Introducing Diffusing Wave Spectroscopy as a Process Analytical Tool for Pharmaceutical Emulsion Manufacturing

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ABSTRACT: Emulsions are widely used for pharmaceutical, food, and cosmetic applications. To guarantee that their critical quality attributes meet specifications, it is desirable to monitor the emulsion manufacturing process. However, finding of a suitable process analyzer has so far remained challenging. This article introduces diffusing wave spectroscopy (DWS) as an at-line technique to follow the manufacturing process of a model oil-in-water pharmaceutical emulsion containing xanthan gum. The DWS results were complemented with mechanical rheology, microscopy analysis, and stability tests. DWS is an advanced light scattering technique that assesses the microstructure and in general provides information on the dynamics and statics of dispersions. The obtained microstructure results showed good agreement with those obtained with bulk rheology. Although no notable changes in the rheological behavior of the model emulsions were observed during homogenization, the intensity correlation function provided qualitative information on the evolution of the emulsion dynamics. These data together with static measurements of the transport mean free path (P) correlated very well with the changes in droplet size distribution occurring during the emulsion homogenization. This study shows that DWS is a promising process analytical technology tool for development and manufacturing of pharmaceutical emulsions. © 2014 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 103:3902–3913, 2014

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INTRODUCTION

Emulsions have a wide range of applications in a plethora of fields, from which we can highlight the food, cosmetic, and pharmaceutical sciences.1–3 These dispersions of two or multiple liquid phases are generally stabilized by a single or several emulsifiers, for example, surfactants, amphiphilic polymers, or small particles. However, when used for the above-mentioned applications, the emulsion composition often comprises several other additives, which introduce a high degree of complexity to the emulsion structure.

To fine-tune the final structure of an emulsion product, it is essential to understand the physical interactions between the different emulsion components as well as the influence of the different processing steps on, for example, the emulsion structure or stability. Therefore, it is of major interest to analytically follow the process of emulsion manufacturing (e.g., homogenization). This aim is in line with the initiatives of “Quality by Design” (QbD) and Process Analytical Technologies (PAT) that are being increasingly implemented to improve product and process understanding as well as quality control.4,5 The QbD approach identifies critical quality attributes and studies how they are correlated with critical material and process parameters. On the basis of such knowledge, a PAT strategy can be defined to monitor the manufacturing process regarding final product quality.

The complex interactions between the different components in the emulsions have been characterized with, for example, light scattering, rheology, microscopy, and turbidity measurements.2 Although some of these techniques can only be used offline, that is, to characterize the final product, others have some potential for real-time monitoring. Especially for process analytics, a technical hurdle is often the high turbidity of typical pharmaceutical emulsions. Herein, the most suitable techniques to monitor changes during emulsion manufacturing would be rheological analysis, or other techniques that allow measurements at high turbidity, such as Raman spectroscopy,6 dielectric spectroscopy,7 ultrasound techniques,8 or diffusing wave spectroscopy (DWS).9

The assessment of the rheological properties is of high importance when characterizing emulsions, because detailed information about the flow behavior, emulsion structure, as well as the nature and strength of the interactions between droplets can be obtained.10–12 The use of mechanical rheometers allows the study of mechanical emulsion properties on a macroscopic scale. Texture and flow properties can be measured through the stress-induced deformation of the samples. Although these properties are also used to assess the emulsion structure, the bulk rheological measurements are often not sensitive to important local changes in the microstructure. Thus, to assess the interactions between the different emulsion components and the variations in emulsion microstructure, microrheological techniques are best suited.13
Several techniques have been used to measure the microrheology of samples, such as single- or multiple-particle tracking atomic force microscopy, dynamic light scattering (DLS), and DWS, which mainly lead to a potential for real-time process analytics. DWS is a powerful optical technique specifically suited to study turbid samples. The method is based on the analysis of the fluctuations of coherent laser light that is scattered multiple times within a sample. DWS is a fast method where the sample is probed by a laser beam over a large frequency range that is partially inaccessible to classical mechanical rheology. These characteristics, together with the ability to measure a sample in its natural unperturbed state, make DWS well suited for on-line measurements in a flow-through cell.

Diffusing wave spectroscopy is an advanced light scattering technique with a well-founded theoretical background. This technique is being increasingly employed in the field of food science, for instance, to follow gelation or acidification, as well as to monitor destabilization mechanisms, such as flocculation and/or creaming or Ostwald ripening. DWS has only recently been introduced into the pharmaceutical field, but to the best of our knowledge not for monitoring of a pharmaceutical manufacturing process.

In this article, DWS was employed as an at-line technique to monitor the manufacturing process of pharmaceutical emulsions. The selected model system was a simple oil-in-water (O/W) pharmaceutical emulsion that was stabilized with a nonionic surfactant and different concentrations of xanthan gum. The evolution of the emulsions during the manufacturing process was investigated using DWS and was complemented with off-line mechanical rheology and microscopy analysis. The results obtained were compared with near-infrared (NIR) analytical centrifugation that was used for off-line stability testing.

MATERIALS AND METHODS

Materials

Xanthan gum, Vanzan NF-C, was supplied by Vanderbilt Minerals, LLC (Norwalk, Connecticut) and almond oil was purchased from Henry Lamotte Oils (Bremen, Germany). Polysorbate 80 (i.e., polyoxyethylene 20 sorbitan monoooleate), methylparaben, and propylparaben were obtained from the local supplier Hänseler (Herisau, Switzerland). All excipients were used as supplied without any further purification.

Methods

Emulsion Preparation

The stock solutions for the continuous emulsion phase were prepared by dissolving 2.0% (w/w) xanthan in distilled water containing preservatives while stirring for 48 h. The solution containing preservatives was prepared by dissolving 0.1% (w/w) propylparaben and 0.5% (w/w) methylparaben at 60 °C in distilled water. The aqueous phase was prepared by diluting the stock solution to the desired xanthan concentration (0.5%–1.0%, w/w) with the solution containing preservatives. Two-hundred gram of this phase were heated to 50 °C and further mixed with a Polytron homogenizer (PT10–35 GT; Kinematica, Luzern, Switzerland) at maximal speed. The oil phase was prepared by mixing 2.0 g of polysorbate 80 with 30.0 g of almond oil, and heating to 50 °C. The oil phase was then added to the aqueous phase while homogenizing. The homogenization process was continued and samples of 12 g were taken at specific time intervals. The total homogenization duration was 10 or 17 min, depending on the final concentration of xanthan gum in the aqueous phase, that is, 0.45% (w/w) or 0.68% and 0.90% (w/w), respectively.

The obtained samples were characterized at a temperature of 25 °C using bright field (BF) microscopy, confocal microscopy, mechanical rheology, DWS, and NIR analytical centrifugation.

BF Microscopy

An Olympus CKX41 microscope (Tokyo, Japan) was used for BF analysis. A small amount of sample was placed on a microscope slide followed by gentle squeezing with a cover slip. To obtain the droplet size distributions, the diameters of approximately 100 oil droplets were analyzed from recorded images using the public domain image analysis software ImageJ (National Institutes of Health, USA). The following bins were used for the droplet size distribution histogram: 0–1, 1–2, 2–3, 3–5, and 5–10 μm. The bins were normalized for the total surface of the histogram to become unity.

Confocal Microscopy

For confocal microscopy, an Olympus FluoView 1000 system was employed. The instrument configuration was based on an inverted Olympus IX61 microscope equipped with a 488 nm laser and a 40x objective with numerical aperture of 0.90. For these experiments, the samples were stained with the lipophilic dye Nile Red. A droplet of the sample was thereafter placed on a cover slip and confocal images were recorded in the bulk at approximately 15 μm from the interface between the cover slip and the sample.

Mechanical Rheology

Mechanical rheology, which allows to probe the bulk rheology of the samples, was performed on a Bohlin Gemini rheometer (Malvern Instruments Ltd., Malvern, UK) equipped with a sand blasted plane-cone geometry (60 mm, 2 °) and a solvent trap. The storage, G′, and loss moduli, G″, were measured in oscillation mode with a constant strain amplitude of 0.05. This value was found to be in the linear viscoelastic range based on the results of an amplitude sweep at 1 Hz.

Diffusing Wave Spectroscopy

Theory and Experimental Setup. Diffusing wave spectroscopy is a multiple light scattering technique that requires highly turbid samples to ensure that the propagation of light in the sample can be approximated by the diffusion equation. In this work, a DWS RheoLab (LS Instruments AG, Fribourg, Switzerland) was used for diffusing wave spectroscopic analysis in transmission mode. A scheme of the apparatus is illustrated in Figure 1.

The laser light (λ = 685 nm) was scattered from a ground glass and collected by a single lens before illuminating the turbid sample. To avoid time-consuming measurements at comparatively low frequencies, that is, long lag times in the intensity correlation function (ICF), the instrument employed the so-called echo technique. The echo data complemented the light scattering results to obtain a complete ICF over a broad range of lag times. In echo mode, the ground glass rotates and
produces a periodically changing speckle pattern. This results in greatly improved statistical ensemble averaging and is particularly important when working with nonergodic or slowly evolving systems. The scattered light was recorded by a single photon counting device and the resulting signal was processed by a digital correlator. The fluctuations of the time-varying scattering intensity, $I(t)$, can then be visualized in the ICF

$$g_2(\tau) = 1 - \frac{\langle I(t)I(t+\tau) \rangle}{[\langle I(t) \rangle]^2}$$

where $\tau$ is a variable lag time. The ICF can be expressed in terms of the mean-square displacement (MSD), $\langle \Delta r^2(t) \rangle$, of the particles. The latter is related to the complex modulus $G^*(\omega)$ via a generalized Stokes–Einstein relation:

$$G^*(\omega) = \frac{k_B T}{\pi a \omega} \langle \Delta r^2(\omega) \rangle = G'(\omega) + iG''(\omega)$$

where $a$ is the particle radius, $k_B$ is the Boltzmann constant, $T$ is the temperature, and $\langle \Delta r^2(\omega) \rangle$ corresponds to the Laplace transform of $\langle \Delta r^2(t) \rangle$. The cumulative signal of multiple light scattering allows monitoring of the average particle dynamics at very short length scales. In line with these considerations, the MSD was used in the generalized Stokes–Einstein relation (Eq. 2) to estimate the storage, $G'(\omega)$, and the loss, $G''(\omega)$, moduli of the samples over a broad range of frequencies. These values are obtained using the software provided by LS Instruments AG.

**Sample Preparation and Calibration.** Samples were analyzed in cuvettes with a thickness, $L$, of 2 mm. To ensure enough turbidity of the xanthan samples for DWS measurements, polystyrene particles (Thermo Scientific, Fremont, California) with a mean size of 430 nm were added to the continuous phase in a concentration of 1% (w/w). This step was not necessary for the emulsion samples collected at the various homogenization times. For the DWS measurements, all samples were equilibrated at 25 °C in the measuring chamber. The measurement time was set to 230 s, including 30 s for echo duration.

The DWS RheoLab is also able to measure the transport mean free path, $l^*$, by comparing the transmitted light intensity (count rate, CR) of a sample to that of a standard sample. In this study, reference standards were selected that contained polystyrene particles (Polysciences Europe Ltd., Eppelheim, Germany) with 600 nm diameter dispersed in water at concentrations of 0.76%, 0.85%, and 1.0% (w/w), respectively. The measured values of CR and $l^*$ of the calibration standards were fitted with $l^* = A^* \text{CR} + B$, which provided $A^* = 0.903 \, \mu \text{m}/\text{kHz}$ and $B = -28.29 \, \mu \text{m}$. This relation allowed the calculation of $l^*$ for the samples based on the transmission measurements.

**NIR Analytical Centrifugation**

Collecting NIR transmission spectra along the length of a sample tube during centrifugation is an offline method to assess emulsion stability. We applied this method using a microprocessor-controlled LUMiSizer® 612 analytical centrifuge (L.U.M. Ltd., Berlin, Germany) to analyze the emulsions obtained at different homogenization durations. Approximately 0.42 g of sample was pipetted into disposable polycarbonate cells with an optical path length of 2 mm. Cells were inserted horizontally in the instrument and centrifuged at 2300 g for 350 min. In this technique, the NIR light ($\lambda = 870 \, \text{nm}$) passes through the complete length of the rotating sample cell and is recorded with a CCD-line sensor continuously during centrifugation. This enables determination of space- and time-resolved sample transmission profiles. In each experimental run, 300 transmission profiles were recorded with a light factor of 1. Using the LUMiSizer® software–SEPView V.5.1 (L.U.M. Ltd.), the range of the cuvette length was individually selected according to the menisci of the samples, and the given clarification profiles. The baselines of the clarification profiles were normalized to zero to subtract static transmission noise and inhomogeneity. This allowed a comparison of samples with different initial turbidity. Results were expressed in terms of the instability index, which is here given by the ratio of the clarification profile at 350 min to the clarification profile at the beginning. The instability index is a dimensionless number between 0 and 1, where 0 corresponds to very stable and 1 indicates very unstable samples.

Figure 1. Schematic representation of the experimental setup for DWS.
RESULTS AND DISCUSSION

Xanthan-Containing Emulsions

The anionic high-molecular-weight polysaccharide xanthan is known for extensive shear thinning (pseudoplastic flow), that is, it exhibits high apparent viscosity at low shear rates and a substantial viscosity loss upon shearing. These properties can be attributed to the formation of a loosely bound gel that is very advantageous in dispersion formulations. Xanthan gum is therefore often used in emulsion formulations both as a thickener and as a stabilizer.\(^*\) The excipient grade used in our study, Vanzan NF-C, produces very clear solutions, which indicates a homogeneous polymer dispersion on the length scale of visible light.

It is important to understand the properties of xanthan gum, as they will largely determine the rheological properties of the emulsions. In its native state, xanthan is in a rather ordered conformation, that is, double helix. Transitions from the ordered to the disordered, random coil, conformation can be achieved by changes in temperature and ionic strength.\(^{33,34}\) This transition is reversible and, under specific conditions, xanthan-ordered conformation can be restored. Depending on thermal treatment of xanthan, different global conformations can be obtained.\(^{35}\) Interestingly, the denaturation of xanthan was reported to make the system no longer sensitive to the thermal history, that is, the behavior of xanthan is then no longer affected by the temperature at which it was prepared.\(^{36}\)

At low polymer concentrations, that is, dilute regime, the individual xanthan molecules diffuse freely and the intermolecular interactions are practically nonexistent. At the so-called overlap concentration (\(c^\#\)), the polymer chains begin to overlap and the entanglements become stronger as the concentration increases. The transition between the semidilute and the concentrated regimes occurs at the critical concentration for aggregation (\(c^{\#\#}\)). All the xanthan concentrations used in this study were well above the \(c^\#\) (\(\sim 0.013–0.080 \text{ mg/mL}\)) and above the \(c^{\#\#}\) (\(\sim 0.11–0.70 \text{ mg/mL}\)). Therefore, the polymer was expected to form a weak gel-like structure in the continuous phase of the emulsion. As the polymer entanglements increase with rising xanthan concentrations, it is reasonable to assume that comparatively longer homogenization durations are also required for higher xanthan concentrations. These theoretical considerations already show the importance to target a constant microstructure rather than constant process parameters in pharmaceutical manufacturing to ensure reproducible product properties. This is in line with QbD targets but it requires availability of an adequate method to assess the microstructure of the product during processing as well as to characterize its final state of homogenization.

Characterization of the Final Emulsions

Emulsion Type, Droplet Size, and Stability

The final composition of the emulsions is shown in Table 1.

<table>
<thead>
<tr>
<th>Emulsion</th>
<th>Xanthan (%)</th>
<th>Polysorbate 80 (%)</th>
<th>Almond oil (%)</th>
<th>Emulsion Type</th>
<th>Stability Index*</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.45</td>
<td>0.90</td>
<td>13.7</td>
<td>O/W</td>
<td>0.65</td>
</tr>
<tr>
<td>B</td>
<td>0.68</td>
<td>0.90</td>
<td>13.7</td>
<td>O/W</td>
<td>0.016</td>
</tr>
<tr>
<td>C</td>
<td>0.90</td>
<td>0.90</td>
<td>13.7</td>
<td>O/W</td>
<td>0.0013</td>
</tr>
</tbody>
</table>

* Determined by means of NIR analytical centrifugation.

The stability of the final emulsions was assessed experimentally by means of NIR analytical centrifugation. The obtained instability indices are also presented in Table 1. The results show that the stability of the emulsions regarding phase separation increased dramatically with increasing xanthan concentration in the continuous phase. As discussed above, the increase in polymer concentration leads to stronger entanglements between polymer chains and thus to stronger gel networks. The gel strength can also be characterized by the polymeric mesh size, that is, the average distance between segments of different polymer chains. The mesh size of highly

Figure 2. Confocal microscopy images showing an area of 41 × 41 \(\mu\text{m}^2\) of the emulsions stained with Nile red. This dye has affinity to the oil phase of the emulsion. The emulsions contain (a) 0.45%, (b) 0.68%, and (c) 0.90% (w/w) xanthan in the continuous phase.
entangled solutions of semiflexible polymers has been described by Hinsch et al.\textsuperscript{39}:

\[ \xi = \sqrt{3/(\nu L_c)} \]  

(3)

where \( \xi \) is the mesh size and \( \nu \) corresponds to the polymer density, that is, the number of polymers with contour length \( L_c \) per unit volume. The typical contour length of xanthan is approximately 2 \( \mu \text{m} \),\textsuperscript{40–42} and the molecular weight of this grade of xanthan has been reported to be approximately \( 2 \times 10^6 \text{ Da} \).\textsuperscript{43} Taking these values into account, the mesh size for the continuous phase networks containing different concentrations of xanthan can be determined. Calculated values in the order of 23, 27, and 33 \( \mu \text{m} \) were obtained for xanthan concentrations of 0.90%, 0.68%, and 0.45% (w/w), respectively. These calculated molecular mesh sizes may, however, differ in the presence of emulsion droplets. The gel network is likely to interact directly with droplets leading to changes in their surface properties and potentially also in their deformation behavior under shear. The latter effect on emulsion rheology has been studied by Saiki et al.\textsuperscript{44} using a model system. However, the molecular mesh size and gel strength are expected to primarily dominate the rheological properties of the continuous phase. In the case of our model emulsions, the most concentrated and densest xanthan gel resulted evidently in some droplet stabilization, thereby hindering coalescence and creaming.

**Rheological Characterization—Comparison Between Mechanical Rheology and DWS**

The rheological characterization is crucial when studying emulsions. The rheology of emulsions is influenced by several factors, such as the composition of the continuous phase, the oil volume fraction, and the physical properties of the interfacial layer.\textsuperscript{12} Several empirical models exist that describe the rheology of emulsions theoretically, depending on different ranges of the dispersed phase volume fraction. For emulsions with rather low volume fractions of oil (intermediate regime), that is, \( 0.01 < \phi < 0.2 \), the Batchelor equation is commonly employed.\textsuperscript{45} This equation accounts for the Brownian motion and interparticle interactions. However, the latter interactions are rather limited at low volume fractions and comparatively larger droplet size. Despite being neglected in the Batchelor model, some influence on the rheology can still arise from the average droplet size and droplet size distribution.\textsuperscript{10,46} For an in-depth introduction to the field of emulsion rheology, the book by Macosko\textsuperscript{17} is, for example, recommended.

Most of what is known about rheological effects on emulsion processing and stability is derived from macroscopic bulk measurements. An exception is, for example, a study of emulsion gels using noncontact acoustic spectroscopy to determine microrheological moduli (\( G' \) and \( G'' \)) in a frequency range that was comparable to that of mechanical measurements (i.e., to a maximum of 100 Hz).\textsuperscript{48}

In our study, the final rheological properties of the selected model emulsions were measured by means of mechanical oscillatory rheology and DWS microrheology. The former technique measures how the system responds to a mechanical perturbation, providing information on the bulk (macro) rheology of the system. DWS microrheology, on the contrary, is a noncontact method that measures the Brownian motion of the particles (droplets) to gain information on the microrheology of the system in a particularly broad range of frequencies. Despite fundamental differences in the experimental principles, it has been reported that good agreement between the data from mechanical rheology and microrheology can be obtained when the size of scattering particles (e.g., droplets) is much larger than the polymer mesh size.\textsuperscript{11,12} This was the case for the herein studied emulsions, where the mean oil droplet size (1–2 \( \mu \text{m} \)) was much larger than the calculated polymer mesh size (20–40 nm), and thus good agreement between the two techniques was theoretically expected. The basic principles of the two techniques and a schematic of the sample microstructure are depicted in Figure 4.

Figure 5 shows the storage (\( G' \)) and loss (\( G'' \)) moduli obtained using the two different techniques. The mechanical experiments covered a frequency range of \( 5 \times 10^{-2}–10^2 \text{ rad/s} \), whereas DWS microrheology extended the range up to \( 10^5 \text{ rad/s} \). In order to obtain \( G' \) and \( G'' \) from the DWS measurements, Eq. 2 was used with the mean droplet radius as the particle radius \( a \). As the experimentally obtained droplet size distributions of the emulsions were rather broad, the direct setting of a mean value for \( a \) as input parameter was avoided. Instead, \( a \) was used as a fitting parameter to reach a reasonable agreement in the overlapping frequency range between the data sets obtained from DWS and mechanical rheometry. The determined values were \( a = 700, 300, \) and 240 \( \text{nm} \) for the xanthan concentrations of 0.45%, 0.68%, and 0.90% (w/w), respectively. At first sight, these values may seem small when compared with the droplet size distribution shown in Figure 3, which would suggest values of \( a \) being on the order of 1 \( \mu \text{m} \). However, it is noteworthy that these droplet size distributions were determined from BF microscopy images where the limited size resolution does not allow detection of submicron droplets. Moreover, the maximal scattering (normalized by volume) is observed for droplet radii of about 200 \( \text{nm} \), which might increase the relative importance of small droplets as compared with the mean droplet size.\textsuperscript{49} The second observation,
Figure 4. Schematic representation of the sample, showing the droplets/particles of radius $a$ distributed within the polymer gel network with mesh size $\xi$. The rheological properties of the sample can be determined by mechanical oscillatory rheology (i.e., the sample is deformed by external forces) and by DWS (i.e., the Brownian motion of the particles is used to probe the surrounding medium).

Figure 5. Storage moduli (black) and loss moduli (red) for emulsions containing (a) 0.45%, (b) 0.68%, and (c) 0.90% (w/w) xanthan in the continuous phase, determined by mechanical rheology (circles) and DWS (lines).

Concerning the obtained values for $a$, is the clear trend to larger values for lower xanthan concentrations. Comparison of the confocal microscopy images (Fig. 2) suggests indeed more small droplets for the 0.90% and 0.68% (w/w) xanthan emulsions than for the 0.45% (w/w) xanthan emulsion. Furthermore, the oil droplets in the latter emulsion showed some aggregation, presumably because of the insufficient stability provided by a comparatively low xanthan concentration. Such aggregates increase the effective droplet size, which would support the trend for larger values of $a$ with lower xanthan concentrations.
In line with these considerations, a good agreement was obtained from DWS microrheology and mechanical rheometry. As expected, a shift toward higher moduli was observed with increasing xanthan concentration in the continuous phase. At comparatively lower frequencies (and xanthan concentrations), a crossover of $G'$ and $G''$ was detected. Herein, $G'$ became predominant and this crossover frequency shifted to lower frequencies when the concentration of xanthan was increased.\textsuperscript{35} This initial crossover was not observed for the highest xanthan concentration, probably because it occurred even below the probed frequency range. The presence of such crossover at low frequencies, which is characteristic for this type of gel, indicates that for long observation times (very low frequencies) the gel can flow again.

One of the advantages of DWS over bulk rheology is that it allows measurements at higher frequencies. Therefore, the use of DWS allowed to identify a second crossover at high frequencies for all emulsions, where $G''$ prevailed over $G'$. This second crossover has been previously observed\textsuperscript{32,50} and it reflects the short-time relaxation and dissipation mechanisms in the system.\textsuperscript{51}

The results indicate that the xanthan-containing emulsions were viscoelastic, with a liquid-like behavior at very low frequencies, a gel-like structure at higher frequencies, and again a dissipation dominated behavior at very high frequencies. Results were in agreement with the view that xanthan was forming a polymeric network and thus a weak gel in the continuous emulsion phase.

**At-Line DWS During Homogenization**

The primary study focus was on monitoring changes in the system during emulsion preparation. It was therefore crucial to first assess how the processing of the aqueous phase affected the rheological behavior of xanthan. Results are shown in Figure 6.

It can be clearly seen that the effect of heating and homogenizing the xanthan solution has only a small, almost negligible, effect on the bulk rheological properties of the continuous phase. The $G'$ moduli decreased slightly with processing, whereas no relevant differences were observed for $G''$. These results are in agreement with the specifications from the manufacturer, according to which the rheological gel properties of xanthan are stable over a broad temperature range.\textsuperscript{32} This may suggest that the used grade of xanthan has been initially heat treated by the excipient supplier so that the rheological properties become more robust regarding a further heat treatment during emulsion manufacturing.\textsuperscript{35} This result was important to better understand the following property changes of the emulsions during homogenization.

**BF Microscopy and Size Distributions**

The observation of the samples under the microscope is a common offline technique to assess the structure and the droplet size of emulsions. Figure 7 shows microscope images for the emulsions containing different xanthan concentrations in the continuous phase. Samples were collected and analyzed at different homogenization times.

It can be seen that for a short homogenization time, very large droplets coexist with small droplets in the emulsion, as it was expected. Longer homogenization times increased substantially the number of small droplets, whereas the number of large droplets became negligible. This can be quantitatively observed in Figure 8, where the homogenization time effect on the droplet size distribution is shown. As expected, a shift toward smaller droplet sizes ($<2 \mu m$) and the number of larger particles decreased considerably.

Mechanical Rheology

The bulk rheological response of the emulsions was measured during the homogenization process. Results presented in Figure 9 show that the rheological properties of the emulsion evolve only little with homogenization duration. Even though drastic changes in the droplet size distribution occurred with homogenization time (Figs. 7 and 8), these changes were here not notably affecting the bulk rheology.

Moreover, the rheological properties of the emulsions were found to not greatly differ from those of the continuous phase. These observations suggest that the bulk rheological properties of the selected model emulsions were mainly determined by the continuous phase, whereas the dispersed phase (e.g., droplet size, interactions between droplets) was clearly of lower importance. As mentioned in a previous section, this result was not uncommon for an emulsion with a comparatively low volume fraction of the dispersed phase. For such an emulsion, a moderate effect of the volume fraction is expected, whereas changes in particle size would barely affect macroscopic rheology because the interactions between droplets are limited.\textsuperscript{11,53} These
Figure 7. Bright-field microscopy images of the emulsions containing (a and b) 0.45%, (c and d) 0.68%, and (e and f) 0.90% (w/w) xanthan in the continuous phase after (a, c, and e) 1 min, (b) 9 min, and (d and f) 17 min homogenization.

considerations explain the insensitivity of the bulk rheological measurements to the observed changes in the emulsion microstructure. Therefore, macroscopic rheology was not suitable to follow changes of the model emulsions during homogenization, apart from being a rather time-consuming offline technique.

Diffusing Wave Spectroscopy
Diffusing wave spectroscopy was used to assess the microrheological properties of the final emulsions. The assumption that the continuous phase would dominate rheological properties was supported by a separate DWS experiment. Therefore, continuous phase alone (at highest xanthan concentration) was evaluated by means of microrheology after the same shear and heat treatment used previously to manufacture the final emulsion. The mechanical moduli showed similar curvatures as well as crossover points compared with the final emulsion (Fig. S1 in the Supporting Information). Thus, experimental results indeed supported the previously mentioned view that the mesh network in the continuous phase dominated the rheological properties of the emulsions.

Besides assessing microrheology, DWS further provides information on the dynamics of the scattering particles. In the particular case of a dilute or semidilute emulsion, droplets represent the scattering particles and their dynamics is determined by the rheological properties of the continuous phase as well as by the size of and interactions between the droplets.

Figure 10 shows the results for the at-line monitoring of the homogenization process for emulsions containing different xanthan gum concentrations in the aqueous phase. A clear trend
Figure 8. Droplet size distributions, obtained from statistical analysis of microscopy images, of the emulsion containing 0.90% (w/w) xanthan in the continuous phase after 1 (black circles), 3 (cyan diamonds), 5 (blue triangles), 9 (grey pentagons), and 17 min (magenta squares) homogenizing. The results represent averages for the bins 0–1, 1–2, 2–3, 3–5, and 5–10 μm.

Figure 9. Bulk rheology moduli, $G'$ (filled symbols) and $G''$ (open symbols), of the continuous phase (blue) and of the emulsion containing 0.90% (w/w) xanthan in the continuous phase after different homogenization times: 1 min (cyan), 8 min (magenta), and 17 min (black).

Figure 10. The measured normalized ICF after different homogenization durations for emulsions containing (a) 0.45%, (b) 0.68%, and (c) 0.90% (w/w) xanthan in the continuous phase.
Subsequently, we defined a reference parameter $t_{2/3}$ as the time for which the normalized ICF decays to 2/3 of its initial value. This reference decay time was determined from the DWS results for all the different homogenization times (Fig. 11). All emulsions exhibited a pronounced drop in $t_{2/3}$ during the initial stage of the homogenization process, and values were then stabilizing toward longer homogenization durations. This qualitative change of the reference decay time $t_{2/3}$ was in the case of the model emulsions mostly reflecting the droplet size effects in the course of homogenization. However, comparing the absolute $t_{2/3}$ values among the different xanthan concentrations (Figs. 11a–11c) was interesting with respect to rheological gel properties. Accordingly, longer $t_{2/3}$ decay times for the more concentrated xanthan gels were a consequence of higher gel stiffness, which slowed down the droplet diffusion.

The DWS transmission measurements further allowed to determine the transport mean free path, $l^*$, which is a critical length scale in the case of diffuse propagation of light. This parameter has widely been employed to study microstructural changes in food systems. $l^*$ can be described as the distance a photon travels in the sample before its direction of propagation is randomized. Therefore, the lower the value of $l^*$, the more turbid is a sample. This value can be calculated using Mie theory for a given droplet size distribution, which considers scattering properties of droplets and of the continuous phase, as well as the wavelength of the probing light. In our system, $l^*$ is theoretically expected to be minimal (i.e., maximal emulsion turbidity) for droplet sizes of the order of 400 nm. At constant droplet volume fraction, both larger and smaller droplet sizes would in theory result in larger values for $l^*$. Thus, during the homogenization process, we expect that an increasing number of relatively small droplets (on the order of 1 or few μm) can directly be monitored by a decreasing $l^*$. The $l^*$ values obtained from the emulsions at different homogenization times are also presented in Figure 11.

The critical length $l^*$ indeed decayed rapidly during the initial homogenization durations and stabilized after a certain time, following a similar trend as $t_{2/3}$. This behavioral similarity of $l^*$ and $t_{2/3}$ is at first sight surprising, because the parameters are essentially different. While $t_{2/3}$ is a measure for the internal dynamics of the sample, $l^*$ describes the optical properties of the sample and is thus a static value. In general, they depend on different sample properties that may change during processing. However, in the herein studied emulsions, the main changes during the homogenization procedure occurred in the droplet size distribution, which dominated both $l^*$ and $t_{2/3}$, and thus offer an explanation for the similar trend observed. It can be presumed that differences between the two parameters would be observed in emulsions that exhibit pronounced rheological changes during the homogenization process. Such an emulsion manufacture would strongly impact the time evolution of $t_{2/3}$ during homogenization. In general, the temporal monitoring of both measures $t_{2/3}$ and $l^*$ allows a better understanding of the emulsion homogenization process than if only one parameter was considered.

As $l^*$ and $t_{2/3}$ are linked to critical quality attributes of the emulsions such as the droplet size distribution and rheological properties, DWS is particularly suited for process monitoring of homogenization. The information obtained by the ICF can be used to ensure targeted properties of the final emulsion. By knowing the ICF profile of the final emulsion, the process can be monitored and stopped once the target values are reached that guarantee emulsion properties to comply with quality specifications.

**Physical Stability of the Emulsions During Homogenization**

As mentioned before, the physical stability (as determined by NIR analytical centrifugation) increased substantially with rising concentrations of xanthan in the continuous phase. In this
section, we compared the evolution of the stability index of the emulsions (offline method) with the at-line DWS measurements. Figure 12 shows the evolution of the instability indices with homogenization time. The results indicate that by increasing the homogenization time, the emulsions became more stable. The degree of stability increase was, however, different for the various xanthan concentrations. In the case of the emulsion containing the lowest xanthan concentration, the increase in homogenization time improved the stability only slightly, as the emulsion was still very unstable even after complete homogenization. On the contrary, the stability of the other emulsions was considerably improved by longer homogenization times, especially for the highest xanthan concentration. These differences in stability depending on the xanthan concentration agreed with the results of the final emulsion characterization.

The increase in emulsion stability with increasing homogenization duration was explained by the decrease in droplet size. However, such a simple trend of stability evolution may not be expected for all kinds of emulsions. The homogenization process can be further complicated, for example, by a gel structure in the continuous phase that is harmed by long homogenization times. Although an optimal homogenization time may be determined during pharmaceutical process development, a time-consuming offline method such as NIR analytical centrifugation is not practical later for production. This emphasizes that changes in the homogenization times can be selected to avoid overprocessing of emulsions. This has a high potential not only for achieving a standardized quality of the final drug product but reduction of process times can further lead to substantial cost savings in production. More research is certainly needed to step from an at-line approach to on-line process monitoring. Further microrheological studies may include more concentrated emulsions for which the rheological properties are less dominated by the continuous phase. Backscattering DWS should be evaluated as well. Even though this measurement mode is not as well defined for calculation of microrheological moduli, it may be technically simpler for process monitoring. This would further advance DWS as long-needed process analyzer for complex and turbid pharmaceutical dispersions.

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REFERENCES

Figure 12. Stability indices determined by offline NIR analytical centrifugation for different homogenization times for emulsions containing 0.45% (black circles), 0.68% (green diamonds), and 0.90% (red squares) (w/w) xanthan in the continuous phase.