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Sensitive real-time measurement of the refractive index and attenuation coefficient of milk and milk-cream mixtures

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ABSTRACT

We demonstrate a first simultaneous measurement of both the refractive index and the attenuation coefficient (defined as the sum of the scattering and absorption coefficients) of highly turbid milk and milk-cream mixtures. We achieve this by observing the real-time reflectance profile of a divergent laser beam made incident on the surface of the milk sample. The experiments were carried out on commercial milk samples with fat volume concentrations of 0.5 or less, 1.6, and 3.3%, and on milk-cream mixtures with fat volume concentrations of 10 and 33.3%, without any dilutions of these samples. We find that the reflectance data are well described, for the first time without any empirical fit-parameters, by Fresnel theory that correctly includes the effect of angle-dependent penetration into the turbid medium on the total internally reflected signal. Therefore, our method provides the most accurate determination to date of the refractive index and attenuation coefficient of milk and milk-cream mixtures. Our sensor is compact, portable, and inexpensive.
Key words: refractive index, scattering coefficient, attenuation coefficient, milk

INTRODUCTION

Real-time accurate optical measurement of the refractive index and attenuation coefficient (i.e., the sum of the scattering and absorption coefficients) offers an attractive, noninvasive method for the monitoring of milk quality and fat content. It is well known that the refractive index of milk is difficult to estimate because of the turbidity (i.e., multiple light scattering) caused by casein micelles, air bubbles, and fat globules (see, for example, Walstra et al., 1999). Attempts have been made to suppress multiple scattering by diluting the milk with a solution that disperses the casein micelles (Walstra et al., 1999) and then estimating the fat content by measurement of the light scattered by the colloidal fat particles in milk. However, these measurements are limited in accuracy by experimental error in the diluting process and theoretical error in modeling the effect of dilution on the optical properties of milk.

A basic question naturally arises: Is there an experimental method to sensitively measure the basic optical properties, such as refractive index and attenuation coefficient, of a highly turbid sample directly without the need to dilute the sample? And, if so, can the data be explained with a valid theoretical model to extract accurate values for the refractive index and attenuation coefficient? We believe, for reasons outlined below, that despite receiving considerable attention for many decades (see, for example, Meeten 1997; Reyes-Coronado et al., 2005; Niskanen et al., 2007, and references therein) the answer to both questions is, surprisingly, no. Specifically, for milk and milk-cream mixtures, the literature suggests that these questions have been of interest for some time (see, for example, Rangappa 1948; Räty and Peiponen, 1999; Jääskeläinen et al., 2001) but remain unanswered.

Traditionally, for transparent fluid samples, the most commonly used method for measuring the refractive index is to determine the critical incident angle at which total internal reflection (**TIR**) occurs for light rays striking the sample. Indeed, this is the principle on which the widely used Abbe refractometer is based: one detects the light transmitted through the sample and observes a dark and a bright region adjacent to each other in the transmitted spot. The dark region is caused by TIR of the incident rays so that the dividing line between the 2 regions yields the critical angle and hence the refractive index. On the other hand, several other critical angle-based refractometers reported recently measure the light reflected from the sample surface (see, for example, Meeten and North, 1995; Bali et al., 2005; McClimans et al., 2006). A distinct "knee" appears in the reflectance versus incident angle curve at the critical angle.

However, no matter whether one measures transmission or reflection, these critical angle-based methods

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fail for highly turbid samples such as milk and milk-cream mixtures because the critical angle is not a well-defined concept for turbid media, as was pointed out recently (Räty and Peiponen, 1999; Niskanen et al., 2007). Experimentally, one observes in the case of the transmission-based Abbe refractometer that the dividing line between the TIR and non-TIR regions becomes indistinct and blurry. In the case of reflection-based critical angle refractometry, a smoothing out of the sharp knee in the reflectance versus incident angle curve is observed (Meeten and North, 1995; Räty and Peiponen, 1999; Jääskeläinen et al., 2001), which precludes a precise determination of the location of the critical angle. It is important to understand the reason behind this smoothening of the reflectance curve in the vicinity of the expected location of the critical angle to satisfactorily model the observed reflectance. An appropriate model has not been proposed to date. Merely empirically defining an effective critical angle that is equal to the angle where maximum change of slope of the reflectance versus incident angle curve occurs, determined by differentiating the reflectance curve (Mohammadi, 1995; this method is also employed in various commercial devices such as the Reichert AR-6 series; Reichert Analytical Instruments, Depew, NY), has been shown to yield significantly erroneous values for the refractive index (Meeten and North, 1995; Meeten 1997). See, for example, Figure 1b in this paper.

Recently, there have been 2 noteworthy attempts (Reyes-Coronado et al., 2005; Niskanen et al., 2007) at measuring the refractive index of turbid media. Reyes-Coronado et al. (2005) demonstrated a first simultaneous real-time measurement of both the refractive index and the attenuation coefficient of a turbid medium by measuring the transmittance of a light beam through a thin prism filled with a colloidal suspension of polystyrene spheres. However, Reyes-Coronado et al. (2005) point out a limitation of their transmission-based approach that is a major drawback from the point of view of milk characterization: their method works only for moderately turbid media with an attenuation coefficient of less than 40 cm^{-1} and is thus inadequate for working with milk and milk-cream mixtures for which the attenuation coefficient ranges from about 40 cm^{-1} for milk with less than 0.5% fat (trade name in the United States: fat-free or skim milk) to almost 1,200 cm^{-1} for a milk-cream mixture with 33% fat (trade name: heavy whipping cream). On the other hand, Niskanen et al. (2007), building upon earlier work by the same group (Räty and Peiponen, 1999; Jääskeläinen et al., 2001), have demonstrated a unique spectrophotometric device capable of measuring the refractive index as well as the attenuation coefficient (though not simultaneously in real-time, unlike the present work) of milk. But this work has the following major drawback (discussed below in more detail in Theoretical Considerations in Materials and Methods): the theory used to model the data is, in the authors' words, empirical. This, in itself, is not necessarily a weakness. However, little or no scientific justification is offered by the authors for their model other than that it has been "observed to be useful" in making their fits agree with the data whenever their measurement "departs strongly" from traditional Fresnel theory. As such, it would not be surprising if the values of the refractive index and attenuation coefficient extracted from their data-fits turn out to be significantly inaccurate. Further, we note that the sensitivity to differential changes in refractive index in Niskanen et al. (2007), Räty and Peiponen (1999), and Jääskeläinen et al. (2001) is limited to 1 part in 10^4 or less, at least an order of magnitude less than what we demonstrate in the present work. Finally, Räty and Peiponen (1999) and Jääskeläinen et al. (2001) present data only for milk, not for milk-cream mixtures.

In this paper, we present a first real-time simultaneous measurement of both the refractive index and the attenuation coefficient of milk and milk-cream mixtures with unprecedented sensitivity. We achieve this by observing the real-time reflectance profile of a divergent laser beam made incident on the surface of the milk sample. The experiments were carried out with commercial milk samples that had fat volume concentrations of 0.5 or less, 1.6, and 3.3% and on samples of highly turbid milk-cream mixtures with fat volume concentrations of 10 and 33.3%, without any dilutions of these samples. The sensitivity of our method to differential changes in refractive index is demonstrated to be at least 1 part in 10^5 (i.e., an order of magnitude better than previous measurements by past workers). It is important to note that we have developed a new model of total internal reflection from a turbid medium based on Fresnel theory that, for the first time, is shown to fit the reflectance data without any empirical fit parameters. Therefore, our method provides a much more accurate determination than ever before of the refractive index and attenuation coefficient of milk and milk-cream mixtures.

MATERIALS AND METHODS

Theoretical Considerations

The basic principle of TIR-based refractive index measurement is depicted in Figure 1a. A sample of refractive index n_{sample} is placed on top of a glass prism of known refractive index n_{prism} ($>n_{sample}$) and a light ray of intensity I_i is made incident on the prism-sample interface so that the angle of incidence at the interface

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