Aggregation and Gel Formation in Biopolymer Solutions

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Abstract: The application of light scattering to concentrated colloidal suspensions has often been considered too complicated due to strong multiple scattering. Here we show that diffusing wave spectroscopy (DWS) permits the characterization of dynamic and static properties of such systems on a large range of time and length scales. In particular we focus on the aggregation and sol-gel transition in different colloidal systems such as latex suspensions or milk. Using DWS we obtain quantitative information about the microscopic dynamics all the way from an aggregating suspension to the final gel, thereby covering the whole sol-gel transition. At the gel point a dramatic change of the particle dynamics from diffusion to a subdiffusive arrested motion is observed. As biopolymer solutions and gels represent one of the most interesting class of gelling systems we have performed a systematic study using solutions of casein micelles which we destabilized and investigated during the sol-gel transition using DWS and rheological measurements. We can find the same behavior as found in concentrated suspensions of monodisperse latex spheres that undergo a sol-gel transition. The changes observed in the microscopic dynamics can be clearly linked to the formation of a macroscopic gel with drastically modified viscoelastic properties.

Keywords: Aggregation · Casein micelles · Colloidal gels · Diffusing wave spectroscopy (DWS) · Sol-gel transition

Introduction

Aggregation and gelation in complex fluids has been a field of intense research for a long time, where both fundamental as well as applied questions are equally important. Applications of gels and sol-gel processing include such different areas as ceramics processing, cosmetics and consumer products, food technology, to name only a few. One generally starts from a solution of subunits (monomers) and the system is then destabilized, which leads to aggregation, cluster formation and gelation. At the gel point a liquid-solid transition is observed which can be characterized by the appearance of a strongly increasing storage modulus,

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G', in rheological measurements. Despite the randomness and the complexity of the sol-gel transition, it has always attracted fundamental researchers due to the unique features of gels and the strong similarities between very different gels. The sol-gel transition displays a very rich behavior of different physical properties that can be characterized by distinct scaling laws [1].

Most of the fundamental research has been carried out on the macroscopic properties of gels, *e.g.* their viscoelastic behavior. However, it is much more difficult to access information about the microstructural properties and furthermore link them to the macroscopic properties of the gel. In this respect colloidal gels are ideal model systems since their building blocks can be very well defined (monodisperse spheres), and they are of convenient size which allows the application of modern scattering techniques. Nevertheless it remains a challenge to link the microscopic system properties, as determined by scattering techniques, to the macroscopic viscoelastic properties as obtained from rheology. New developments both in light scattering techniques and in theory now for the first time allow to bridge this gap even for very concentrated and turbid systems [2–6].

Ideal systems to investigate sol-gel transitions are well-defined suspensions of charge stabilized colloidal particles. Because of the many possibilities to easily change the strength and the range of interactions between colloidal particles they offer unique ways to study the effects of the interactions on the phase behavior and the microstructure and dynamics. It is the goal of our research in Fribourg to probe and understand these relationships. A major effort is devoted in particular to the characterization of the diffusive motions of particles in stable concentrated suspensions as well as to the onset of aggregation and sol-gel transition after destabilization, and the link between diffusive and elastic properties of the systems. In our research we combine experimental (scattering techniques, rheology, and optical microscopy) and theoretical investigations (classical theories for colloid structure, dynamics and stability and Brownian dynamics and Monte Carlo computer simulations).

The Sol-Gel Transition in Model Suspensions

The coagulation of charge stabilized concentrated latex dispersions with volume fractions typically in the range of $0.1 \leq \Phi \leq 0.4$ is induced through an increase of the ionic strength or by a change of pH towards the so-called isoelectric point (IEP) of the particles where they become neutral (Fig. 1) [4–7]. The onset of aggregation, the subsequent sol-gel transition and evolution of the gel can be followed *in situ* by means of scattering experiments. As we address particularly the high concentration regime where classical techniques such as dynamic (single) light scattering fail, we have to use other approaches. A modern multiple light scattering technique – Diffusing Wave Spectroscopy (DWS) – is of particular use in this high concentration regime.

DWS is a powerful tool to obtain information about the local dynamical properties [2][3]. In a typical DWS experiment, coherent laser light impinges on one side of a very turbid sample, and the intensity fluctuations of the light transmitted through the sample are then analyzed. DWS works in the limit of very strong multiple scattering, where a diffusion model can be used in order to describe the propagation of the light across the sample (Fig. 2a) [2]. Using such a diffusion approximation one can then determine the distribution of scattering paths and calculate the temporal autocorrelation of the intensity fluctuations analogous to a classical photon correlation experiment (Fig. 2b). DWS provides unique information on particle motion, and it is for example possible to quantitatively determine the average mean-square displacement (MSQD) of the scattering particles, $\langle \Delta r^2(\tau) \rangle$, from the measured intensity autocorrelation function over a very broad time scale 10^{-6} s $\leq \tau \leq 10^4$ s (Fig. 2c). DWS can thus probe particle motion on very short length scales, and it has for example been demonstrated that it can measure motions of particles of the order of 1 μm in diameter on length scales of less than 1 nm [2]. This allows very important information on the dynamics of particle suspensions and gels to be obtained, that can for example directly be compared with the results from computer simulations.

Fig. 2b shows typical snapshots out of a sequence of correlation functions $g(\tau)-1$ during aggregation and gelation of a latex particle suspension [5]. For ergodic systems, the mean square displacement of
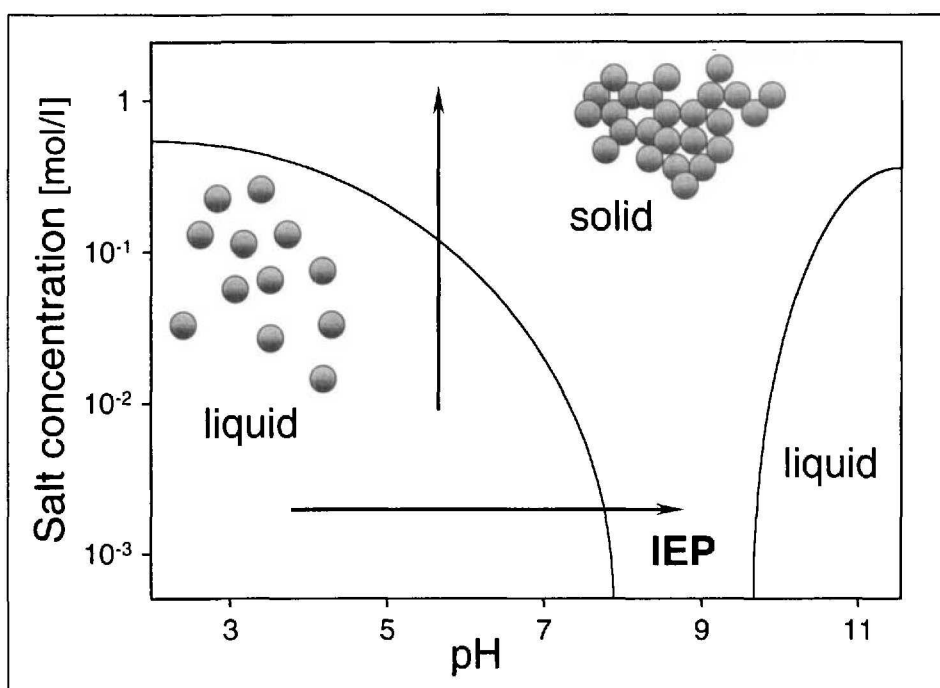

Fig. 1. Schematic representation of the two different paths in the destabilization og charge stabilized amphotericparticles leading to aggregation and gel formation.
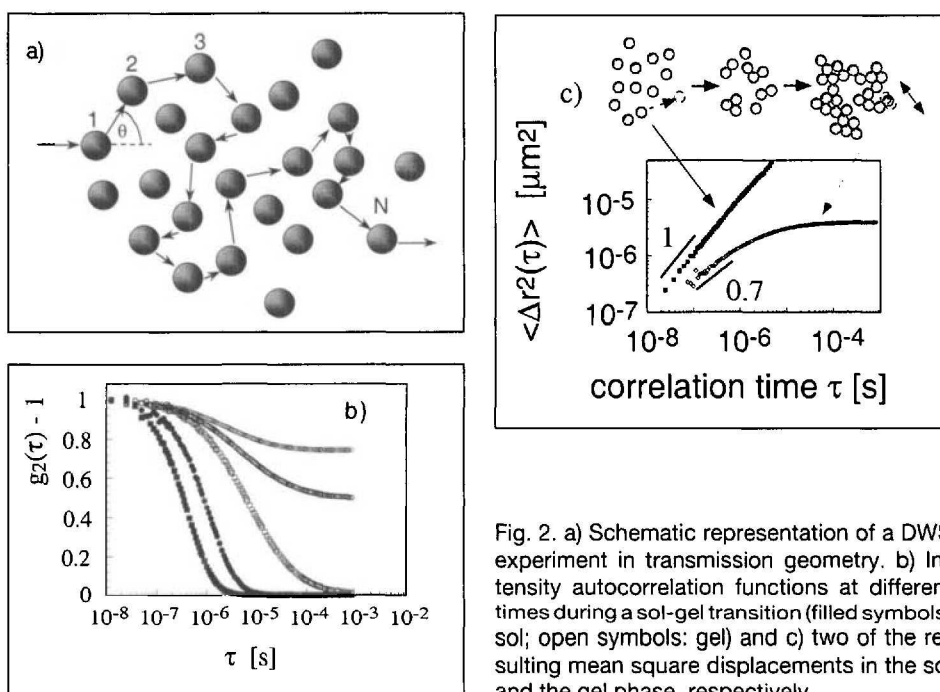

Fig. 2. a) Schematic representation of a DWS experiment in transmission geometry. b) Intensity autocorrelation functions at different times during a sol-gel transition (filled symbols: sol; open symbols: gel) and c) two of the resulting mean square displacements in the sol and the gel phase, respectively.

the correlated Brownian particles can be successfully modeled by means of an averaged short-time diffusion coefficient. This leads to a correlation function well approximated by a single exponential decay [2]. However, at later stages in the aggregation process the clusters fill the entire sample volume and gelation occurs. Because of the high volume fraction the network is very stiff, and as a consequence the trapped particles can only execute limited, very slow motions about their fixed averaged positions. After

about ten days the gel changes only very little and we take that time as the terminal state. The correlation function now exhibits a distinctly different decay, which is described by a stretched exponential with an arrested decay leading to a plateau [5].

This dramatic change in the local particle dynamics becomes even more clearly visible when looking at the time dependence of the corresponding mean square displacement $\langle \Delta r^2(\tau) \rangle$. The particle dynamics in the initial stable suspen-

sion as well as in the aggregating suspensions prior to the gel point (solid symbols) exhibit the typical characteristics of free particle diffusion due to Brownian motion. This is reflected by an almost exponential decay of the correlation functions and leads to a linear dependence of $\langle \Delta r^2(\tau) \rangle$ on time (Fig. 2c). However, at the gel point a quite dramatic change in the particle dynamics occurs, and the short time behavior changes from Brownian to a subdiffusive motion (open symbols). The MSQD is now well described by a stretched exponential. At short times this results in a power law behavior $\langle \Delta r^2(\tau) \rangle \sim \tau^p$ with an exponent $p \approx 0.7$ (see Fig. 2c) [5].

The Sol-Gel Transition in Biopolymer Solutions: From Milk to Yogurt

Acidified milk products such as yogurt are popular and important food products. For such acid-induced biopolymer gels the process variables have an enormous impact on the gel-process as well as on the physical properties of the final gel. However, we currently lack a clear understanding for the relationship between the microscopic structural dynamic properties of the macromolecular constituents and the resulting macroscopic mechanical and rheological properties of the gel. In milk the main part of the proteins, the caseins, are organized into micelles, which are sterically stabilized by a layer or 'brush' of κ-casein molecules. The κ-caseins extend their C-terminal part into the solution, causing the micelles to repel each other on close approach. A number of studies have demonstrated that the casein micelles have properties very similar to those of sterically stabilized hard sphere suspensions ([8][9] and references therein). To destabilize these micelles and to induce the gelation process one can either enzymatically remove the stabilizing hairs or acidify the milk. In Fig. 3 a schematic comparison between the resulting interaction potential of sterically stabilized casein micelles and charge stabilized colloidal particles is shown.

Here we now demonstrate the close analogy between the yogurt making process and the sol-gel transition in colloidal model suspensions. We show that one can obtain very detailed information about aggregation and gelation in complex food emulsions from an application of optical methods such as DWS.

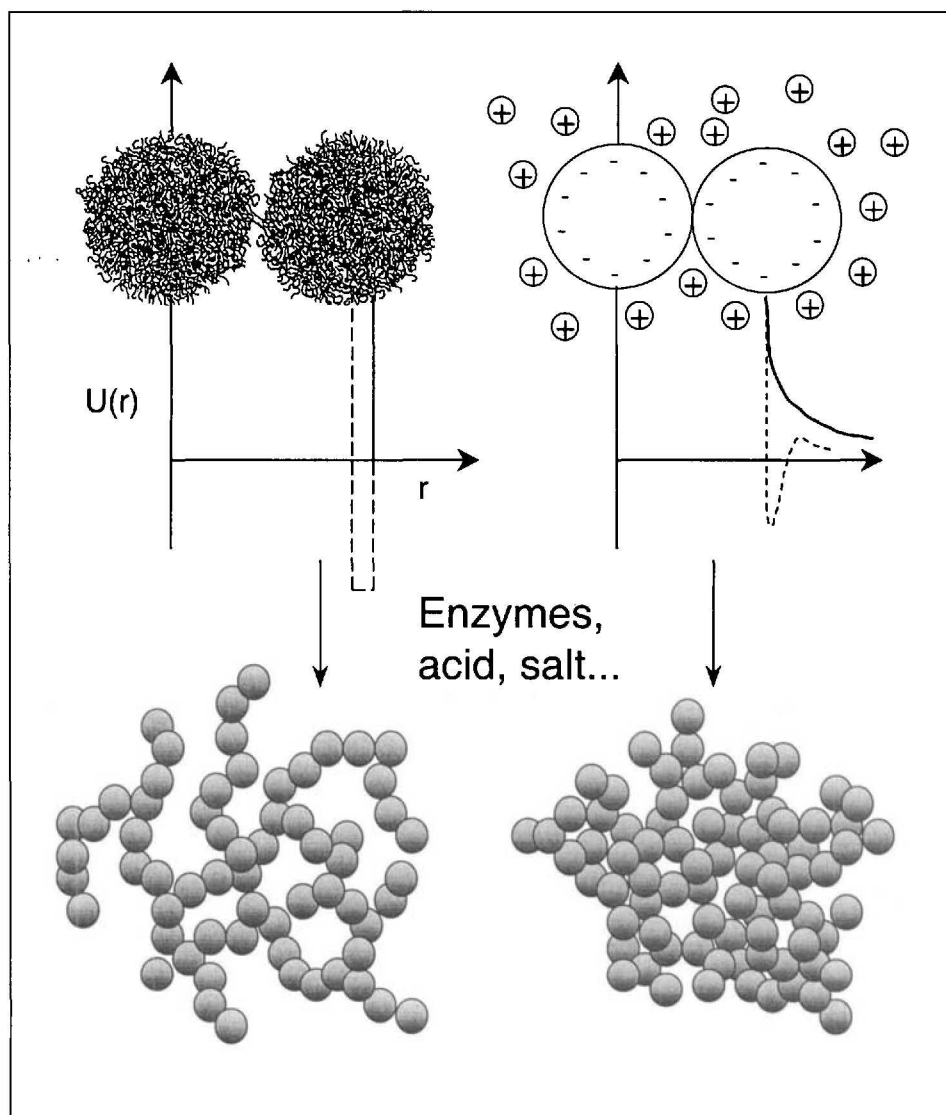To induce the yogurt formation in different milks (fat-free milk and fat-con-

Fig. 3. Schematic representation of the potential of sterically stabilized particles (casein micelles) and charge stabilized particles. The dashed lines represent the potential after the collapse or release of the hairy layer and the screening of the charges by the addition of salt or variation of the pH, respectively.

taining milk), we added glucono-δ-lactone (GDL), which hydrolyses in milk and shifts the pH to lower values. This leads to a 'collapse' of the stabilizing brush, which now is no longer able to prevent aggregation of the particles. Details on the experimental procedures and the instrumentation and data analysis used are given elsewhere [5][6][10][11].

Typical DWS results from measurements on fat-free milk at different stages of the gelation process are given in Fig. 4. Shown are the correlation functions $g(\tau) - 1$, and a plot of the corresponding mean square displacements $\langle \Delta r^2(\tau) \rangle$ is shown as an inset. The open symbols are used for data from liquid-like samples corresponding to times prior to the gel formation, and filled symbols are used for measurements in the gel. The first measurement right after the addition of GDL (open circles) reflects the diffusion

of the free, stabilized casein micelles. Initially we observe a faster decay of the correlation function, which corresponds to an increase of the diffusion coefficient (open triangles). This is due to a decrease of the particle size and the modified interaction potential because of the brush collapse caused by the addition of GDL. As the casein micelles are no longer stabilized, they then start to aggregate, which can be seen from the slowing down of the particle diffusion due to the formation of clusters.

The particle dynamics in the stable milk as well as in the aggregating suspensions prior to the gel point (open symbols) exhibit the typical characteristics of free particle diffusion due to Brownian motion. This is reflected by an almost exponential decay of the correlation functions and leads to a linear dependence of the MSQD on time (indicated with a line

with slope one in Fig. 4). However, at the gel point a quite dramatic change in the particle dynamics occurs, and the short time behavior changes from Brownian to a subdiffusive motion (filled symbols). The MSQD is now well described by a stretched exponential. At short times this results in a power law behavior $\langle \Delta r^2(\tau) \rangle \sim \tau^p$ with an exponent $p \approx 0.7$ (Fig. 4). This is the same behavior as already found in concentrated suspensions of almost mono-disperse polystyrene spheres that undergo a sol-gel transition as discussed above.

The same experiments have been repeated for the fat-containing milk, and the results are summarized in Fig. 5. The correlation functions and the corresponding mean square displacements are plotted at different times during the destabilization process from the stable milk to the final gel. In contrast to the fat-free milk, the initial increase of the casein micelle diffusion coefficient caused by the collapse of the hairy layer cannot be observed any more due to the contribution from the additional population of fat droplets. However, we clearly observe the slowing down of the average diffusion coefficient due to cluster formation, and the crossover of the particle dynamics at the gel point. This is again visible in the short time behavior of $\langle \Delta r^2(\tau) \rangle \sim \tau^p$, where we again find a decrease of the exponent p from 1 for the stable milk (open symbols), to about 0.7 for the gel (filled symbols).

In rheology it is common practice to define the gel point as the point where the elastic properties (represented by the storage modulus G') dominate over the viscous properties (represented by the loss modulus G") ([9] and references therein). A comparison between the time-resolved rheological measurements and the DWS experiments demonstrates that the qualitative change in microscopic particle dynamics indeed coincides with a dramatic change in the macroscopic viscoelastic properties of the samples at the gel point. This is shown in Fig. 6 and 7, where the storage (G') and loss (G") moduli measured at a single oscillation frequency and the exponents p obtained from DWS are plotted as a function of time. In the case of the fat-free milk (Fig. 6) we observe a steep increase of G' at approximately 2.2 h after the GDL addition, indicating the transition from a sol to a gel (Fig. 6a). Fig. 6b shows that at the same time the exponent p drops from 1 to about 0.7.

The results obtained for fat-containing milk are plotted in Fig. 7, and they qualitatively show the same feature. How-
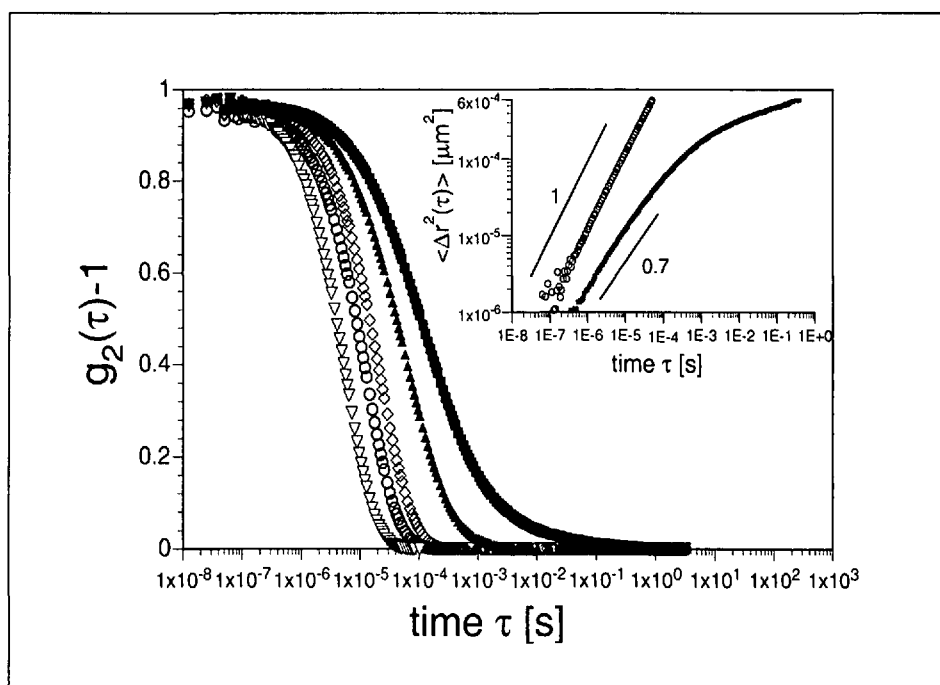

Fig. 4. Intensity autocorrelation functions $g_2(\tau) - 1$ from DWS experiments and as an inset the corresponding mean square displacements $\langle \Delta r^2(\tau) \rangle$ for fat-free milk at different stages of the aggregation and gelation process. The open symbols correspond to times prior to the gel point, the filled symbols are measurements in the gel (symbols ordered with increasing time: open circles, open triangles, open diamonds, filled triangles, filled squares).
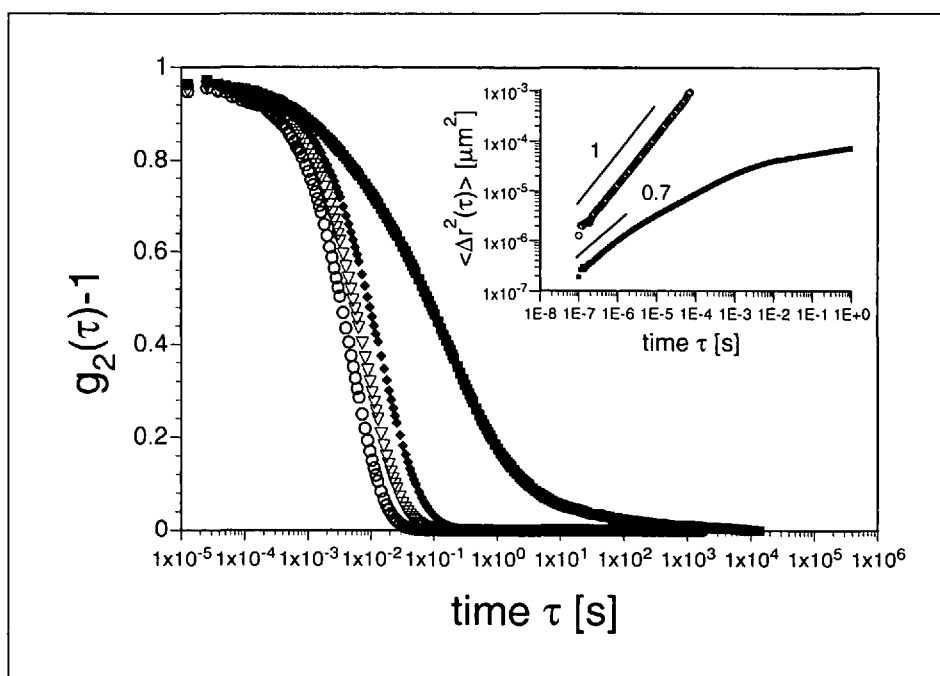

Fig. 5. Intensity autocorrelation functions $g_2(\tau) - 1$ from DWS experiments and as an inset the corresponding mean square displacements $\langle \Delta r^2(\tau) \rangle$ for fat-containing milk at different stages of the aggregation and gelation process. The open symbols correspond to times prior to the gel point, the filled symbols are measurements in the gel (symbols ordered with increasing time: open circles, open triangles, filled triangles, filled squares).

ever, the resulting values of G' are significantly higher when compared to the fat-free milk, and the sol-gel transition appears to be broader. This is visible in the time evolution of G' and G" (Fig. 7a) as well as of the exponent p (Fig. 7b). We currently lack a detailed and quantitative

understanding of the effects of particle polydispersity on the sol gel transition in attractive particle suspensions. Nevertheless our results clearly demonstrate that the sol-gel transition in fat-containing milk exhibits the same similarities in the link between microscopic dynamics and

macroscopic elastic properties as the much simpler model systems latex suspensions and fat-free milk.

However, we can go far beyond a simple qualitative comparison of the DWS and rheology experiments. It is in fact possible to extract qualitative information on the viscoelastic properties of the gel. This has first been demonstrated for dilute latex particle gels by Krall and Weitz [12]. Using fractal arguments they suggested that the constrained dynamics observed in fractal gels is due to a hierarchy of elastic modes within the fractal cluster, covering a wide range of relaxation times. Each gel segment is embedded in successively larger sub-clusters up to the entire cluster radius R_c and thus its thermal motion is coupled to the motion from any larger segment. The maximum displacement δ^2 and the characteristic relaxation time of the correlation function τ_c determined from DWS are then related to the elastic modulus of the gel [12]. Thus it is possible to compare the moduli extracted from light scattering to the moduli measured with classical rheology. We indeed find excellent agreement between the light scattering data and the rheological measurements even at high particle volume fractions where the concept of a fractal gel breaks down [13]. One can even extend this approach and determine the full frequency dependence of the loss and storage moduli $G'(\omega)$ and $G''(\omega)$ over a very wide range of frequencies [10][11].

This optical microrheology approach is not limited to idealized particle gels, but can also be applied to much more complex systems such as yogurt. This opens up a completely new field of applications of non-invasive light scattering techniques for the characterization of complex systems or process control in various areas such as ceramics or food processing.

Acknowledgements
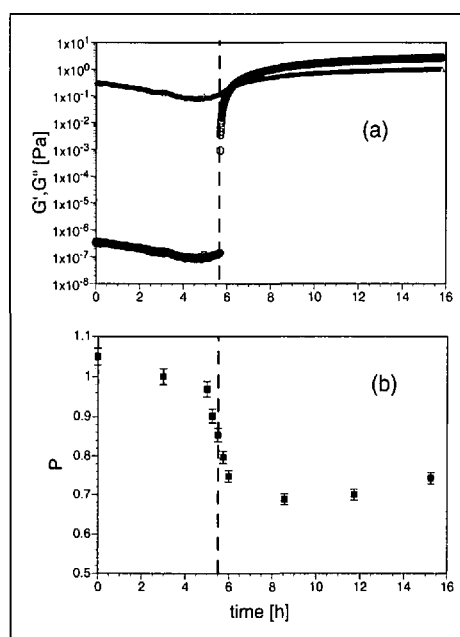
Received: January 15, 2001

Fig. 6. Comparison of results from classical rheology and DWS during the yogurt-making process in fat-free milk. (a) Time evolution of the storage modulus G'(t) (big gray circles) and the loss modulus G''(t) (small black triangles) obtained from an oscillating rheological measurement (1Hz, 1% amplitude). (b) Time evolution of the exponent p obtained from DWS. The enormous increase in G' and the drop of the exponent coincide (dashed line) and indicate a link between microscopic particle dynamics and macroscopic sol-gel transition and viscoelastic properties.
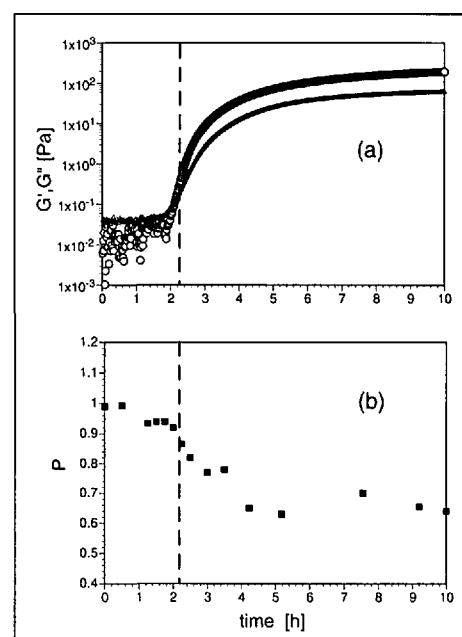

Fig. 7. Comparison of results from classical rheology and DWS during the yogurt-making process in fat-containing milk. (a) Time evolution of the storage modulus G'(t) (big gray circles) and the loss modulus G''(t) (small black triangles) obtained from an oscillating rheological measurement (1Hz, 1% amplitude). (b) Time evolution of the exponent p obtained from DWS (redrawn after [10]).

Chen, J.S. Huang, P. Tartaglia, Kluwer Academic Publishers, Dordrecht, 1992, pp 731.

[3]   T.G. Mason, H. Gang, D.A. Weitz, J. Opt. Soc. Am. A 1997, 14, 139.
[4]   C. Urban, S. Romer, F. Scheffold, P. Schurtenberger, Macromol. Symp. 2000, 162, 235.
[5]   a) S. Romer, F. Scheffold, P. Schurtenberger, Phys. Rev. Lett. 2000, 85, 4980; b) F. Scheffold, S.E. Skipetrov, S. Romer, P. Schurtenberger, submitted to Phys. Rev. E.
[6]   C. Urban, S. Romer, F. Scheffold, P. Schurtenberger, Prog. Colloid Polym. Sci. 2000, 115, 270.
[7]   T.J. Graule, F.H. Baader, L.J. Gauckler, J. Mater. Educ. 1994, 16, 243.
[8]   a) C. Holt, D.S. Horne, Neth. Milk Dairy J. 1996, 50, 85; b) C.G. de Kruif, Int. Dairy J., 1999, 9, 183; c) J.A. Lucey, H. Singh, Food Research Int. 1998, 30, 529.
[9]   D.S. Horne, Int. Dairy J. 1999, 9, 261
[10] A. Stradner, S. Romer, C. Urban, P. Schurtenberger, Prog. Colloid Polym. Sci. 2001, in press.
[11] S. Romer, C. Urban, A. Stradner, C.G. de Kruif, P. Schurtenberger, submitted to Food Hydrocolloids.
[12] A.H. Krall, D.A. Weitz, Phys. Rev. Lett. 1998, 80, 778.
[13] S. Romer, H. Bissig, A. Stradner, F. Scheffold, V. Trappe, P. Schurtenberger, manuscript in preparation.

[1]   C.J. Brinker, G.W. Scherer, 'Sol-Gel Science: the Physics and Chemistry of Sol-Gel Processing', Academic Press, San Diego, 1990.
[2]   D.A. Weitz, J.X. Zhu, D.J. Durian, D.J. Pine, in 'Structure and dynamics of strongly interacting colloids and supramolecular aggregates in solution'; Ed. S.-H.