Measuring Silica Nanoparticles via Fluorescence Anisotropy

Introduction

Silica is currently one of the most important industrial materials, whose nanoparticles are formed via a sol-gel process. They are non-toxic and commercially valuable, for they are used widely in sensors, scintillation detectors, and household products. Future uses may involve metabolic sensors and drug-delivery vehicles. Measuring the size of silica nanoparticles and pores is still difficult and unreliable, despite the multibillion-dollar production of silica worldwide.

In a sol-gel process, the *sol*, a liquidlike solution, is converted via a nanoparticle colloid stage into a *gel*, a highly porous structure filled with solvent. Drying the gel can produce solid glass for photonics and sensors and, when ground finely, as cleansers, polishers, adhesives, and printing agents. The molecular details of structure and dynamics for sol-gel formation are still poorly understood.

In this *Technical Note*, Dr. David Birch and colleagues at Strathclyde University in Scotland examined the formation of silica gels[1].

Theory

Fluorescence spectroscopy can uncover molecular structure and dynamics of sol-gels. A fluorophore's Brownian rotation causes fluorescence depolarization, and provides information on local mobility of the fluorophore. The changes in steady-state or time-resolved anisotropy observed during sol-gel polymerization from initial mixing to beyond the sol-to-gel transition, t_g, are related to viscosity.

Anisotropy, $\langle r \rangle$, is defined as[2]

$$\langle r \rangle = \frac{I_{VV} - G * I_{VH}}{I_{VV} + 2 * G * I_{VH}} \qquad \text{Eq. 1}$$

where G, the "G factor," is

$$G = \frac{I_{HV}}{I_{HH}} \qquad \text{Eq. 2}$$

I_{HV}, I_{HH}, I_{VV}, and I_{VH} are intensities for the relative polarizer orientations **H**orizontal and **V**ertical. Four measurements of intensity, permutations of the polarizers' orientations, are needed to determine $\langle r \rangle$. Fig. 1 shows the method.

Anisotropy provides information on molecular size and shape, local viscosities of a fluorophore's environment, and changes in sizes of polymers and other macromolecules. Thus anisotropy measurements are ideal for examining the sol-gel process.

[1] D. Birch and C.D. Geddes, "Sol-gel particle growth studied using fluorescence anisotropy: An alternative to scattering techniques", *Phys. Rev. E* **62**(2), 2000, 2977–2980; C.D. Geddes, D. Birch, "Nanometre resolution of silica hydrogel formation using time-resolved fluorescence anisotropy", *J. Non-Cryst. Sol.* **270**(2000), 191–204; C.D. Geddes, *et al.*, "1- and 2-Photon Fluorescence Anisotropy Decay in Silicon Alkoxide Sol-Gels: Interpretation in Terms of Self-assembled Nanoparticles", *J. Phys. Chem. B* 2002 (**106**) 3835–3841; J. Karolin, *et al.*, "Nanoparticle metrology in sol-gels using multiphoton excited fluorescence", *Meas. Sci. Technol.* **13** (2002) 21–27.

[2] Joseph R. Lackowicz, *Principles of Fluorescence Spectroscopy*, 3rd ed., New York, Springer, 2006, pp. 353–354, 361–364.

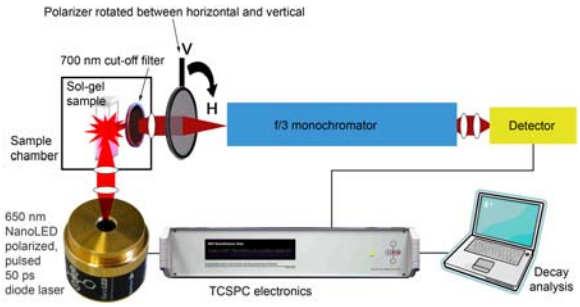

Fig. 1. *Experimental set-up. Vertical (V) and horizontal (H) orientations of the polarizer are shown. The NanoLED's output is itself polarized, thus it may be rotated to give H and V orientations.*

The anisotropy with respect to time, $r(t)$, for silica hydrogels is best described with two rotational correlation times, τ_{r1} and τ_{r2}, written as

$$r(t) = (1-f)r_0 e^{\left(\frac{-t}{\tau_{r1}}\right)} + fr_0 e^{\left(\frac{-t}{\tau_{r2}}\right)} \qquad \text{Eq. 3}$$

where r_0 is the initial anisotropy, and f is the fraction of fluorescence caused by fluorophores bound to silica. Therefore $1 - f$ is the fraction of free fluorophore in the sol. From the Stokes-Einstein equation, τ_{r1} gives the sol's microviscosity $\eta_1 = 3\tau_{r1}kT/4\pi r^3$, where r is the hydrodynamic radius of the dye. Using η_1 and τ_{r2} gives the silica particle's mean hydrodynamic radius.

If the fluorescence lifetime, τ_f, is much faster than τ_{r2}, the anisotropy decay is analogous to the hindered rotation of a fluorophore in a membrane or protein. This gives a residual anisotropy,

$$r(t) = (1-f)r_0 e^{\left(\frac{-t}{\tau_{r1}}\right)} + fr_0 \qquad \text{Eq. 4}$$

Experimental method

Fluorescence anisotropy-decay curves were collected using our time-correlated single-photon counting (TCSPC) FluoroCube lifetime spectrofluorometer (Fig. 2). Overall instrumental response was ~100 ps FWHM. Two-photon-excited rhodamine 6G (R6G) fluorescence was chosen via a 800-nm cutoff filter, removing the laser's fundamental. Laser power-dependence of the fluorescence confirmed its two-photon nature.

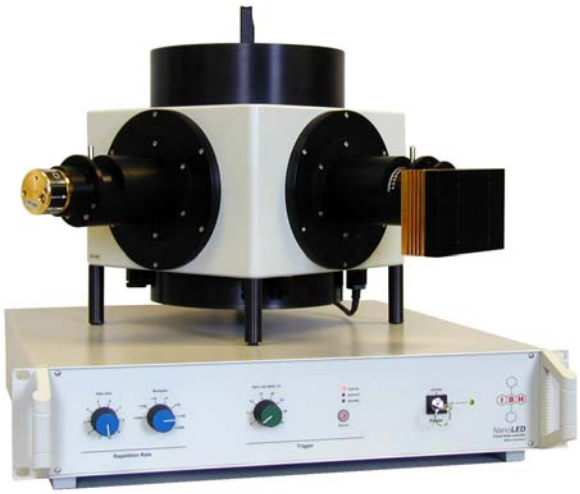

Fig. 2. *The FluoroCube TCSPC lifetime spectrofluorometer.*

For the time-resolved one-photon-excited fluorescence experiments, a Ti:sapphire crystal generated white light. A 500 ± 10 nm interference filter selected the excitation. Fluorescence emission was observed through a 550-nm long-pass filter. The temperature was held to 20 ± 1 °C. Typical measurement times for the anisotropy decay were ~30 min, in order to accumulate a maximum count per channel of 10 000–20 000 in the difference function $I_{VV} - G^*I_{VH}$.

Impulse reconvolution analysis of fluorescence anisotropy-decays was performed using our IBH software. Magic-angle polarization, 54.7°, was

chosen for all R6G fluorescence lifetime measurements.

Results

Table 1. Two-photon anisotropy-decay analysis.

Polymerization time (min)	τ_{r1}/ps	τ_{r2}/ns	r_0	χ^2
3986	347	0.971	0.501	1.12
15941	340	1.42	0.488	1.21
18611	345	1.01	0.512	1.23
20176	280	1.14	0.506	1.14
23156	319	1.48	0.490	1.44
24546	298	1.39	0.546	1.20
25656	319	1.67	0.534	1.26
27376	364	1.73	0.530	1.15
28626	289	1.46	0.481	1.20
30231	296	1.59	0.540	1.27
31836	330	1.54	0.514	0.97
33156	381	1.86	0.540	1.19
34566	293	1.77	0.502	1.32
35766	319	2.18	0.498	1.15
36131	258	1.57	0.495	1.12
37371	298	1.92	0.501	1.35
38751	313	2.09	0.453	1.42
40266	318	1.91	0.489	1.17
44706	297	1.85	0.488	1.24

Data for R6G, with two rotational times, for a ~22% SiO_2, pH = 2.3 TMOS sol-gel. λ_{exc} = 800 nm.

Two-photon excited anisotropy-decay gives enhanced dynamic range of r_0. Analysis using two rotational correlation times (Eq. 3) is shown in Table 1. Note the excellent χ^2 values. Fig. 2 presents a typical impulse reconvolution fit of Eq. 3 for one data set during the tetramethyl orthosilicate (TMOS) alcogel polymerization. The two-correlation-time model was more appropriate than one correlation time. Adding an additional *g* term for dye fixed in a gel gave no significant improvement in χ^2.

One-photon excited anisotropy-decay curves are shown in Fig. 3, this time for a hydrogel prepared from sodium silicate. For all these curves, fitting to Eq. 3 gave a better fit (smaller χ^2). Eq. 3 provided r_0 values closest to the theoretical maximum (0.4) and constant as *f* increased with time.

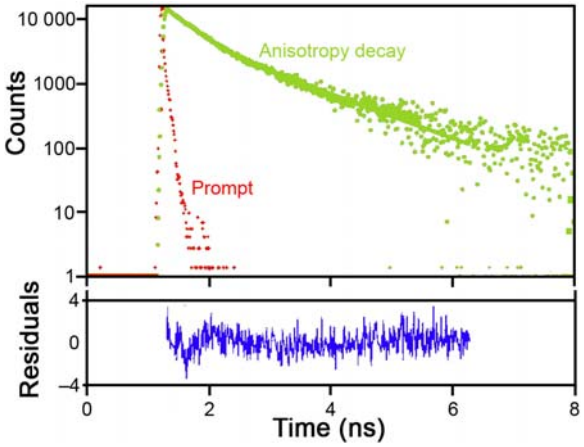

Fig. 2. *Impulse reconvolution fit of two rotational times to the data for the R6G-doped TMOS sol after a polymerization time of 40 266 min, using two-photon excitation at 800 nm. The prompt (<100 ps FWHM) and weighted residuals are also shown. Channel width = 8.7 ps.*

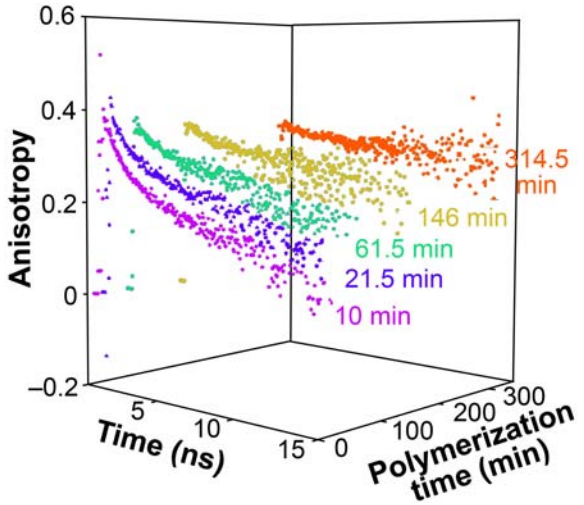

Fig. 3. *Fluorescence anisotropy-decay curves versus polymerization time for a 15.3% SiO_2 and 0.27* N *sodium silicate hydrogel.*

Discussion

The TMOS sol's particle radius increased from 0.8 nm to nearly 1.1 nm

after one month of polymerization, that is, before t_g, ~ 57 500 ± 1500 min. This 0.3 nm growth is small, yet measurable via lifetime anisotropy-decay spectroscopy (Fig. 4).

There is a distribution of particle sizes in this system, plus perhaps more than one microviscosity (e.g., vicinal and bulk). Particles need to be considered when recording fluorescence anisotropy in sols, whether before, at, or soon after t_g, though at or soon after t_g the majority of a sol-gel's volume is still fluid. Gelation, therefore, is only the *minimum* amount of material used to span the container to form a solid network.

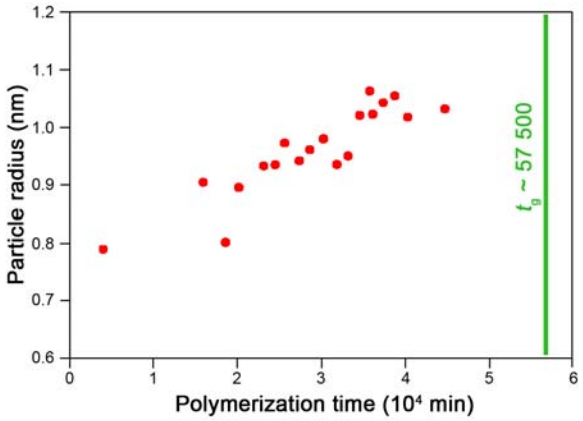

Fig. 4. *Silica particle radius versus polymerization time for the TMOS sol determined from* τ_{r2} *and the microviscosity from* τ_{r1} *(Eq. 3). Error in particle radius is ~ ± 0.1 nm.*

Conclusions

Birch, *et al*., have shown that fluorescence anisotropy-decay in sols with two rotational correlation times can be explained by the existence of silica particles. This interpretation allows monitoring sol-gels *in situ* with sub-nanometer resolution. Because a fluorophore immobilized in a gel does not depolarize fluorescence, one can study particle size well after the sol-to-gel transition time, a major advantage over scattering techniques (e.g., with neutrons or X-rays) where gel-scattering distorts the scattering function.

Moreover, the rapid data-acquisition needed to acquire anisotropy-decay data within time-spans as short as a few minutes is aided greatly by the high-repetition rate of the NanoLED excitation sources from HORIBA Jobin Yvon.

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