Elucidating local viscosity using fluorescence lifetime measurements

Certain fluorescent molecules, known as molecular rotors, can be employed to estimate the local (nanoscale) viscosity in microheterogeneous systems by measurement of their fluorescence lifetime. This can be advantageous over the usual fluorescence anisotropy method, as the measurement is simpler and faster to perform. This is demonstrated using the HORIBA Scientific TemPro fluorescence lifetime system to monitor the gelation of silica produced using the sol-gel technique.

Silica produced by the sol-gel technique
The use of the sol-gel method allows a silica glass-like material to be formed at relatively low temperature (ambient, unlike ~1800°C for normal glass). The materials produced can be of good optical quality and quite porous (pore size on nanoscale), with large internal surface areas (eg ~300m²/g). They also exhibit some biocompatibility and hence have found many applications, ranging from optical biosensor use (using entrapped biomolecules) to hosts for laser dyes or other optically addressable molecules. The sol-gel method usually makes use of a metal alkoxide and starts from a colloidal solution (sol) which is a precursor for a porous networked material (gel). The sol to gel transition is accompanied by marked change of viscosity, both bulk and microscopic, as bonds are formed between the colloidal particles as the network develops. Further consolidation of this matrix can then occur, accompanied by shrinkage in the materials overall size, in a process referred to as ageing. The versatility of this method is such that it can be used to produce monolithic structures or thin films.

Molecular rotors
A well known feature of fluorescence is the sensitivity of a fluorophore to its microenvironment (ie viscosity change). Coupled with the advantage of using the fluorescence lifetime over fluorescence intensity (former is absolute, while latter is a relative measure) means that by careful fluorophore selection it is possible to monitor viscosity of the sol to gel transition. Normally a fluorescence anisotropy measurement, where rotational molecular diffusion is monitored, maybe considered, but there are fluorophores that undergo a viscosity dependent intermolecular rearrangement that effects their fluorescent lifetime. This makes the decay acquisition faster by reducing the number of measurements. Two such molecules, made use of in this work, are shown below (see references for details).

In order to make use of this phenomenon a “calibration” is made by measuring their fluorescence lifetimes in solutions of known viscosity (this should produce a linear calibration on a log-log plot of lifetime vs viscosity). The fluorescence decays, measured on a TemPro, and a plot for the average lifetime of molecule 1 at different viscosities is shown in the figure below.

Monitoring the viscosity of the sol to gel transition
The two fluorophores were chosen for this work as they exhibit different solubility. 2 is hydrophilic, thus they should be complementary in reporting viscosity changes within different nanosized regions of the network during the sol to gel transition.
The initial gelation period can occur in minutes (illustrated above), but the ageing process can occur over weeks. As the lifetime is an absolute measure, it allows results from both of these processes to be compared. This would be difficult using the fluorescence intensity as correction would be required for fluctuations in the excitation source intensity and changes in geometry of the sample if it shrinks during the ageing process.

The low deadtime of the timing electronics of the TemPro when combined with high repetition rate excitation sources make it ideal for applications involving high throughput or measurements requiring fast data acquisition. In this case a filter based TemPro using NanoLED laser diode excitation was utilised in order to maximise the collection of the fluorescence photons. In two separate experiments the time-resolved fluorescence decays for probes 1 and 2 were measured after incorporation in a reaction mixture including tetraethyl orthosilicate and phosphate buffered saline solution in order to form a silica monolith, with the conditions and procedure the same in both cases.

The decay data was analysed using DAS6 software and required the sum of three exponential decays for both probes 1 and 2. For probe 1, the average lifetime was ascertained, while for 2 two of the lifetime components were ascribed to aggregates (because of the hydrophobic environment) with the other related to the monomeric form. It is this lifetime that was used to estimate the viscosity. Making use of the recovered lifetime values and the “calibration” plots for both probe molecules it was possible to estimate the nanoscale viscosity sensed by each. This is shown in the following plot.

Since 1 is more hydrophilic than 2 it is likely to associate with the water rich region inside the pores of the network. The hydrophobic probe 2 would be expected to associate with the polymeric silica network, with exposure to the aqueous environment causing some probe aggregation. The plot above is indicative of a two stage process, initial gelation followed by early ageing. 2 only seems to sense the initial formation of the polymeric silica network, while 1 reports a continued slower increase in viscosity, after the initial faster rate, that can relate to contraction of the pore structure as the material consolidates.

The results shown in this note are based on the following papers,


Acknowledgement

Marina Kuimova and Gokhan Yahioglu (Imperial College London and PhotoBiotics London respectively) are thanked for provision of compound 2