Analysis of Light Scattered by Turbid Media in Cylindrical Geometry

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ABSTRACT: The angle dependence of the transmitted light through a cylindrical turbid sample (latex suspension, developing milk gel, draining/coarsening milk, and protein foams) in a standard light scattering setup was analyzed in terms of the transport mean free path length or scattering length ($l^*$) (a measure for the turbidity) and the absorption length ($l_{abs}$). By variation of the concentration of an absorbing dye, the independence of $l^*$ and $l_{abs}$ was demonstrated. The resulting value of the specific extinction coefficient of the dye was found to be in fair agreement with direct spectroscopic determination and practically identical in milk and latex suspensions. The validity of this technique for obtaining $l^*$ was demonstrated by monitoring the acid-induced gelation of milk. The possibility to simultaneously determine $l^*$ and $l_{abs}$ was used to follow the time development of a draining and coarsening protein foam which contained an absorbing dye. It was shown that $l_{abs}$ can be used as a measure for the volume fraction of air in the foam. This method of monitoring the transmission of multiple light scattering provides an easy way to determine $l^*$ and, specifically for foams, quantitative data dominated by the bulk of the foam.

INTRODUCTION

Light scattering by optically dense, turbid colloidal systems can be used to obtain information on their microstructure and microrheology by analyzing the time autocorrelation function of the fluctuations in the transmitted or backscattered light. This approach, diffusing wave spectroscopy (DWS), is an easy to use and versatile technique to get qualitative information (and quantitative information in the case of a well-defined particle size) on changes and processes taking place inside turbid media.1–6 In particular, in food research, DWS has been used frequently, for the obvious reason that food is nearly always turbid and susceptible to changes in rheology and microscopic structure.7–9 DWS analyzes the fluctuation in time of the transmitted or backscattered light intensity without taking into account the time averaged transmitted intensity. However, the latter can also be used for obtaining structural information if it is measured at different positions relative to the direction of the incoming light beam.

Light photons entering a turbid medium are scattered many times before they leave the medium. They are considered “diffusing” when the characteristic distance between randomizing scattering events, usually called $l^*$, is at least an order of magnitude smaller than the macroscopic dimension of the medium.5 In the case of a constant intensity of incoming photons, the static light intensity distribution inside a turbid medium can be calculated by solving the time independent diffusion equation for the geometry in question.10 The boundary conditions are determined by the shape of the container and the reflectivity of the inner container wall.

The shape of the angle dependence of the transmitted intensity is determined by the scattering length or transport mean free path length $l^*$, and, in the case of sufficient absorption, the absorption length $l_{abs}$. Therefore, $l^*$ and $l_{abs}$ may become simultaneously accessible by analysis of the angle dependence of the transmitted intensity.

The availability of $l_{abs}$ in a turbid medium is in particular interesting for the study of foams. During aging, foams drain and coarsen. If an absorbing colorant is added to the foam liquid, the change in absorption length is a measure for the change in the volume fraction of air. By monitoring the angle dependence of transmission of light, the coarsening in the bulk of the foam can be studied without relying on observations from the part of the foam bordering a transparent wall.

Usually, the DWS setup used consists of a flat rectangular cuvette and a laser beam as the source of photons. The multiply scattered light is detected on the side of the incoming beam (back scattering mode) or on the other side of the sample (transmittance mode). In the work presented here, a cylindrical cuvette is used. The angle dependence (relative to the incoming beam) of multiply scattered transmitted light is measured. The configuration is that of a standard multiangle light scattering setup. A cylindrical geometry and light detection in a direction perpendicular to the axis of the cylinder offers the advantage that no correction is needed for the angle dependent line of view or parallax. This approach should be compared with that in refs 11 and 12, where the state of the polarization of statically backscattered light is analyzed using Mueller matrices, resulting
in particle sizes and polydispersity. In the present method, 
polarization is fully averaged out, and the resulting size 
information is contained in Iₙ, together with the volume fraction 
and the optical contrast.

Foams have been intensively studied with DWS. When 
a coarsening, drained foam is made from a transparent solution, 
without strongly scattering particles, the randomizing scattering 
length lᵣ could be linked to an average of the bubble size. 
However, to our knowledge, the angle dependent transmission 
of light by a colored foam has not been used before for 
monitoring the time dependence of a foam.

This purpose of the paper is to introduce and test the method of 
angle dependent transmission in the presence of an absorbent and 
to demonstrate its applicability for the study of foams.

## THEORY

When a turbid medium is irradiated with a laser beam, the beam 
penetrates a distance lᵣ into the medium. Beyond this distance, the 
photons spread into the medium by a process which can be 
treated as diffusive. The distance lᵣ is the distance beyond which 
the diffusing photons lose all correlation with their initial position. 
lᵣ is related to the diffusion constant of the photons, given by

$$D_i = \frac{lᵣ c}{3}$$

(1)

in which c is the velocity of light in the medium.

If the medium is sufficiently turbid, or, in other words, if 
lᵣ ≪ R (with R the cylinder radius) and lᵣ ≪ L (with L the 
filling height), the light transmitted at angle θ (relative to the 
transient beam, see Figure 1) by a cylindrical sample can be 
calculated by solving the static diffusion equation for a 
cylindrical geometry

$$\Delta \Phi(\vec{r}) - \frac{3}{lᵣ lᵣ} \Phi(\vec{r}) = -\frac{3}{lᵣ} \delta(\vec{r} - \vec{r}_0)$$

(2)

where Φ(\vec{r}) is the fluence (the flux per unit area of photons), 
lᵣ is the absorption length, and \vec{r}_0 is the position of the source 
(i.e., a distance lᵣ from the wall inside the sample). The boundary conditions are the location of the source and a zero 
fluence at r = R + rₑ (with rₑ the so-called extrapolation length 
and assumed to be equal to ARᵣ). A is given by the reflectance 
of the inside of the container (quartz surrounded by toluene):16

$$A = \frac{2}{3} \frac{1 + R_{\text{eff}}}{1 - R_{\text{eff}}} \approx 0.70$$

(3)

$$T(\theta, z₀) = 1 - \frac{(1 - \bar{I}) \cos(\pi - \theta)}{4\pi R^2} \sum_{k=-\infty}^{\infty} \left( \alpha_k R + \frac{1}{r_k} \right) \cos(m - \theta) Iₗₘ(\alpha_k R - \alpha_k R:\bar{I}:Iₗₘ(\alpha_k R + A\alpha_k R:\bar{I}:Iₗₘ(\alpha_k R + A\alpha_k R:\bar{I}:Iₗₘ(\alpha_k R + A\alpha_k R))$$

(6)

with Iₗₘ(x) and Kₘ(x) the modified Bessel functions of the first and 
second kind, respectively.

Further,

$$\lambda_k = \frac{k\pi}{L - 2AIₗₘ}$$

(7)

$$\alpha_k = \sqrt{\mu eff^2 + \lambda_k^2}$$

(8)

(9)

$$r_{l,k} = \left[ \frac{1 + (1 - \bar{I})^2 - 2(1 - \bar{I}) \cos(\pi - \theta)}{2kL + 4A\lambda_k^2} \right]^{1/2}$$

(10)

Figure 1. Left: Schematic drawing of a sample tube with filling height 
L, a source of diffusing light at r = R - lᵣ, and a distance z₀ below the 
surface of the sample. The incoming beam defines the radial direction 
θ. Right: Schematic drawing of the experimental setup showing the 
incoming laser beam, turbid sample, scattering angle θ, and 
detector.
such that the presence of practical concentrations of absorbing colorant) are made using a vertically positioned, containers for the macroscopic movies were the same cylindrical quartz toluene bath at 20 °C. The sample container was made of optical quartz, with a wall thickness of 1 mm. The inner diameter was 8 mm. The angle between 17 and 148° will be shown below, typical values of $\mu_l$ are addressed. In practice, only pixel coordinates $x$ and $y$ are inserted into the sample container without trapping air and other quantities with a tilde are in units of $R$.

$\mu_{eff}^{-1}$ is the distance in the upward and downward direction over which the diffusing light spreads. When $I^*$ and $I_{abs}$ are obtained from eq 6, their values should be considered as an average over $\mu_{eff}^{-1}$ in the upward and downward direction. As will be shown below, typical values of $I^*$ and $I_{abs}$ (in the presence of practical concentrations of absorbing colorant) are such that $\mu_{eff}^{-1}$ is typically 1.2–4 mm.

Although eq 6 is valid for any $I^* < R$, it was found that, with decreasing $I^*$, an increasing number of terms in the double summation is necessary for convergence. Below a certain value of $I^*$, standard numerical precision becomes insufficient for the calculation. The smallest feasible $I^*$ is dependent on $R$, and is about 0.1 mm in the present case of $R = 4$ mm. The number of terms in the summation in $k$ was 50, and that in the summation in $m$ was 150. A higher value for $k$ had no effect. A higher value for $m$ gave rise to floating point errors (in Matlab R2007b).

For larger $R$, the smallest $I^*$ feasible for calculation is larger. Choosing $R$ less than 4 mm leads to shorter photon diffusion paths, and limits the range of suitable systems to those that are very turbid. For $R = 4$ mm, the value used in this work, the range of accessible $I^*$ values is between 0.1 and 0.8 mm, with the latter value equal to 1/10th of the sample diameter.

### EXPERIMENTAL SECTION

**Light Scattering and Image Analysis.** The setup used was a standard multiangle light scattering setup (ALV-CGS4, Langen, Germany) for static and dynamic light scattering. The wavelength of light used was 532 nm. Figure 1 shows schematically the setup.

The intensity of transmitted light was measured at 32 equally spaced angles between 17 and 148° for a static system (milk and latex suspension), and at eight equally spaced angles between 17 and 136° for an aging foam. In the latter case, fewer angles were available because of the slow detector movement relative to the development of the foam structure.

Very large angles (i.e., close to the incoming beam) were avoided in order to prevent an effect of constructive interference\cite{8,19} in back-scattered light.

The sample container was made of optical quartz, with a wall thickness of 1 mm. The inner diameter was 8 mm. The filling height was 40 mm. The sample container was inside a temperature controlled toluene bath at 20 °C.

Macroscopic and microscopic movies of the development of foam volume and structure were made using a Dino-Lite 7013 digital microscope with a long working distance (5–50 cm). The sample containers for the macroscopic movies were the same cylindrical quartz containers as those used for the light scattering experiments. Microscopic movies of foams, for which transient light and a flat wall are desirable, were made using a vertically positioned, flat 80 × 50 × 2 mm² glass container. Time lapse movies were made with a time step of 30 s during 90 min. Figure 2 shows examples of the images obtained and used.

In order to deduce a measure for the mean bubble size from the polidisperse size distribution in the microscopic images (Figure 2B), the gray value spatial correlation function was used

$$c(X, Y) = \frac{\langle (\text{gray}(x_0, y_0) - \text{gray}(X, Y)) \cdot \text{gray}(X, Y) \rangle_{x_0,y_0}}{\langle \text{gray}(x_0, y_0) \cdot \text{gray}(x_0, y_0) \rangle_{x_0,y_0}}$$

where $\langle \cdot \rangle_{x_0,y_0}$ is the average at constant pixel distances $X$ and $Y$ when pixel coordinates $x_0$ and $y_0$ are varied such that all pixels in the image are addressed. In practice, only pixel–pixel distances of 10% of the image size are relevant, because this covers all distance scales present. $c(R_0)$ is the radial average of $c(X, Y)$

$$c(R) = \frac{\sum_i c(X_i, Y_i) \cdot x_i^2 + y_i^2 - R_i^2}{\sum_i x_i^2 + y_i^2}$$

with $c(X_i, Y_i)$ being the value of the pixel at $X_i, Y_i$ in the 2D gray value correlation chart and $\delta$ the Kronecker delta. Figure 3 shows examples of the gray value correlation functions. The first maximum, typically near 0.15 mm, was taken as a measure for the mean bubble size, and used for comparison with $I^*$.

**Sample Preparation and Materials.** Foams were prepared from a solution in demineralized water of ovalbumin (Sigma, grade II) at a concentration of 10% w/w. The solution of ovalbumin was centrifuged before foaming at 5000g for 30 min to remove light scattering aggregates and heated at 35 °C for 30 min in order to get a more stable foam. The protein solution was foamed in the barrel of a syringe cappuccino machine that fitted inside the syringe barrel. By applying the plunger in the syringe, the foam was directly pumped into the bottom of the sample container through a PVC tube from the nozzle of the syringe to the bottom of the container. In this way, the foam could be inserted into the sample container without trapping air of the gray value correlation functions. The first maximum, typically near 0.15 mm, was taken as a measure for the mean bubble size, and used for comparison with $I^*$.

For all calculations, both for image analysis and fit procedures, Matlab 7.5.0 (R2007b) was used.

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pockets. Typically, the initial foam volume in the container was 3 mL. The initial volume fraction of air in the foam made in this way was between 0.65 and 0.8. It was measured by weighing the container before and after filling, and dividing this mass by the volume.

The colorant used to provide absorbance of light was a mixture of amaranth and Ponceau 4R (obtained from IFF, Hilversum, The Netherlands), with a mass attenuation coefficient of 0.023 mg−1 L mm−1 at 532 nm.20 A similar picture is obtained from a latex suspension at 532 nm.20 Amaranth dye and Ponceau 4R have very similar spectra in the relevant wavelength range.21 Monodisperse latex particles with a specified diameter of 430 nm in a suspension with a volume fraction of 32% were obtained from AkzoNobel, Arnhem, The Netherlands, and diluted in 0.01 M NaCl to 4.82%. From dynamic light scattering measurements, the diameter was found to be slightly smaller, 395±0.02 nm. The latter value was used for calculations of $l^*$. Glucono δ-lactone was purchased from Sigma.

Semiskimmed and full fat milk were bought at the local supermarket. They contained 1.5 and 3% fat, respectively.

RESULTS AND DISCUSSION

Determination of $l^*$ and $l_{abs}$. The simultaneous determination of $l^*$ and $l_{abs}$ is only useful if scattering and absorption of light take place independently, and if the absorption is only determined by the colorant and not affected by the presence of the colloidal particles. These conditions were tested by measuring the angle dependent transmission at fixed colloid concentration and increasing concentration of light absorbing colorant. Two systems were chosen, a 4.82% (w/w) suspension of monodisperse latex spheres and semiskimmed milk.

Figure 4 shows as representative examples the transmission of semiskimmed milk and latex suspension as a function of the angle, in the presence and absence of amaranth dye. In order to obtain $l^*$ and $l_{abs}$, $T(\theta; l^*, l_{abs})$ given by eq 6 multiplied by an instrument constant was fitted to the experimentally determined intensity of transmitted light. In eq 6, maximum values of $m_1$ and $l_1d$ were 150 and 50, respectively. Increasing the maximum values of $m$ and $k$ did not change the results of the fit procedure. In Figure 4, it is shown that the fits are satisfactory.

![Figure 4. Angle dependent transmission, normalized on the scattering intensity of toluene, of semiskimmed milk with and without amaranth dye and 4.82% latex with amaranth dye. The full curves are fits of eq 6.](image)

<table>
<thead>
<tr>
<th>conc. of amaranth dye (mg/L)</th>
<th>$l^*$ (mm)</th>
<th>$l_{abs}$ (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.55 (±0.02)</td>
<td>1.12 x 10^4</td>
</tr>
<tr>
<td>0.23</td>
<td>0.57 (±0.02)</td>
<td>202 (±10)</td>
</tr>
<tr>
<td>0.80</td>
<td>0.56 (±0.02)</td>
<td>69 (±4)</td>
</tr>
<tr>
<td>0.96</td>
<td>0.55 (±0.02)</td>
<td>58 (±4)</td>
</tr>
<tr>
<td>1.4</td>
<td>0.54 (±0.02)</td>
<td>28 (±2)</td>
</tr>
<tr>
<td>2.8</td>
<td>0.56 (±0.02)</td>
<td>13.4 (±1)</td>
</tr>
</tbody>
</table>

where $l^*$ was also found to be independent of the concentration of the dye and equal to 0.16 (±0.03) mm. This is in fair agreement with the value of 0.15 mm obtained from

$$l^* = \frac{k^6}{\pi \rho \int_0^{2\pi} P(\rho) Q^3 \, dQ}$$

using the near field Mie solution23,24 for the form factor $P(\rho)$. Here $Q$ is the modulus of the scattering vector, $n$ the refractive index of the solvent, $k = 2\pi/\lambda$, $\lambda$ is the wavelength in vacuum, and $\rho$ is the number density of latex particles. The calculated value, however, is very sensitive to the size of the particles and polydispersity, and a precise agreement cannot be expected without experimental measures which are outside the scope of the present work.

For

$$l_{abs} \gg \frac{3R^2}{l^*} \approx 10^2 \text{ mm}$$

and

$$\eta_{GDL} \ll \frac{1}{\eta_1} \approx \frac{1}{R}$$

the data will be very weakly dependent on $l_{abs}$. Therefore, values of $l_{abs}$ larger than 1000 are considered unreliable.

Figure 5 shows $l_{abs}$ and $l^*$ in milk and latex dispersion for various concentrations of amaranth dye. In both of the turbid systems, $l_{abs}$ decreases with increasing dye concentration, while $l^*$ is practically constant. The value of $l_{abs}$ appears to be the same in both systems at the same concentration of dye, which is more clearly seen in Figure 6. The amaranth dye turns out to not be affected by replacing polystyrene spheres by casein micelles (the major colloidal material in semiskimmed milk), and may therefore be expected to have the same absorption properties in other protein systems, such as an ovalbumin foam.

Figure 6 shows that $l_{abs}^{-1}$ in both milk and latex are on the same linear trend with increasing dye concentration. From the slope of this trend, the specific extinction coefficient at 532 nm was found to be 0.026 ± 0.003 mg L−1 mm−1. This is in agreement with 0.023 mg L−1 mm−1 deduced from data reported in the past.20

**Determination of $l^*$ during Aggregation.** In order to validate the method of obtaining $l^*$ from the angle dependence of the transmission, $l^*$ was monitored in full fat milk during acidification by 2% GDL, in the absence of absorbing color. $l_{abs}^{-1}$ was therefore set at zero during the fit procedure of eq 6 to the transmission. The transmission was measured at eight angles at time intervals of 3 min. Acidification leads to destabilization of the casein micelle suspension and ultimately to the formation of a gel. As shown in Figure 7, the resulting $l^*$ shows with decreasing pH the expected sharp increase at pH 5.2, when the isoelectric point of the micelles is reached. Before
that, there is a shallow minimum, ascribed to the collapse of κ-casein “hairs” responsible for the steric repulsive interaction between the micelles. This collapse causes the micelles to shrink. By shrinking, the scattering by the micelles becomes more evenly spread in all directions, which shortens $l^*$. This pH dependence is in accordance with particle size and viscosity measurements. From these observations in acidifying milk, it turns out that values of $l^*$ can be obtained from the angle dependent light transmission which are consistent with other techniques to monitor the degree of aggregation.

**Application to Foam Drainage.** Once the method for determining simultaneously $l^*$ and $l_{abs}$ has been established, and the expected relation between $l^*$ and developing structure demonstrated, the method can be used for following in time the liquid volume fraction in a draining foam, provided that the liquid absorbs part of the diffusing light. As a test case, the angle dependent transmission of a fresh foam of a 10% (w/w) ovalbumin solution and of skimmed milk, both with 4.18 mg L$^{-1}$ of dye, was followed in time. According to the relation derived from Figure 6, which was assumed to be valid at 4.18 mg/L, i.e., beyond the range it was actually measured, $l_{abs}$ is 9.2 mm for this concentration. This value of $l_{abs}$ corresponds to a zero air volume fraction. Increase of $l_{abs}$ in foam is interpreted as an increase of the air volume fraction $\phi$ due to draining (accompanied by some coarsening). The relation between the values of $l_{abs}$ in the foam and the solution prior to foaming ($l_{abs,foam}$ and $l_{abs,sol}$, respectively) is proposed to be

$$\frac{1}{l_{abs,foam}} = (1 - \phi) \frac{1}{l_{abs,sol}}$$

(18)

Figure 8 shows an example of the development in time of the transmission of the foam at a selection of four angles. Because
the foam behaves with respect to scattering like a solid, speckles are not averaged out by thermal motion. The time development of the transmission data was therefore smoothed by fitting a smoothing spline to it.

In spite of the low number of angles available (i.e., eight), the fit of eq 6 to the data gave satisfactory low-noise trends. Figure 9 shows $I^*$ and $I_{abs}$ and the volume fraction of air $\phi$ in fresh foam deduced from $I_{abs}$ for a duration of ~1 h of draining and some coarsening. For comparison, a sample of the same foam in the same container was also monitored in a time lapse movie. The volumes of the drained fluid and the remaining foam were registered. Together with the mass of the foam, $\phi$ could be calculated. For ovalbumin foam, $\phi$ started at 0.78 in the freshly made foam and increased in 60 min to about 0.90. The time dependences of $\phi$ derived from the transmission and from direct observation are in general agreement. However, the transmission suggests a less regular increase. This is due to the fact that the transmission reflects a weighted average over the volume, with the region close to the source and detector contributing the most. Bubble collapse or rearrangements in later stages of draining in this region can be expected to have a direct effect on the measured transmission and cause an irregular time dependence.

Fresh foam of skimmed milk appeared to contain less air, and the volume fraction of air increased to only 0.87 before leveling off and decreasing slightly. This decrease may be due to an accumulation of draining fluid in the bottom of the container, affecting the measured absorption. The increasing level of drained fluid may lower the volume fraction of air just above it as a consequence of capillary rise. With the standard multiangle light scattering setup used for this work, it is not possible to vary the position of the point of irradiation. This problem is less important for “dryer” foams, such as ovalbumin foam. $I^*$ appears to be nearly constant, or slowly decreasing in time. This is seen both in the transmission results and in the results from image analysis of microscope images. Although precise agreement between the values of $I^*$ and the results from image analysis cannot be expected, their trend in time can be compared. The interpretation of a changing $I^*$ in a foam made from a clear, not turbid liquid is usually made in terms of an increase in bubble size. A value of $I^*$ showing hardly a consistent time dependence instead of an increase in time, as is seen for drained, coarsening foam, may be explained by the fact that the foam studied here is draining and not (strongly) coarsening. In a draining foam, the number and size of bubbles is not changing, in contrast to later stages, where bubbles start to coalesce or develop by Ostwald ripening. The rather constant bubble size can be directly concluded from the microscope images in Figure 2B at early times. Only after longer times, $I^*$ increases sharply, to values larger than 1 mm, and therefore outside the validity of the diffusion approach (results not shown). A more detailed interpretation involving the bubble size distribution and interactions and the separate roles of lamellae and plateau borders is not yet available.

**CONCLUSIONS**

It was shown that the turbidity ($I^*$) and the absorbance ($I_{abs}$) of turbid colloidal systems containing an absorbing color can be obtained in a cylindrical geometry from the angle dependent transmittance of light. The specific absorbance of the colorant was found to be the same in protein and latex suspensions, indicating fulfillment of a necessary condition for the validity of...
the method. The applicability of this method to obtain structural information from the bulk of the turbid medium was demonstrated by following the acidification of milk, and the time development of the transmittance of a draining colored protein and milk foam. The increase in time of the absorbance length was interpreted to be due to an increase in the volume fraction of air. This turned out to increase from 80 to 90% during 40 min after preparing the foam. It was found to be only weakly time dependent during draining.

**AUTHOR INFORMATION**

**Notes**
The authors declare no competing financial interest.

**ACKNOWLEDGMENTS**

André Liemert gratefully acknowledges the financial support by the Carl Zeiss Foundation, Germany.

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