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Raman correlation spectroscopy: A method for studying chemical composition and dynamics of disperse systems

Wolfgang Schrof,* Jürgen F. Klingler, Stanislaw Rozouvan, and Dieter Horn
Polymers Laboratory, Department of Polymer and Solid State Physics ZKM, BASF-Aktiengesellschaft, D-67056 Ludwigshafen, Germany

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Raman correlation spectroscopy—intensity fluctuation spectroscopy of Raman-scattered light from a small sample volume—has been demonstrated. A modified confocal Raman microscope is used to observe number fluctuations of colloidal particles caused by Brownian motion. Correlation analysis of the fluctuations yields mobility and number density of the particles. In mixed systems, particles are differentiated according to their chemistry by selecting characteristic Raman bands for detection. Cross correlation of the signals from different Raman bands has been demonstrated. This method extends the domain of optical fluctuation spectroscopies to Raman scattered light, combining the chemical identification obtained by Raman scattering with the structural and dynamical information obtained by correlation spectroscopy. [S1063-651X(98)50403-5]

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In the last three decades, optical fluctuation spectroscopies have become indispensable tools for studying dynamic phenomena in colloidal systems. The most widespread of these methods are dynamic light scattering (DLS, or photon correlation spectroscopy, PCS), first demonstrated 33 years ago [1] and fluorescence correlation spectroscopy (FCS), now 25 years old [2]. Today, DLS instrumentation is readily available, and this method and a number of its variants are used in many fields ranging from macromolecular chemistry to biophysics to industrial quality control of submicron particle sizes [3,4]. Even after 30 years, DLS is still seeing fundamental development. An example is x-ray PCS [5], which promises an extension of the length scales accessible to DLS. FCS has only recently seen a surge in interest, especially in biophysical applications [6,7]. This surge, caused by technical advances, can be expected to continue, since commercial FCS instrumentation is now available.

The common element of these optical fluctuation spectroscopies is that the sample is studied by analyzing fluctuations in the observed signal. These fluctuations are caused by and therefore contain information about dynamic processes in the sample, like Brownian motion, agglomeration processes, or chemical reactions. Signal analysis is commonly done in the time domain by autocorrelation of the signal or in the frequency domain by Fourier transforming the signal.

We report the development of a method that employs fluctuations of the intensity of individual bands in the spectrum of Raman scattered light from a small sample volume. This method, which we named Raman correlation spectroscopy (RCS), extends the domain of optical fluctuation spectroscopies to Raman scattered light, thus combining the chemical identification obtained by Raman scattering [8] with the structural and dynamical information obtained by correlation spectroscopy. RCS uses number fluctuations of Raman scattering particles within a small observation volume defined by confocal optics. In this respect, RCS is analogous to FCS.

The instrument employed is a modified confocal Raman microscope [9] with high optical throughput [Fig. 1(a)]. The sample is illuminated with a 10 mW HeNe laser through a microscope objective (magnification 100×, numerical aperture 0.8). Light Raman scattered in the backward direction is collected by the objective and passes through a notch filter for suppression of Raleigh scattering and a confocal pinhole onto a single grating (600 lines/cm) for spectral dispersion of the Raman bands. To follow fast temporal fluctuations the original cooled charge-coupled device detector is replaced by a linear array of 40 individual optical fibers with a diameter effective for light collection of 200 μm and a center-to-center distance of 230 μm between adjacent fibers, corresponding to 30 cm^{-1} of Raman shift. Two Si avalanche photodetectors are plugged to various detection fibers, allowing selection of Raman bands [Fig. 1(b)]. The signals of the detectors [Fig. 1(c)] are fed into a hardware correlator to generate autocorrelation and cross correlation functions (ACF and CCF) [Fig. 1(d)].

The fluctuations observed in RCS are particle number fluctuations of Raman scattering particles in the observation volume. In its theoretical description, RCS is analogous to FCS, where the observed intensity fluctuations are caused by number fluctuations of fluorescent particles or molecules. A direct result of a autocorrelation RCS experiment is an ACF $G_2(t)$, defined as

$$G_2(t)=\frac{1}{T}\int_0^T I(t')I(t+t')dt', \tag{1}$$

with $I(t)$ being the observed Raman intensity and T the duration of the measurement. According to FCS theory [6,7,10] this ACF is given by

$$\lim_{T\to\infty} G_2(t)=1+\frac{c^2}{N}\left(\frac{1}{1+\frac{t}{\tau}}\right)\left(\frac{1}{1+\frac{t}{s^2\tau}}\right)^{0.5} \tag{2}$$

*Author to whom correspondence should be addressed.

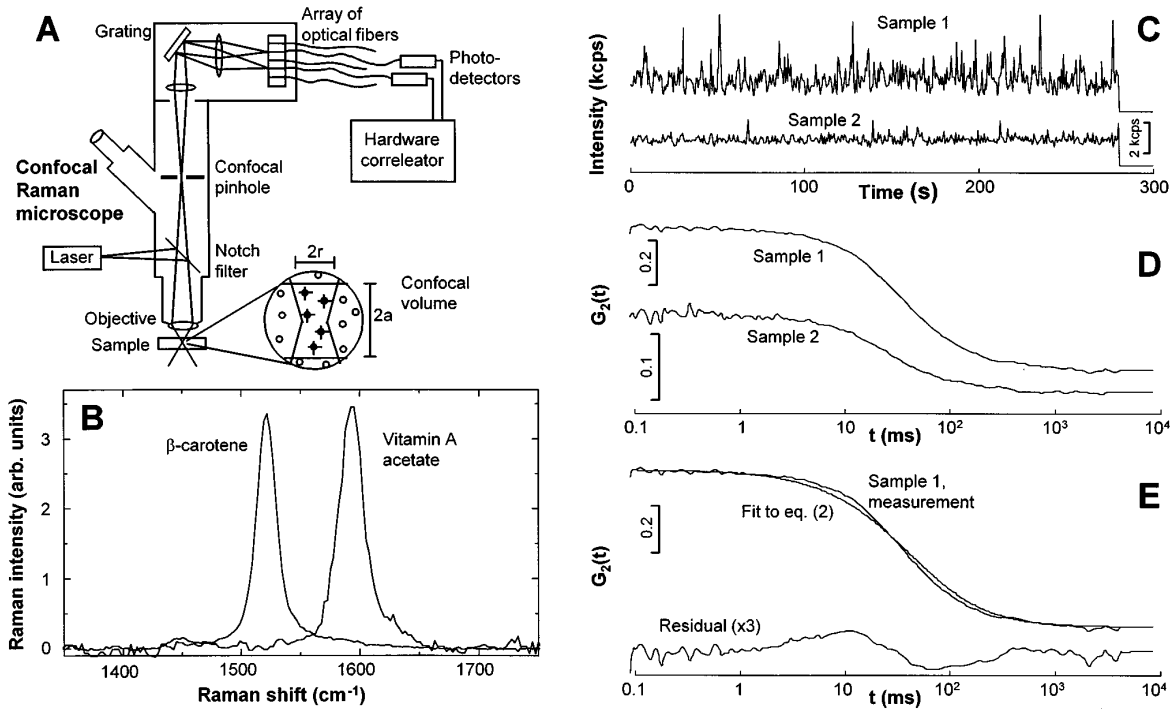

FIG. 1. Overview of the RCS technique. (a) Instrumental setup. (b) Raman spectra of the carotenoids of this study. (c) Raman intensity traces at 1510 cm^{-1} from two different β-carotene dispersions. Sample 1: particle diameter 220 nm; sample 2: 150 nm, 1 wt % β-carotene in suspension. (d) ACFs resulting form the intensity traces. (e) Comparison of a fit of Eq. (2) to the ACF of sample 1, the residual has been magnified 3× [16].

with the characteristic ACF decay time $\tau = r^2/4D$, the confocal volume shape parameter $s = a/r$, and the background intensity correction factor $c = 1 - I_B/I$. Here N is the average number of Raman scattering particles in the observation volume, D their thermal diffusion coefficient, a and r the extensions of the confocal volume along the optical axis and perpendicular to it [Fig. 1(a)]. I_B is the background intensity and I the time averaged intensity during the measurement. Equation (2) has two adjustable parameters, N and D, for a given ACF. The geometry parameters a and r and the background intensity I_B can be measured independently. For spherical particles undergoing Brownian diffusion, the Diffusion coefficient D is related to the particle diameter d by the Einstein equation $d = kT/3\pi\eta D$ (3), where k is the Boltzmann constant, T the absolute temperature, and η the viscosity of the medium.

As model substances we studied colloidal suspensions of carotenoid particles. Carotenoids have a large Raman scattering cross section due to their high content of C═C double bonds. The submicron sample suspensions are prepared using the technique of carotenoid micronization [4]. In short, the carotenoids are dissolved in a water-miscible solvent at high temperatures followed by a rapid quenching of the solution in water containing gelatin as a protective polymer. This process results in stable colloidal suspensions of the carotenoids that can be spray dried and resuspended in water. Resulting particle sizes are on the order of 100–300 nm.

The samples are put in rectangular glass capillaries and placed under the microscope objective of the RCS setup. Raman spectra similar to bulk carotenoids [11] are observed, with C═C double bands in the region of 1500–1600 cm^{-1} [Fig. 1(b)]. Typical Raman intensities observed in the optical fiber adjusted to the Raman maximum of the samples are 2000–5000 cps [Fig. 1(c)] at a sample concentration of 0.5–1 wt % carotenoid, with a background intensity I_B of 300 cps and a dark count rate of the detector of 80 cps. The typical ACF collection time is 300 s. The ACFs are fitted to Eq. (2). Here a value of the shape parameter $s = 1.0$ gives the best overall fit results and is used for all fits. The decay of the measured ACF is consistently steeper than the best possible fit from Eq. (2) [Fig. 1(e)]. This is probably due to the non-negligible size of the carotenoid particles as compared to the extension of the confocal volume. A similar effect can be seen in FCS on particles of comparable size.

A fit of Eq. (2) to the ACFs of two different β-carotene suspensions [Fig. 1(d), DLS-particle diameters $d_1 = 220$ nm, $d_2 = 150$ nm] yields their characteristic decay times τ and number of particles N ($\tau_1 = 46$ ms, $\tau_2 = 34$ ms, $N_1 = 1.9$, $N_2 = 10.8$). From the known particle diameters and concentrations, the observation volume of the present RCS setup can be deduced to have a radial extension $r = 0.7\ \mu$m and a confocal volume of 2.0 fl.

To demonstrate the influence of viscosity on the Brownian diffusion of a carotenoid suspension, equivalent carotenoid samples (1 wt % β-carotene, DLS-particle size 220 nm) are prepared in different water/glycerol mixtures. With increasing glycerol content, the viscosity of the medium increases and slows down the Brownian dynamics. This can be seen in the measured intensity versus time traces [Fig. 2(a)] and the resulting ACFs [Fig. 2(b)]. Assuming a constant particle size of the carotenoids, Eq. (2) and Eq. (3) can be used to calculate the relative increase in viscosity in the samples. The results for this apparent viscosity from the RCS experi-

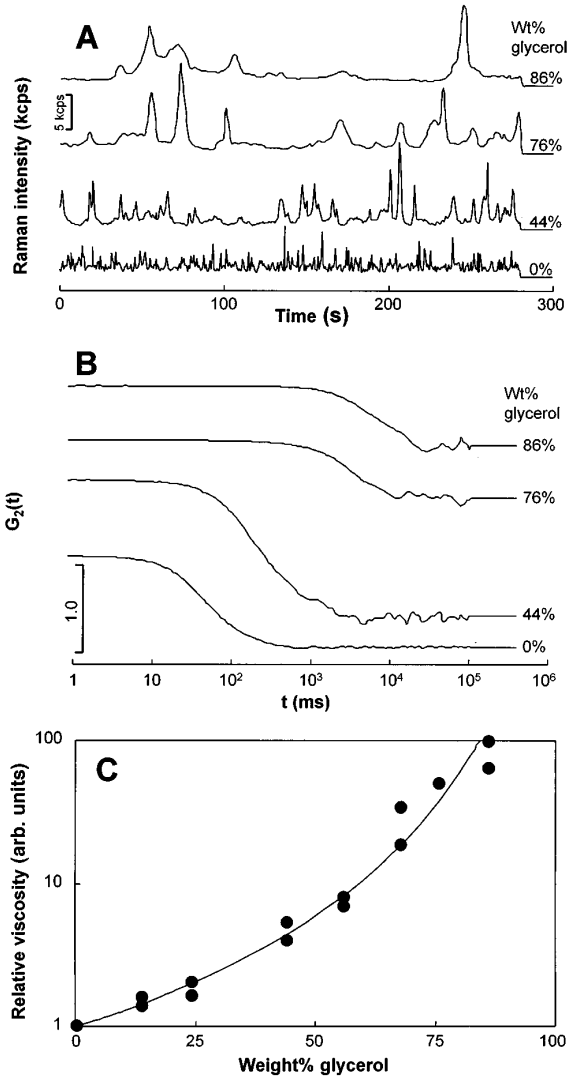

FIG. 2. Influence of medium viscosity on the dynamics of carotenoid particles (β-carotene, particle diameter 220 nm, 1 wt % β-carotene in suspension) Parameter of the different curves is the glycerol content, varied from 0 to 86 wt %. (a) Raman intensity traces at 1510 cm^{-1}. (b) Resulting ACFs. (c) Relative viscosities of the medium, calculated from the ACFs using Eqs. (2) and (3) (●), compared to the viscosity of water glycerol mixtures from the literature (line) [12,16].

ments correspond well to the literature values [12] for the viscosity of water/glycerol mixtures [Fig. 2(c)].

With RCS, the dynamic behavior of one species of particles can be selectively monitored in a mixed particle system. This is demonstrated adding a small number (1:90 number ratio) of β-carotene particles (DLS diameter 220 nm) to polystyrene (PS) lattices (DLS diameter 49 nm, pH 8.5, negligible Raman activity at 1510 cm^{-1}). The ACF of the mixture shows the same dynamics as the ACF of a pure β-carotene sample with twice the β-carotene content (Fig. 3). The ratio of the particle numbers N found in the mixture and in the pure sample is 0.48 [after background correction after Eq. (2), 0.61 without background correction]. This experiment shows that the carotenoid particles can be selectively measured in the mixture and their dynamics are changed only slightly by the presence of the PS lattices.

When two detection channels tuned to different Raman

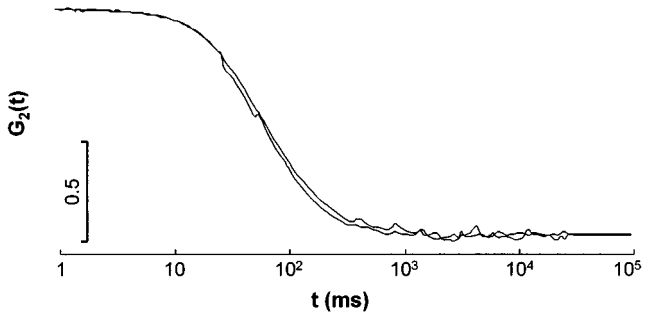

FIG. 3. ACFs of β-carotene particles (particle diameter 220 nm, measured at 1510 cm^{-1}), pure (lower curve, 1 wt % β-carotene in suspension) and in the presence of polystyrene lattices (upper curve, latex particle diameter 49 nm, particle number ratio latex/β-carotene 90, 0.5 wt % β-carotene in suspension). The ACF of the sample with the lattices has been scaled by a factor 0.61 to match the height of the ACF of the pure sample [16].

shifts are used in the RCS setup, species with different Raman spectra can be observed simultaneously. In addition to their individual dynamics, the simultaneous recording allows the sensitive detection of correlated fluctuations (caused, e.g., by complex formation) between the two species via crosscorrelation of the signals.

We studied a 1:3 mixture of β-carotene and vitamin A acetate. With the first detection channel of our RCS setup tuned to 1525 cm^{-1} and the other to 1585 cm^{-1} (2 fibers distant in the linear fiber array), the spectra of β-carotene and

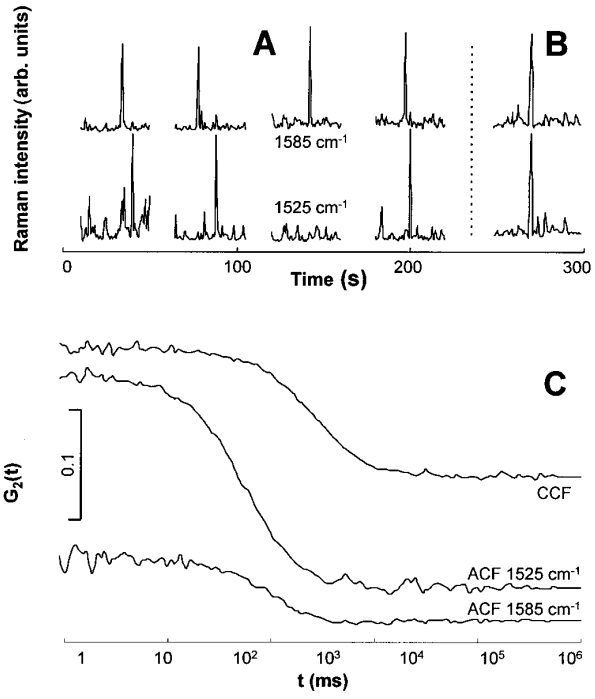

FIG. 4. Dual channel experiments with mixtures of β-carotene and vitamin A acetate. Channel 1 is set to 1525 cm^{-1}, channel 2 to 1585 cm^{-1}. (a) Intensity traces (channel 1 lower, channel 2 upper curves) showing the channels fluctuating independently. (b) Intensity trace showing a simultaneous signal in both channels. (c) Autocorrelation and cross-correlation functions from the mixture [16].

vitamin A acetate particles can be recorded in the individual channels with little overlap [Fig. 1(b)]. When recording both channels simultaneously, the individual intensity versus time traces in the two channels fluctuate independently most of the time, with distinct peaks in the intensity occurring at different times for the individual channels [Fig. 4(a)]. The ACFs of the individual detection channels are identical to the ones seen in pure samples. Sometimes, however, a large signal can be seen in both detection channels exactly simultaneously [Fig. 4(b)]. The CCF of the two detection channels shows that the component causing the simultaneous signals in both channels has slower dynamics than the two original carotenoids [Fig. 4(c)].

The nature of this component is not clear as of now. Both the formation of a small amount of agglomerates between β-carotene and vitamin A acetate particles or the presence of a small amount of larger fluorescent particles in the suspension are possible explanations.

Future improvement of the RCS technique will have to center on obtaining better sensitivity. While Raman scattering is intrinsically weak, much weaker than, e.g., fluorescence, and RCS therefore will probably never reach single molecule sensitivity, our current RCS setup leaves room for several orders of magnitude of improvement. Such improvements can be made using a higher laser power for illumination, dedicated microscope objectives with narrower beam waists and better collection efficiency, and—most important—a dedicated Raman spectrograph. In our current setup we lose much Raman intensity, because only a small part of the line focus generated by the grating is actually collected with the optical fiber array. An ideal instrument design would collect all the light from a whole Raman band (spatially and with respect to the frequency domain) onto the detector. Such sensitivity improvements will greatly boost the practicability of the method and could give RCS an advantage over FCS for problems where fluorescent labeling of the components is not desired or where there are more components to distinguish than is practical by fluorescent labeling.

We also expect variants of the RCS technique to develop, like scanning RCS [13] as a technique to quantify the distribution of different chemical species and inhomogeneities in samples, analogous to scanning FCS [14,15]. Another possibility is Raman fluorescence cross correlation spectroscopy. There, one detection channel could monitor a specific Raman band intrinsic to one component of the sample, the other channel a more sensitive fluorescence signal of, e.g., a labeled additive.

We expect that with the arrival of improved, dedicated RCS instrumentation, a number of fundamental and applied problems will benefit from this new technique with its potential to study dynamics of selected chemical species. For instance, many industrial products like coatings, pigments, and pharmaceutical formulations are complex colloidal systems with many open questions about the interactions of the individual components. A technique allowing one to distinguish these components in their native state without labeling and monitoring their behavior selectively will be an important step forward in this field. The present experiments demonstrate that Raman correlation spectroscopy has the potential to become such a technique.

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