Many attempts have been made to determine the stability of emulsions by detecting changes in the properties of an emulsion over time. Monitoring gravitational separation (e.g., creaming and sedimentation) generally involves measuring the height of the interface between a dropletdepleted serum layer and a droplet-rich creamed layer after standing (McClements 2005). The direct observation of droplet flocculation or coalescence requires the use of an optical or electron microscope (Jokela et al. 1990; Brooker 1995). To experimentally characterize the aggregation phenomena of emulsion (e.g., flocculation or coalescence), the change in droplet size distribution is measured as a function of time using particle-sizing techniques, such as light scattering, ultrasonic spectrometry, nuclear magnetic resonance and/or electrical pulse counting (McClements 2005).

Creaming, where the dispersed phase has a lower density than the continuous phase, is a common source of instability for emulsions. Techniques generally used to detect creaming, one of physical destabilizations, are monitoring the change in the height of the boundary between the creamed and serum layers with naked eyes. However, there are several problems with monitoring the resistance of an emulsion to gravitational separation by determining the height of the creamed or serum layer: (1) it provides information only about the location of the boundaries between the different layers; and (2) it may not provide information about the location of the boundaries between the different layers if the boundaries are diffused or if the layers are optically opaque (McClements 2005). Another techniques used to detect creaming are analytical instruments more accurate and reliable than the observation by naked eyes. However, most of dispersions in foods are quite concentrated and opaque, resulted in the dilutions of dispersed samples. The results obtained from the diluted sample are sometimes quite different from the concentrated dispersion. Therefore, the light-scattering detection method has received significant attention as a new method for monitoring gravitational separation (Davis 1996; Chanamai and McClements 2000c). Current optical scanning instruments can evaluate the optical characteristics of an emulsion. It is possible to measure both the size and concentration of droplets at any height by measuring the intensities of transmitted and backscattered light through a vertical glass vial containing samples of the emulsion using a single apparatus without a dilution. Thus, it is possible to detect destabilization phenomena much earlier and easily than using conventional approaches (Mengual et al. 1999; Lemarchand et al. 2003). A novel instrument (TURBISCAN LAB, Formulaction, L'Union, France) has been developed to be able to analyze physical destabilization of concentrated colloidal systems and dispersions. According to the manufacturer, this instrument works on multiple light scattering in both transmission and backscattering mode, in order to analyze highly concentrated dispersions. The photons from a pulsed near-infrared light source ($\lambda = 880 \text{ nm}$) are sent into the dispersions. These photons, after being scattered many times by the particles (or droplets) in the dispersion, emerge from the dispersion and are detected by the two detectors located at 180° and 45° from the light source. Because backscattering is directly related to the photon transport mean free path, backscattering intensity depends on particle size and concentration. In addition, the particle size change could be predicted by monitoring the change in the intensity of backscattered light. Therefore, monitoring the change in backscattering intensity could be very useful to observe the physical destabilization of concentrated dispersions.

In the present study, the determination of emulsion stability was carried out using the backscattering light detection method, and this method was compared with the volumetric method, which is commonly used to determine the resistance of an emulsion to gravitational separation. Therefore, the objective of this study was to investigate the validity of the backscattering light detection method based on backscattering profiles of an o/w emulsion system.

MATERIALS AND METHODS

Materials

Food-grade perilla oil was kindly supplied by Nongshim Co., Ltd. (Seoul, Korea). Tween 60 (polyethylene [20] sorbitol monostearate) was supplied by Ilshinwells Co., Ltd. (Seoul, Korea). Distilled and deionized water (DDW) were used to prepare the emulsion. All chemicals were analytical reagent grade.

Preparation of the o/w Emulsion

To prepare the oil phase containing emulsifier, the desired amount of Tween 60 (as an emulsifier) was added to 8 g of perilla oil. Next, the premix was homogenized using a highspeed blender (Ultra-Turrax T25, Ika, Staufen, Germany) at 8,000 rpm for 2 min at 25C. After Tween 60 was sufficiently homogenized, 12 mL of DDW was added to the Tween 60-dispersed perilla oil. An emulsion was prepared by homogenizing the mixture of perilla oil and aqueous phases using a high-speed blender (Ultra-Turrax T25) at 8,000 rpm for 2 min at 25C. The effect of the initial emulsifier concentration on the stability of the emulsion was determined by preparing oil phases containing different concentrations of Tween 60 (from 100 to 500 μ mol). The perilla oil and emulsifier concentrations used in this study and the procedure used to prepare the emulsion were established based on a preliminary analysis of the optimal conditions for the microencapsulation of perilla oil.

Determination of the Stability of the Emulsion by Measuring the Backscattering Profile

An analysis of the stability of the emulsion was carried out by determining the variation in backscattering (Δ backscattering, Δ BS) using a TURBISCAN LAB optical analyzer. The equation

$$Backscattering = \left(\frac{1}{\lambda^*}\right)^{1/2} \tag{1}$$

was applied, where λ^* is the photon transport mean free path in the emulsion. The value of λ^* was calculated as follows:

$$\lambda^* = \frac{2d}{3\phi(1-g)Q_{\rm s}} \tag{2}$$

where Φ and *d* are the volume fraction and mean diameter of the oil droplets, respectively. Q_s and *g* represent the scattering efficiency and asymmetry factors, respectively, which are optical parameters given by the Lorenz-Mie theory (Bohren and Huffman 1998).

The prepared emulsion was transferred immediately into a flat-bottomed cylindrical glass vial (70 mm in height and 25 mm in internal diameter) to a height of 5 cm. The vial was then placed in the instrument, which utilizes a nearinfrared ($\lambda = 880$ nm) electroluminescent diode as the light source. Two synchronous optical sensors periodically recorded the intensities of transmitted and backscattered lights at 40 μ m intervals at 25C while moving along the entire length (~55 mm) of the vial. The transmission detector receives the light that goes through the emulsion (180° from the incident beam), whereas the backscattering detector receives the light scattered by the emulsion at 45° from the incident beam. The obtained variation of backscattering was used to create the creaming profile (i.e., Δ BS versus storage time along the sample height).

The light-scattering detection method is based on variation in droplet size and droplet concentration at various heights, which produce variation in the intensity of transmitted and backscattered light. The percentage of transmitted and/or backscattered light is measured as a function of emulsion height and storage time by scanning up and down the sample, and is represented graphically based on increases or decreases in the intensity of backscattered light. Monitoring the change in the intensity of transmitted light could be useful for the relatively transparent and/or translucent dispersions, and the change in the intensity of backscattered light is used for the opaque dispersions with average particle size. Because our emulsions were quite opaque, ΔBS was mainly monitored in our study.

Determination of the Emulsion Stability Index by the Volumetric Method

To evaluate the emulsion stability index (ESI) by the volumetric method, the volumetric method proposed by Chang (1994) was slightly modified. The freshly prepared emulsion was poured into 100 mL of glass volumetric cylinder and allowed to stand at 25C. The emulsion stability was evaluated by visually monitoring the development of the clarified serum layer at the bottom of the emulsion with storage time. Then, the ESI was calculated as follows:

$$ESI = \left(1 - \frac{V_{\rm w}}{V_{\rm e}}\right) \times 100 \tag{3}$$

where V_e is the volume of the o/w emulsion and V_w is the volume of the separated bottom layer after the desired storage period.

Statistical Analysis

All experiments were performed in triplicate; mean values and standard deviations are given. A simple linear regression was performed to determine the relationship between the backscattering light detection and volumetric methods using SigmaPlot Software (Systat Software, Inc., Chicago, IL).

RESULTS AND DISCUSSION

Effect of the Emulsifier Concentration on the ΔBS Profile

Figure 1 shows the Δ BS profiles of the Tween 60-stabilized o/w emulsions as a function of storage time and emulsion height. As shown in Fig. 1, the Δ BS profiles showed a continuous decrease to -46, -46, -43 and -39% of the negative peaks for emulsions stabilized with 200, 300, 400 and 500 μ mol of Tween 60, respectively. With the storage time, the negative peak became deeper and wider. This indicates the occurrence of the phase separation. The migration of oil droplets from the bottom to the top of emulsion sample could lead to a progressive reduction of oil droplet



FIG. 1. BACKSCATTERING PROFILES OF PERILLA OIL-IN-WATER EMULSIONS STABILIZED WITH 200 (A), 300 (B), 400 (C) AND 500 μ MOL (D) TWEEN 60

Data are reported as a function of storage time (0-11 h) and sample height (from 0 to 50 mm). a, emulsion stored for 0 h; b, emulsion stored for 1 h; c, emulsion stored for 3 h; d, emulsion stored for 5 h; e, emulsion stored for 7 h; f, emulsion stored for 9 h; g, emulsion stored for 11 h.

concentration at the bottom of the emulsion sample, and a concurrent decrease in the intensity of backscattered light resulted in the appearance of negative peak. The migration of oil droplet could be due to the lower density of perilla oil (930 kg/m³) than the continuous phase (water [1,000 kg/ m³] in our study). Also, the negative peak also appeared at a sample height over 40 mm. These negative peaks did not show any pattern like the gradual decrease or increase as increment in storage time or emulsifier concentration. The foam layer on top of the emulsion could be a main reason for the appearance of the negative peak at a sample height over 40 mm. These results were demonstrated by the decrease in ΔBS (negative peak) in the lower region of the emulsion and slight increase in ΔBS (positive peak) in the upper region of the emulsion. This phenomenon could be due to gravitational separation, as reported by Chanamai and McClements (2000a,b). The droplets moved upward

during storage, and a creamed (droplet-rich) layer was formed.

When creaming or sedimentation takes place in an emulsion, the ΔBS varies with the height of the emulsion and with storage time. If the emulsion is stable, no discernible change in ΔBS is observed over the entire vial during storage. The droplets move upward over time, and the droplet concentration in the lower region gradually decreases, permitting more light to be transmitted through the emulsion. Thus, the ΔBS decreases with storage and shows a negative peak.

Determination of the ESI by the Backscattering Light Detection Method

The initial droplet diameters were 8.4, 7.3, 7.0 and 7.5 μ m for the emulsions stabilized with 200, 300, 400 and 500 μ mol of Tween 60, respectively. The initial droplet

diameter in the emulsion stabilized with 100 μ mol of Tween 60 was not determined because of the instability of the emulsion (an oil layer formed on top of the emulsion just after homogenization). This may indicate an insufficient amount of emulsifier to completely cover all of the droplets formed during homogenization. Increasing the emulsifier concentration decreases the initial droplet size because the droplets are coated with emulsifier more rapidly within the homogenizer, thereby reducing re-coalescence (Qian and McClements 2011). As described earlier, the emulsion preparation with various Tween 60 concentrations (from 100 to 500 μ mol) was preliminary conducted. The beginning of phase separation was observed in the 100 μ mol Tween 60-stabilized emulsion immediately after emulsification. However, just emulsified emulsions with 200, 300, 400 and 500 μ mol of Tween 60 did not show phase separation as quick as emulsion stabilized with 100 μ mol of Tween 60. Therefore, the emulsions stabilized with 200, 300, 400 and 500 μ mol of Tween 60 were used for further analysis.

When an oil droplet has a lower density than the continuous phase, the droplet moves upward (Becher 1985; Hiemenz and Rajagopalan 1997). Therefore, the creaming rate of the emulsion can be predicted using a mathematical model, and the velocity (v_{Stokes}) with which an oil droplet moves upward through the continuous phase can be expressed by Stoke's law:

$$v_{\rm Strokes} = -\frac{2gr^2(\rho_2 - \rho_1)}{9\eta}$$

where *r* is the oil droplet radius; *g* is the acceleration due to gravity; ρ_1 and ρ_2 are the density of the dispersed (perilla oil, 930 kg/m³) and continuous phases (DDW, 1,000 kg/m³), respectively; and η is the shear viscosity of the continuous phase (1 mPa/s).

Using the values given earlier, the oil droplets in the emulsions prepared in this study were expected to cream at a rate ranging from 6.7 to 9.6 mm/h with the emulsifier



FIG. 2. CHANGES IN EMULSION STABILITY INDEX (ESI) CALCULATED BY VOLUMETRIC METHOD AND SUM OF ΔBACKSCATTERING (BS) AT THE CLARIFICATION LAYER, DEPENDING ON TWEEN 60 CONCENTRATION

(A) 200 μ mol Tween 60-stabilized emulsion. (B) 300 μ mol Tween 60-stabilized emulsion. (C) 400 μ mol Tween 60-stabilized emulsion. (D) 500 μ mol Tween 60-stabilized emulsion.



FIG. 3. THE FIT CURVE FOR CORRELATIONSHIP BETWEEN BACKSCATTERING LIGHT DETECTION METHOD AND THE VOLUMETRIC METHOD (A) 200 μmol Tween 60-stabilized emulsion. (B) 300 μmol Tween 60-stabilized emulsion. (C) 400 μmol Tween 60-stabilized emulsion. (D) 500 μmol Tween 60-stabilized emulsion.

concentration used in this study. An emulsion in which the calculated creaming rate is less than 1 mm/day can be considered to be stable toward creaming (Dickinson 1992). Therefore, the prepared emulsion could not avoid gravitational separation.

The ESI of the o/w emulsion determined by the backscattering light detection method was compared with that determined by the volumetric method. In the volumetric method, the ESI is determined by observation of the boundary between the droplet-rich and droplet-depleted layers. Therefore, the height of the clarified layer at the bottom of an emulsion has a negative relationship with the ESI obtained by the volumetric method. However, determining the stability of an emulsion based on changes in the height of the clarified layer during storage is problematic. If the oil droplets in an emulsion are not uniform in size, the boundary between the layers will be diffused or the layers will be optically opaque, and the height of the clarified layer determined by the backscattering light detection method will not match the ESI determined by the volumetric method. Therefore, the measurement of the area of the negative ΔBS profile was calculated with the sum of ΔBS values at the clarified layer at the intervals of 0.04 mm from the bottom. Because of the creaming phenomenon, the ΔBS at the clarified layer (at the bottom of the emulsion) was negative. Figure 2 shows the ESI determined by the volumetric method and sum of ΔBS at the clarified layer as a function of storage time. As shown in Fig. 2, in all cases, the ESI values produced by the volumetric method were similar to the sum of ΔBS at the clarified layer. These results indicate that the decrease in ESI was nearly equal to the increase in area of the negative peak for ΔBS .



FIG. 4. RELATIONSHIP BETWEEN THE SUM OF Δ BS AT THE CLARIFIED LAYER AND ESI FROM THE VOLUMETRIC METHOD

, 200 μmol Tween 60-stabilized emulsion; ◆, 300 μmol Tween
60-stabilized emulsion; ▼, 400 μmol Tween 60-stabilized emulsion; ◄,
500 μmol Tween 60-stabilized emulsion. ESI, emulsion stability index;
ΔBS, Δbackscattering.

Relationship between the Sum of ∆BS at the Clarified Layer and the ESI as Determined by the Volumetric Method

The values for the sum of ΔBS at the clarified layer matched well with the ESI determined by the volumetric method (Fig. 3). Linear relationships between the sum of ΔBS at the clarified layer and ESI determined by the volumetric method were obtained with correlation factors (R^2) of 0.9990, 0.9933, 0.9980 and 0.9980 for the emulsions formed with 200, 300, 400 and 500 μ mol of Tween 60, respectively. Also, Fig. 4 shows a linear relationship $(R^2 = 0.9530)$ between the sum of ΔBS at the clarified layer and ESI determined by the volumetric method, independent of the concentration of the emulsifier. New methods for the evaluation of emulsion stability have been developed by many research groups (Garti et al. 1981; Latreille and Paquin 1990). Garti et al. (1981) established a new method based on electrical conductivity measurements, and Latreille and Paquin (1990) further developed the method by pairing centrifugation with conductivity measurement. These methods for the determination of emulsion stability are easy and fast, and they correlate well with stand stability tests. However, the method of Latreille and Paquin (1990) can destroy the emulsion sample during measurement because of the centrifugation step. Moreover, electrical conductivity measurements can only provide information about emulsion stability (not oil droplet size). However, the backscattering light detection method can provide information about emulsion stability and changes in oil droplet size. This indicates that the sum of ΔBS at the clarified layer can be used as a new indicator of emulsion stability.

CONCLUSIONS

In our study, the validity of the backscattering light detection method for the evaluation of stability in o/w emulsions was examined. The backscattering light detection method has been compared with other well-known methods for determining the ESI (volumetric method), and a good correlation between the backscattering light detection method and the volumetric method has been reported. Compared with other optical analytical methods, including microscopy, particle size and potential, determination of the ESI by the backscattering light detection method is advantageous because it is nondestructive; in particular, no sample dilution is needed. Most emulsion and colloidal systems are quite concentrated and opaque. Therefore, these systems fall outside the range of commonly used instruments. However, the backscattering light detection method allows the analysis of physical destabilization in concentrated emulsion and colloidal systems. Moreover, it provides more useful information about particle size variation (flocculation and coalescence) during destabilization. Thus, the backscattering light detection method is a simple and sensitive technique for the determination of the ESI using an o/w emulsion.

ACKNOWLEDGMENTS

This work was financially supported in part by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (NRF-2011–0010786) and by the High Value-added Food Technology Development Program (313021-3) by the Ministry of Agriculture, Food and Rural Affairs, Republic of Korea.

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