Experimental report

12/09/2023

Proposal: 9-13-1044 **Council:** 10/2022

Title: Diffusion of small medically relevant proteins in gels

Research area: Other...

This proposal is a new proposal

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Samples: FmocFF gel in D2O set using NaOD and glucono-delta-lactone (GDL), Insulin, Lysozyme; neutral pH

Instrument	Requested days	Allocated days	From	To
IN16B Si 111 BATS	3	3	04/04/2023	07/04/2023

Abstract:

The increasing use of biologics as therapeutic agents brings pharmaceutical benefits such as selectivity and reduced side effects. However, they can be administered only upon injection in liquid formulations with the drawback of a fast uptake, leading to a high frequency of administration. In this regard, gels are attracting attention as pharmaceutical delivery vehicles, because of their higher viscosity compared to liquid formulations guaranteeing a delayed drug release. Supramolecular gels accomplish this task of a tunable viscosity more easily than the classical polymeric gels.

Previously, we have established the solvent diffusion in gels and the comparison to the pure bulk for different gels, as well as the diffusion of small guest molecules therein. We now propose to record for the first time the (sub-)nanosecond self-diffusion of Insulin, the peptide hormone used for the treatment of diabetes, and Lysozyme, an enzyme indicated for antimicrobial therapies, within an FmocFF peptide supramolecular gel. We will probe direct and indirect effects, through hydrodynamics, of confinement.

This proposal is part of an InnovaXN PhD thesis involving the industrial partner AstraZeneca.

Experimental Report 9-13-1044: "Diffusion of small medically relevant proteins in gels"

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Scientific case

This experiments is part of the overall endeavour to study drug diffusion in supramolecular gels on the picosecond timescale. Previously, we have investigated the dynamics of two painkillers (paracetamol and ibuprofen sodium) in bis-urea gel and of ibuprofen sodium in FmocFF gel, a peptide-based hydrogel. These works on two small model molecules have shown how the specific fiber surface of the gels actively influence drugs diffusion. Specifically we observed an enhanced solvent and drug diffusivity in bis-urea supramolecular gel in comparison to the pure bulk state while in the case of the pepide FmocFF gel drug diffusion is slower than in the respective bulk. We here record for the first time the (sub-)nanosecond self-diffusion of Insulin, the peptide hormone used for the treatment of diabetes, and Lysozyme, an enzyme indicated for antimicrobial therapies, within an FmocFF peptide supramolecular gel; this work increases the complexity of the probe and it moves towards a more industry-oriented scientific case. This work, for which publication is expected, will be part of the doctoral thesis "Diffusion in supramolecular gels for drug delivery" in collaboration with AstraZeneca.

Experiment

We collected QENS data with the following six samples.

- D_2O/NaOD 0.1M in D_2O 9:1 + GdL (4.5 wt%)
- FmocFF gel (0.5 wt%) + D_2O/NaOD 0.1M in D_2O 9:1 + GdL (4.5 wt%)
- Lysozyme (2.5 wt%) in D_2O/NaOD 0.1M in D_2O 9:1 + GdL (4.5 wt%)
- Lysozyme (2.5 wt%) in FmocFF gel (0.5 wt%) + D_2O/NaOD 0.1M in D_2O 9:1 + GdL (4.5 wt%)
- Insulin (2.5 wt%) in D_2O/NaOD 0.1M in D_2O 9:1 + GdL (4.5 wt%)
- Insulin (2.5 wt%) in FmocFF gel (0.5 wt%) + D_2O/NaOD 0.1M in D_2O 9:1 + GdL (4.5 wt%)

The amount of sample was 0.7 ml for a double walled cylindrical Al cans. For the interest of time we started by measuring the pure solvent and gel samples: this solution allowed us to prepare the loaded gel one day before the measurement thus giving enough time to have a stable (around 12 h for the gel to set) yet not aged sample. We followed the same procedure even for the bulk solvent samples in order to make the batches as much comparable as possible. The FmocFF gel sets by pH change that has to decrease from a value of 10.4 to 4 via the slow hydrolysis of the glucono-δ-lactone (GdL). Two main challenges had to be faced: the first one was to dissolve the hydrophobic gelator at 0.5 wt% in 0.1M NaOD in D2O. This has been done with an alternation of stirring through vortex and sonication at room temperature until the solution turns into clear. Once the clear solution was reached the GdL was addedd directly on the Al can, after the solvent sample had been transferred into the sample holder. The second problem regarded the solubility of the two drugs. Previously on BATS experiment with small molecule drugs we worked with 5 wt% of drug but in this case we couldn't go more than 2.5 wt%. This reflected in a weak difference between the signal of the solvent and the gel state with the main difference mostly concerning the signal around the elastic peak, as expected being the gel network basically solid. Although for the global centre of mass diffusion a difference is observed, especially in the case of Lysozyme, the information of the local dynamics on the two drugs was lost, due to the overlapping wings of the QENS signal Fig.1(a) and 1(b). Each measurement took around 4 hours, the best trade off between having an acceptable signal to noise ratio and enough time to measure the samples at 3 temperatures (T = 285, 295 and 310 K) enabling us to generate an Arrhenius plot and thus test the validity of our modelling. The gel state was verified by the traces left on the inner Al cylinder.

Data analysis: Data reduction was performed with a Python script designed by Dr.Christian Beck based on functions available on Mslice. Reduced data are included in the energy range of -150μeV $\leq\Delta\hbar\omega\leq$ +150μeV with a q range 0.2 $\leq$q$\leq$ 1.9 $\mathring{A}^{-1}$. The data analysis so far consisted in fitting the unloaded solvent and gel sample with the following equation:

$$S_{solvent}(q,\omega) = b(q)L(\gamma_{solvent}(q),\omega) + c(q)\delta(\omega) + \alpha\omega + \beta \quad (1)$$

where $L(\gamma_{solvent}(q),\omega)$ is the Lorentzian describing solvent diffusion, $b(q), c(q), \alpha$ and β are scalars. Here the Dirac function $\delta(\omega)$ stands for the elastic contribution to the QENS signal and it includes every component too

slow to be resolved by the instrument resolution (immobile solvent fraction, gelator network and the empty can). The solvent fit parameters have been fixed and passed to the respective loaded sample data in the following model function:

$$S(q,\omega) = a(q)L(\gamma_{solute}(q),\omega) + \varphi \cdot b(q)L(\gamma_{solvent}(q),\omega) + d(q)\delta(\omega) + \alpha\omega + \beta \tag{2}$$

where $\alpha(q)$, $\gamma_{solute}(q)$ and $d(q)$ were free parameters and φ the scaling factor representing the excluded volume by the guest molecule. Herein d(q) also takes into account the elastic contribution from the guest molecule fraction immobile to energy resolution. An example spectrum is reported in Fig.1(c). To get the diffusion coefficients and gauge the impact of gel fibers I plot the Half width at half maximum values versus q^2 Fig.1(d). Before fitting with a diffusion model (jump or fickian diffusion) data have to be further refined especially in the first 4 q values. However even from these preliminary results drugs diffusion seems to be slower in the gel with respect to the pure solvent state.

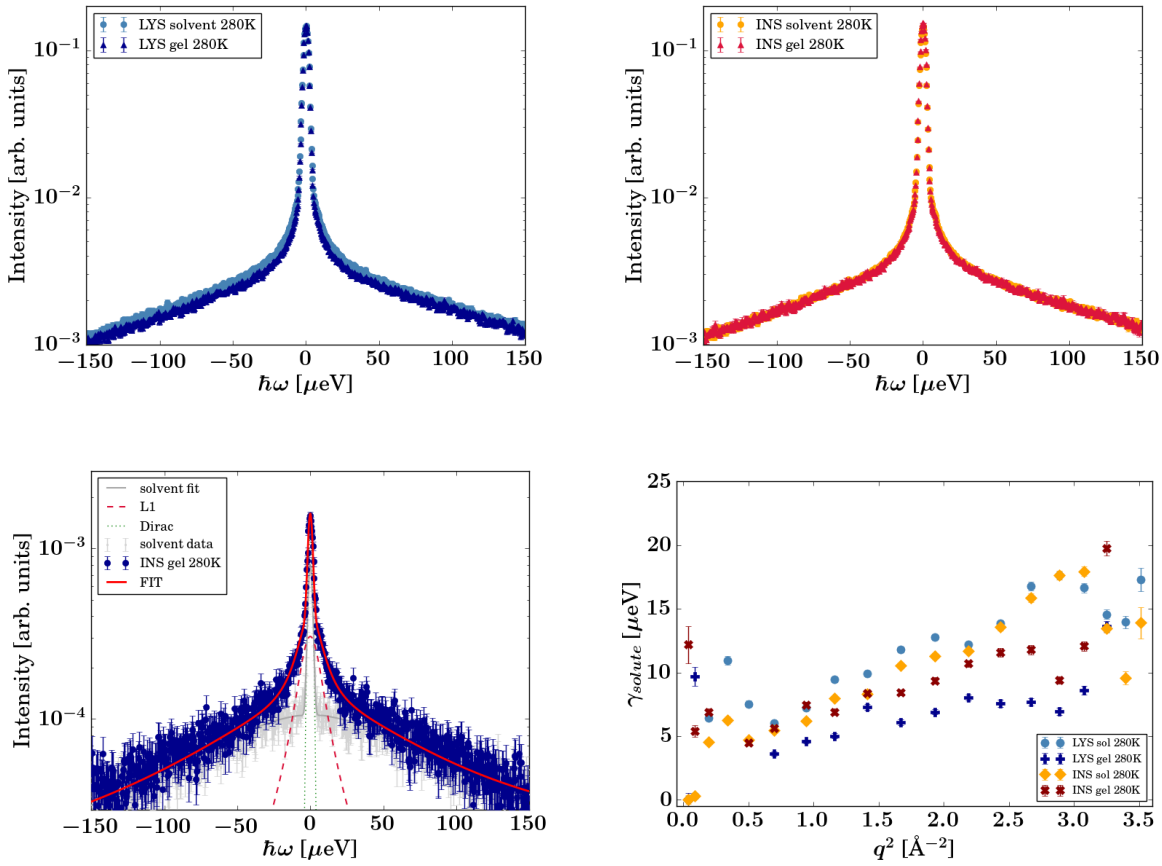

Fig. 1. (a) QENS spectrum of Lysozyme and (b) Insulin summed over q to improve the statistic.(c) Example spectrum of 2.5 wt% Lysozyme in 0.5 wt% FmocFF gel at q=1.2 Å^{-1} and fit according to eq.2; (d) Half width at half maximum $\gamma_{solute}(q)$ versus q^2 for Lysozyme and Insulin in FmocFF gel and pure solvent at T=280K.