Experimental report

02/08/2022

Proposal: 9-13-972 **Council:** 10/2020

Title: Understanding the microscopic properties and drug diffusion kinetics of long-acting peptoid-peptide drugdelivery implants for HIV/AIDs

Research area: Engineering

This proposal is a new proposal

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Experimental team: Ralf SCHWEINS

Local contacts: Ralf SCHWEINS

Samples: D2O
PP1-5% wt, ALP 10.0 U/mL, pH 8
PP1CAB-5% wt, ALP 10.0 U/mL, pH 8
PP2CAB-5% wt, ALP 10.0 U/mL, pH 8
PP2-5% wt, ALP 10.0 U/mL, pH 8

Instrument	Requested days	Allocated days	From	To
D11	2	2	11/06/2021	12/06/2021
			09/09/2021	10/09/2021

Abstract:

Patients struggle to adhere to complex regimens of HIV medicines, which require a cocktail of drugs to be taken several times daily. Our research group aims to overcome this issues by creating a long-acting drug releasing injection to deliver HIV drugs for 28 days. This hydrogel implant, composed of biologically stable peptide-like molecules, termed peptoid-peptides, forms in response to enzymes present within the skin. This hydrogel implants has the advantage of reducing rapid drug release, to gradually deliver HIV drugs over 28 days, thereby improving patient adherence to medication and provide clinical HIV treatment/prevention. This project will explore the role of gel fibre structure and entangled gel fibre networks on both i) mechanical (gel formation, rheology, gel strength) and ii) drug release properties (drug cleavage from peptoid-peptide, drug diffusion). Small angle neutron scattering will be used to probe the properties of gel fibres. Four different peptoid-peptides will be studied, both concentration of gelator and enzyme trigger will be varied to provide SANS data that will be valuable in optimising the peptoid-peptide structure for 28 day delivery of HIV drugs.

Background: To fulfil the need for a convenient and effective long-acting formulation to deliver drugs to HIV/AIDS patients over a sustained period, we have developed an injectable *in situ* forming peptide and peptide-mimetic hydrogel implants for the delivery of HIV/AIDS drugs for ≥28 days. Our formulations include an enzyme-responsive self-assembling low molecular weight D or L-α peptide hydrogelator, namely phosphorylated (naphthalene-2-ly)-acetyl-diphenylalanine-lysine-tyrosine-OH (NapFFKY[p]-OH), to which zidovudine is conjugated covalently via an ester linkage. This forms a powder that can be readily dissolved in aqueous buffer to create an injectable solution.

Aims/Objectives: To understand the macroscopic properties of gels and the relationship between their underlying structure and the diffusion kinetics of the HIV/AIDS drug zidovudine using small angle neutron scattering (SANS) on D11 and to link to rheological and drug release studies performed in our lab at Queen's University Belfast.

Experimental: Small angle neutron scattering (SANS) was used to determine the morphology of the structures underpinning the hydrated peptide gel network. Both drug and non-drug conjugated forms of peptides L-α (NapFFKY-OH and NapFFKYG-OH) and D-enantiomer (NapffkY-OH and NapffkYG-OH) were studied. Peptide hydrogels (2% w/v) were formulated in 2 mm path length UV spectrophotometer grade quartz cuvettes (Hellma) but using deuterated water (D_2O) rather than PBS. SANS measurements were performed using the D11 instrument at the Institut Laue – Langevin, Grenoble, France. Scattering from the primary assembled structures (most commonly fibres) and the network were collected over a wide Q range [$Q = 4\pi\sin(\theta/2)/\lambda$] of 0.001 to 0.5 $Å^{-1}$ and three sample-detector distances (1.4 m, 8 m, and 39 m). Peptide samples were placed in a temperature-controlled sample rack during measurements. Data obtained was reduced to 1D scattering curves of intensity vs. Q using the facility provided software. Scattering from D_2O controls, empty cuvette, electronic background were subtracted from the data and the full detector images normalized. SasView software version 5.0.4 was used to fit instrument-independent data to several models with and without a power law applied, including: a cylinder, an elliptical cylinder, a flexible cylinder, a flexible elliptical cylinder, and hollow cylinders. Utilizing chi squared, the best fitted model for peptide hydrogels was the flexible cylinder elliptical model with Power Law applied. Chi squared was still relatively high in this case and some flexibility is required. A summary of the fitting parameters are shown in Table 1. The data fits are shown in Figure 1.

Results and Discussion: Peptides form gels that are proven to effectively scatter neutrons and data obtained can be used determine structure of fibrous hydrogel networks [1, 2]. Figure 1 demonstrates that 2% w/v peptide hydrogels (dotted line) for L-α, D-enantiomer and zidovudine attached variants closely fit model data for flexible cylinder elliptical model with the Power Law applied (straight line). The fibre radius of each is: 1.495 nm, 2.038 nm, 1.939 nm, 1.959 nm respectively for non-zidovudine attached peptides (NapFFKY-OH, NapFFKYG-OH, NapffkY-OH, NapffkYG-OH) and 1.998 nm, 2.279 nm, 2.261 nm, and 2.152 nm for zidovudine conjugated variants (NapFFK(AZT)Y-OH, NapFFK(AZT)YG-OH, Napffk(AZT)Y-OH, Napffk(AZT)YG-OH). These observations are consistent with previous studies on dipeptide NapFF peptide hydrogels formed by a glucono-δ-lactone (GdL) pH triggered approach [3]. SANS data also showed the composition of the gel fibres are similar at low Q. Therefore, the differences in gel stiffness when drug is attached is likely due to entanglement of fibres, rather than the composition of fibres themselves or their secondary structures. Length of these fibres are also very large (Table 1), which is also a common property of entangled gel fibres. The presence of entangled gel fibres also suggests there is a large component of gel stiffness/strength that can be controlled by external conditions, for example the gelation process. This may allow a change in gelation or formulation parameters to optimize material specifications, most notably gel strength and therefore drug release kinetics, for long-acting drug delivery. It has been previously demonstrated that varying the peptide gel formulation method, for example enzyme, pH, temperature or salt

(calcium) triggered, can be effective in altering the structure of fibrous hydrogel networks and its mechanical properties [4].

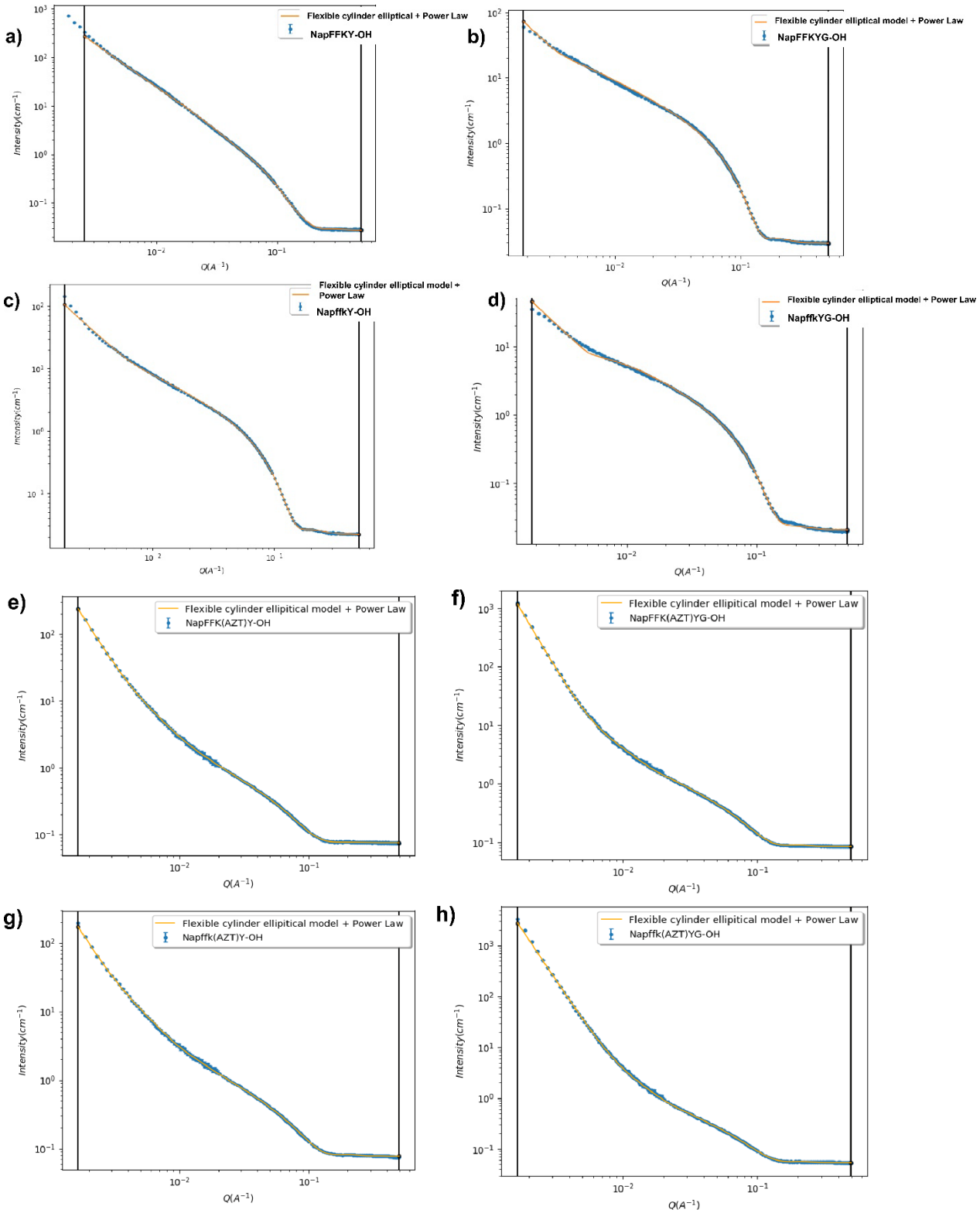

Figure 1. SANS data for 2% w/v peptide hydrogels a) NapFFKY-OH, b) NapFFKYG-OH, c) NapffkY-OH, d) NapffkYG-OH, e) NapFFK(AZT)Y-OH, f) NapFFK(AZT)YG-OH, g) Napffk(AZT)Y-OH, h) Napffk(AZT)YG-OH (dotted line). Solid line relates to model data for flexible cylinder elliptical model. Scattering data collected over a wide Q range [Q = $4\pi\sin(\theta/2)/\lambda$] of 0.001 to 0.5 $Å^{-1}$ and three sample-detector distances (1.4 m, 8 m, 39 m).

Table 1. SANS fitting parameters summary.

Parameter \Peptide hydrogel (2% w/v)	NapFFKY-OH	NapFFKYG-OH	NapffkY-OH	NapffkYG-OH	NapFFK(AZT)Y-OH	NapFFK(AZT)YG-OH	Napffk(AZT)Y-OH	Napffk(AZT)YG-OH
Scale	0.0046856E-5 +/- 1.2111	0.0059716 E-5 +/- 1.5383	0.0037313E-5 +/- 1.1722	0.0054887E-5 +/- 1.5409	7.6759E-07 +/- 16.451	2.89E-08 +/- 0.59263	3.60E-08 +/- 0.24386	2.02E-07 +/- 6.8836
Background (cm^{-1})	0.027338 +/- 3.0788e-5	0.029379 +/- 2.9467e-5	0.020866 +/- 2.896e-5	0.02208 +/- 2.911e-5	0.075657 +/- 2.75E-05	0.083826 +/- 2.88E-05	0.076342+/- 2.78E-05	0.05353 +/- 2.24E-05
Length (Å)	75680 +/- 11099	2.9899e+15 +/- 3.3481e+15	7.0584e+43 +/- 6.6517e+43	1.8917e+5 +/- 33290	752.55 +/- 50.063	2863.7 +/- 1.00E+08	2368.6 +/- 1.00E+08	1466.2 +/- 1.00E+08
Khun length (Å)	75.838 +/- 0.38383	1001.5 +/- 9.9707	670.27 +/- 3.9572	560.66 +/- 3.0707	494.45 +/- 20.793	3.39E-21 +/- 2.03E+06	8.27E-25 +/- 2.82E+06	3.63E-17 +/- 8.90E-08
Radius (Å)	14.954 +/- 0.020758	20.378 +/- 0.028828	19.393 +/- 0.035733	19.585 +/- 0.030648	19.977 +/- 0.11472	22.787 +/- 0.022169	22.607 +/- 0.02427	21.52 +/- 0.02728
Axis ratio	2.1361 +/- 0.0068177	1.454 +/- 0.0041078	1.5634 +/- 0.0055837	1.4657 +/- 0.004574	1.4721 +/- 0.016232	9.8719 +/- 0.11499	17.872 +/- 0.37564	7.8643 +/- 0.083226
SLD ($x10^{-6}$ $Å^{-2}$)	2.143	2.143	2.143	2.143	2.143	2.143	2.143	2.143
SLD solvent ($x10^{-6}$ $Å^{-2}$)	6.39	6.39	6.39	6.39	6.39	6.39	6.39	6.39
χ^2	18.758	19.805	13.397	12.027	2.8005	6.309	7.4149	5.2904

Conclusions: A large component of gel strength can be controlled by external conditions and this may allow a change in formulation parameters to optimise material specifications, most notably gel strength and drug release kinetics for long-acting drug delivery. We have submitted the results for NapFFKY[p]-OH peptides as part of a research paper to a journal publication, it is currently under review.

References:

[1] E.R. Cross, S. Sproules, R. Schweins, E.R. Draper, D.J. Adams, Controlled tuning of the properties in optoelectronic self-sorted gels, Journal of the American Chemical Society, 140 (2018) 8667-8670.

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[3] E.R. Draper, B. Dietrich, K. McAulay, C. Brasnett, H. Abdizadeh, I. Patmanidis, S.J. Marrink, H. Su, H. Cui, R. Schweins, A. Seddon, D.J. Adams, Using Small-Angle Scattering and Contrast Matching to Understand Molecular Packing in Low Molecular Weight Gels, Matter, 2 (2020) 764-778.

[4] C. Colquhoun, E.R. Draper, R. Schweins, M. Marcello, D. Vadukul, L.C. Serpell, D.J. Adams, Controlling the network type in self-assembled dipeptide hydrogels, Soft Matter, 13 (2017) 1914-1919.