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A Low Molecular Weight Hydrogel with Unusual Gel Ageing

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SUPPORTING INFORMATION

Synthetic Procedures

All chemicals were obtained from Sigma Aldrich and were used as received. Deionized water was used throughout.

2-Thiophene phenylalanine ethyl ester:

2.13 g (0.00927 moles) of L-phenylalanine ethyl ester hydrochloride was dissolved in dichloromethane (30 mL) with 5.10 mL (0.0463 moles) of N-methylmorpholine (NMM). This was then added slowly to a solution of 1.37 mL (0.00927 moles) of 2-thiophene acyl chloride in dichloromethane (20 mL) whilst stirring at 0 °C. This was allowed to react for 16 hours before being washed with water (3 x 100 mL) and once with acidic water (20 mL). The organic layer was then dried over magnesium sulphate then the solvent was removed *in vacuo*. The white solid was then washed in hot petroleum ether to remove any unreacted thiophene acyl chloride and carboxylic acid formed by hydrolysis. A typical yield was around 80 %. ^{1}H NMR (d_6-DMSO): 8.75 (d, 1H, NH, J = 10 Hz), 7.75 (dd, 1H, ArH J = 5 and J = 1 Hz), 7.71 (dd, 1H, ArH, J = 5 and J = 1 Hz), 7.15-7.45 (m, 6H, ArH), 4.60 (m, 1H, CH), 4.10 (q, 2H, CH_2, J =7 Hz), 3.05-3.20 (m, 2H, CH_2) and 1.13 (t, 3H, CH_3, J = 7 Hz) ppm. ^{13}C NMR (d_6-DMSO): 173.0 (O-C=O), 162.0 (C=O), 139.1 (ArC), 139.0 (ArC), 137.5 (ArC), 129.0 (ArC), 128.7 (ArC), 128.4 (ArC), 127.9 (ArC), 126.5 (ArC), 75.9 (C-O), 61.0 (C-N), 54.0 (CH_2) and 13.5 (CH_3) ppm. MS: ES$^+$: $[M+H]^+$ Accurate mass predicted 304.0929. Found 304.1010.

2-Thiophene phenylalanine:

Deprotection of the L-phenylalanine was carried out by dissolving 2-thiophene phenylalanine ethyl ester in a THF: water mixture of 30 mL: 10 mL. LiOH (0.35 g) was then added and the solution allowed to react for two hours until no precipitate was formed when a small portion was added into water. Then, 100 mL of water was added and the pH lowered to between 3-4. The THF was then removed *in vacuo* and the solid precipitate collected, washed with water and dried under vacuum to give a white solid. Typical yield was between 85–95 %. ^{1}H NMR (d_6-DMSO): 12.85 (bs, 1H, OH), 8.74 (d, 1H, NH, J = 10 Hz), 7.85 (dd, 1H, ArH, J = 5 and J = 1 Hz), 7.75 (dd, 1H, ArH, J = 5 and J = 1 Hz), 7.13-7.33 (m, 6H, ArH), 4.57 (m, 1H, CH) and 3.00-3.25 (m, 2H, CH_2) ppm. ^{13}C (d_6-DMSO): 173.1 (O-C=O), 161.0 (C=O), 139.5 (ArC), 138.0 (ArC), 131.0 (ArC) 129.2 (ArC), 128.6 (ArC), 128.2 (ArC), 128.0 (ArC), 126.3 (ArC), 54.0 (C-N) and 36.2 (CH_2). MS: ES$^+$: $[M+Na]^+$. Accurate mass calculated: 298.0508. Found 298.0507.

2-Thiophene diphenylalanine ethyl ester:

0.944 g (0.0041 moles) of L-phenylalanine ethyl ester hydrochloride was dissolved in dichloromethane (30 mL) with 0.676 mL (0.00615 moles) of NMM. This was then added slowly to a solution of 1.13 g (0.0041 moles) 2-thiophene phenylalanine and

0.585 mL (0.00451 moles) of isobutylchloroformate in dichloromethane (20 mL) whilst stirring at 0 °C. This was allowed to react for 16 hours before being washed with water (3 x 100 mL) and once with acidic water (20 mL). The dichloromethane was then dried over magnesium sulphate then the solvent was removed *in vacuo*. A typical yield was between 70 – 80 %. ^{1}H NMR (d_6-DMSO): 8.62 (d, 1H, NH, *J* = 10 Hz), 8.56 (d, 1H, NH, *J* = 10 Hz), 7.77 (dd, 1H, ArH, *J* = 5 and *J* = 1), 7.73 (dd, 1H, ArH, *J* = 5 and *J* = 1), 7.12-7.35 (m, 11H, ArH), 4.72 (m, 1H, CH), 4.49 (m, 1H, CH), 4.04 (q, 2H, CH_2, J = 7 Hz), 2.90-3.10 (m, 4H, 2 x CH_2) and 1.10 (t, 3H, CH_3, *J* = 7 Hz) ppm. ^{13}C (d_6-DMSO): 171.5 (O-C=O), 171.1 (O-C=O), 161.0 (C=O), 131.0 (ArC), 129.0 (ArC), 128.5 (ArC), 128.1 (ArC), 128.0 (ArC), 127.8 (ArC), 126.3 (ArC), 126.1 (ArC), 61.0 (CH_2), 54.3 (C-N), 53.7 (C-N), 37.0 (CH_2), 36.6 (CH_2) and 13.8 (CH_3) ppm. MS: ES+: [M+Na]+. Accurate mass calculated: 473.1505. Found 473.1508.

2-Thiophene diphenylalanine:

Deprotection of the $_{L}$-phenylalanine was done by dissolving 2-thiophene diphenylalanine ethyl ester in a THF: water mix of 30 mL: 10 mL. LiOH (0.35 g) was then added and allowed to react for two hours until no precipitate was formed when a small portion was added into water. Then 100 mL of water was added and the pH lowered to between 3-4. The THF was then removed *in vacuo* and the precipitate was collected, washed well with water and dried under vacuum to give a white solid. A typical yield was between 80-90 %. ^{1}H NMR (d_6-DMSO): 13.80 (bs, 1H, OH), 8.60 (d, 1H, NH, *J* = 10 Hz), 8.32 (d, 1H, NH, *J* = 10 Hz), 7.81 (dd, 1H, ArH, *J* = 5 and J = 1), 7.73 (dd, 1H, ArH, *J* = 5 and J = 1), 7.12 – 7.36 (m, 11H, ArH), 4.70 (m, 1H, CH), 4.43 (m, 1H, CH), 3.10 (m, 2H, CH_2) and 2.95 (m, 2H, CH_2). ^{13}C (d_6-DMSO): 172.6 (O-C=O), 171.3 (O-C=O), 160.9 (C=O), 139.1 (ArC), 129.2 (ArC), 128.5 (ArC), 128.2 (ArC), 128.0 (ArC), 127.8 (ArC), 126.4 (ArC), 126.2 (ArC), 52.4 (CN), 53.9 (CN), 37.0 (CH_2) and 36.7 (CH_2). MS: ES^{+}: $[M+Na]^{+}$. Accurate mass calculated: 445.1192. Found 445.1188.

Instruments

Nuclear Magnetic Resonance Spectroscopy (NMR)

NMR spectra were recorded using a Bruker DPX-400 spectrometer operating at 400 MHz for ^{1}H NMR and 100 MHz for ^{13}C, in deuterated DMSO. For NMR spectra of dried turbid and transparent gels, gels were freeze-dried using a Labconco freezone 4.5 dryer with a condenser temperature of -50 °C and a shelf temperature of 20 °C, then re-dissolved in deuterated DMSO.

Hydrogel Formation

A pH switch method was used to form the hydrogels. All gels were prepared at a concentration of 2.5 mg/mL of gelator unless otherwise stated. The gelator was added to 2 mL of water with an equimolar amount of sodium hydroxide (0.1 M, aqueous). For solutions prepared with potassium carbonate and potassium hydroxide again an equimolar amount of 0.1 M solutions were used. The solution was stirred until all the gelator was dissolved. This solution was then transferred to a vial containing a 2.5 mg/mL of glucono-δ-lactone (GdL) and shaken gently. This was then left to stand to allow gelation to occur within a few hours.

For degassed gelation, the gelator solution at high pH was placed in a sealed 25 mL round bottomed flask and nitrogen was bubbled through the solution for an hour. The solution was then transferred *via* a degassed syringe to a 14 mL vial filled with nitrogen containing a pre-weighed amount of GdL. The vial was sealed with a rubber seal and wrapped in parafilm.

For degassed gel turbidity measurements in a quartz cuvette a pre-weighed amount of GdL was added before being sealed with a rubber seal and filled with nitrogen. It should be noted that for these two measurements that the rubber seals were not perfect fits for the cuvette or the vial and so leaking was likely (as seen in Figures S5 and S6). This experiment was repeated again by degassing a solution of the gelator at high pH in a 25 mL round bottom flask. A pre-weighed amount of GdL was added this time to a 10 mL round bottom flask which was degassed and sealed with a rubber seal which fitted the flask tightly. The solution was transferred to this round bottom flask *via* a degassed syringe and nitrogen was blown over the top of the solution for 10 minutes before removing the nitrogen supply and covering any holes from the needles with vacuum grease and parafilm. This data is shown in Fig. S7.

For gelation under carbon dioxide, the gelator solution was placed in a vial with a rubber seal and a balloon of carbon dioxide placed on the top.

Rheological Measurements

Dynamic rheological and viscosity measurements were performed using an Anton Paar Physica MCR101 rheometer. A cup and vane measuring system was used to perform the frequency and parallel plates for time sweeps. For frequency tests, 2 mL of the gels were prepared in 7 mL Sterilin vials and left for 24 hours at room temperature for the turbid gels and for three days for the transparent gels before measurements were performed. For time sweeps, 2 mL of gel was prepared on the plate and, once the top plate was lowered, mineral oil was placed around the plate to prevent solution from drying out. All experiments were performed at 25 °C.

Time sweeps: Time sweeps were performed with a 50 mm plate with a plate gap of 0.8 mm. Tests were performed at an angular frequency of 10 rad s^{-1} and with a strain of 0.1 %.

Frequency sweep: Frequency scans were performed at 1 rad/s to 100 rad/s under a strain of 0.5 %. The shear modulus (storage modulus (G') and loss modulus (G″)) are read at 10 rad/s. These measurements were done within the viscoelastic region were G′ and G″ are independent of strain amplitude.

Strain sweep: Strain scans were performed from 0.1 % to 100 % with a frequency of 10 rad/s. The critical strain was quoted as the point that G' starts to deviate for linearity and ultimately crosses over the G″, resulting in gel breakdown.

pH Measurements

A FC200 pH probe (HANNA instruments) with a 6 mm x 10 mm conical tip was used for pH measurements. The stated accuracy of the pH measurements is ±0.1. The pK_a values of the gelators were determined *via* the addition of GdL as reported previously.[1] Measurements were recorded every 5 minutes. The temperature was maintained at 25 °C during the titration by using a circulating water bath.

Turbidity Measurements

Turbidity measurements were carried out using a Thermo Scientific Nanodrop 2000/2000c spectrophotometer. The spectrophotometer was used in cuvette mode where samples were prepared in quartz cuvettes with a path length of 1.0 cm. Gels were prepared as previously mentioned. Once GdL was added to the cuvettes spectra were recorded from 250 nm to 800 nm. For the first 3 hours, a spectrum was recorded every 10 minutes. After this, a spectrum was recorded every hour until the solution became clear. The change in turbidity was measured by the absorbance at 600 nm.

SEM Imaging

SEM images were obtained using a Hitachi S-4800 FE-SEM at 3 KeV. Gel was deposited onto glass cover slips which were fixed onto aluminium SEM stubs with carbon tabs and left to dry for 24 hours. The samples were gold coated for 3 minutes at 15 mA prior to imaging using a sputter coater (EMITECH K550X).

Fourier Transformed Infrared Spectroscopy

IR spectra were collected on Bruker FTIR at 2 cm^{-1} resolution by averaging over 64 scans. Hydrogels were prepared as previously mentioned. The hydrogels were loaded onto a CaF_2 windows, and another CaF_2 window was placed on the top of the gels. Each spectrum was background subtracted. The peak intensities were obtained by fitting the spectra with PEAKFIT software using Gaussian functions. The fitting coefficients were all above 0.98.

Mass Spectroscopy

Measurements were carried out using a Micromass LCT Mass Spectrometer in positive mode at 40 V in methanol. Samples were run by the University of Liverpool mass spectrometry service.

Optical Microscopy

Optical microscope images were collected using a Nikon Eclipse LV100 microscope with a Nikon TU Plan ELWD 50x/0.60 lens attached to an Infinity2-1C camera. Gels were prepared as described above. Whilst still a solution the gelator/GdL mixture was transferred into a 1 cm rubber O ring with had been glued onto a microscope slide (Shown inset in Figure S2.). A cover slip was then placed on top to prevent the gel from drying out. Images were taken whilst the gel was turbid and then again once the gel had become transparent.

Figures

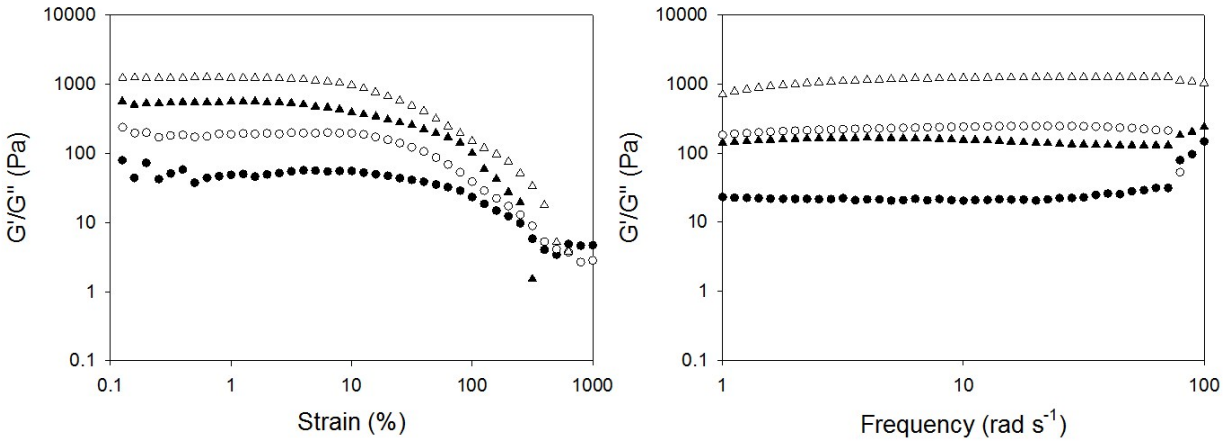

Figure S1. Strain sweeps (left) ran at 10 rad/s-1 and frequency sweeps ran at 0.5 % strain of a turbid gel (represented by circles), and a transparent gel (represented by triangles). Open shapes show G' and full shapes show G".

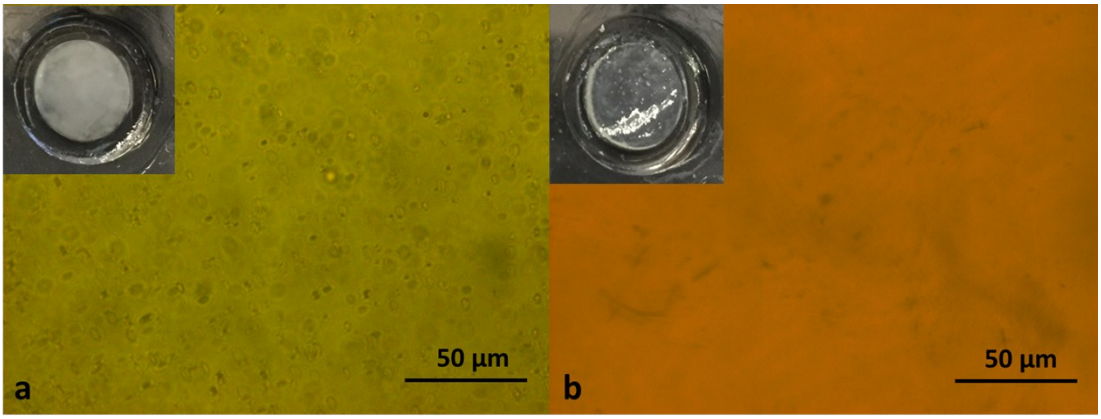

Figure S2. Optical microscope images showing the (a) turbid gel after one day and (b) transparent after two days. The insets show a photograph of the gel formed inside a cover o-ring, showing that the gel is turbid in (a) and transparent in (b).

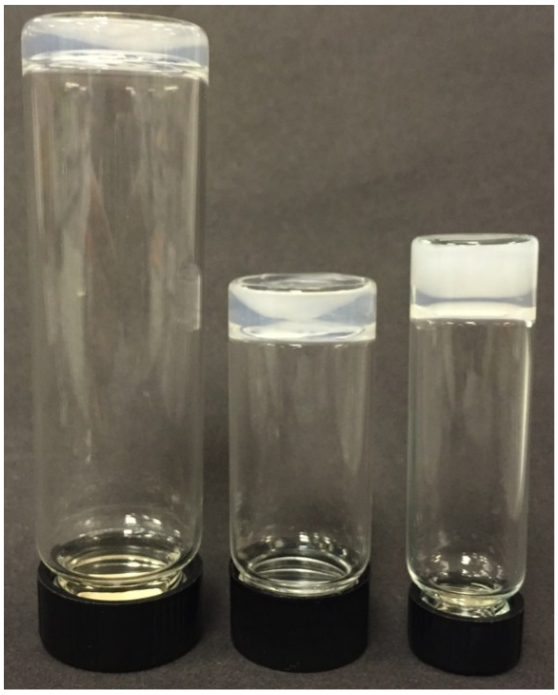

Figure S3. Photograph showing gels with a decreased air-water interface from left to right. In all cases, the same volume of gel was used. The gels with an increased surface area show increased rate of turbid to transparent transition.

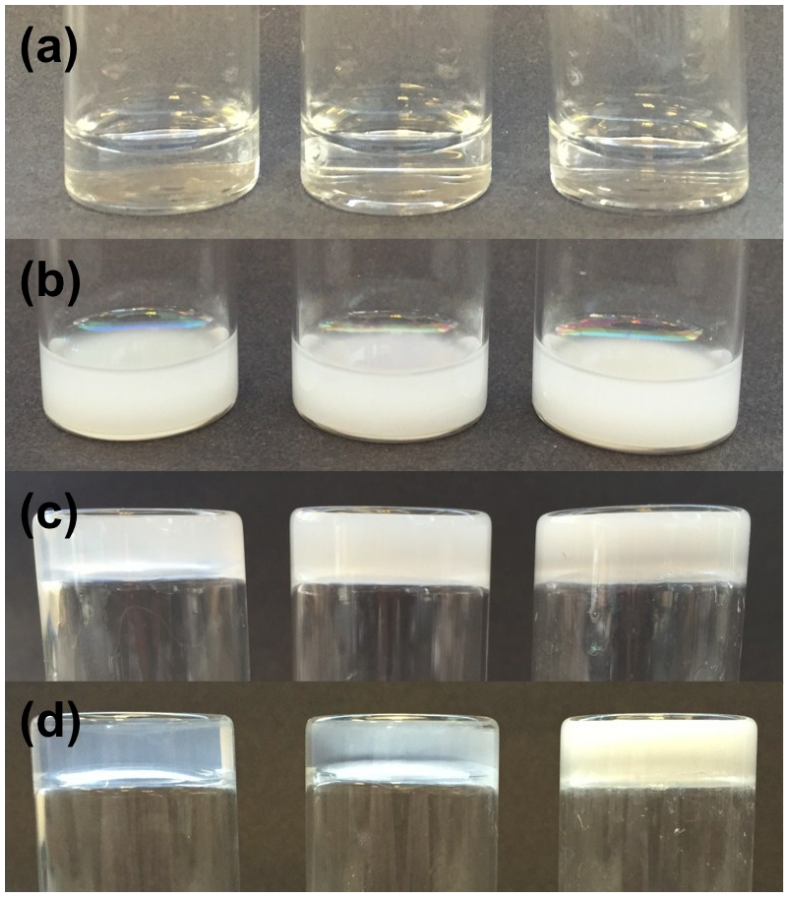

Figure S4. From left to right, solution of 2.5 mg/mL, 3.5 mg/mL and 4.5 mg/mL of gelator (a) immediately after GdL addition (b) after 1 hour (c) after 24 hours and (d) after 48 hours.

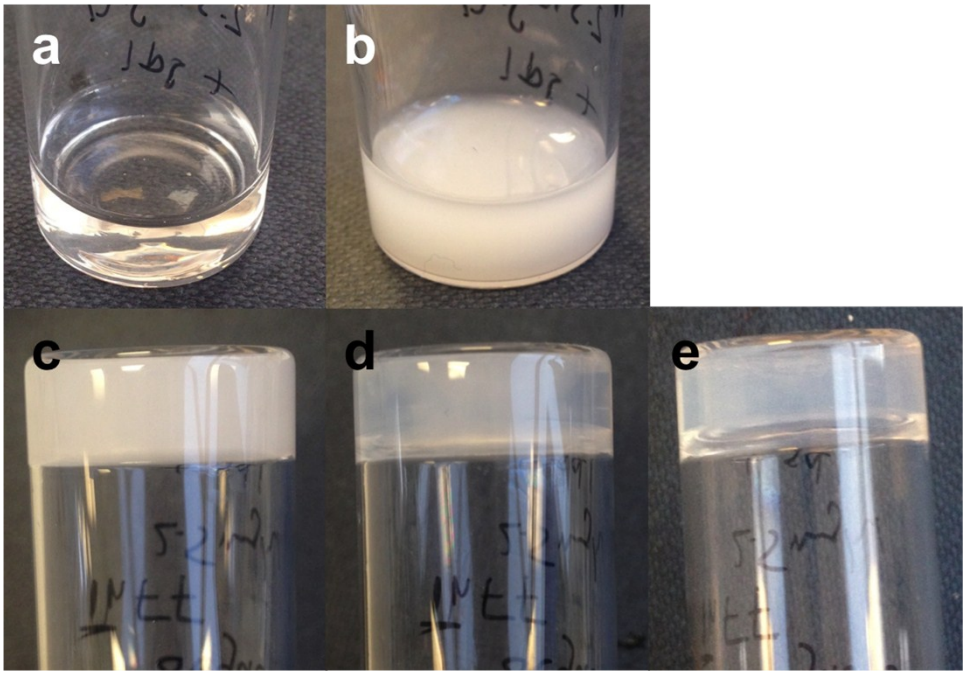

Figure S5. Photographs of gelation carried out under nitrogen (a) immediately after GdL addition (b) after one hour (c) after 1 day (d) after 2 days (e) after 4 days.

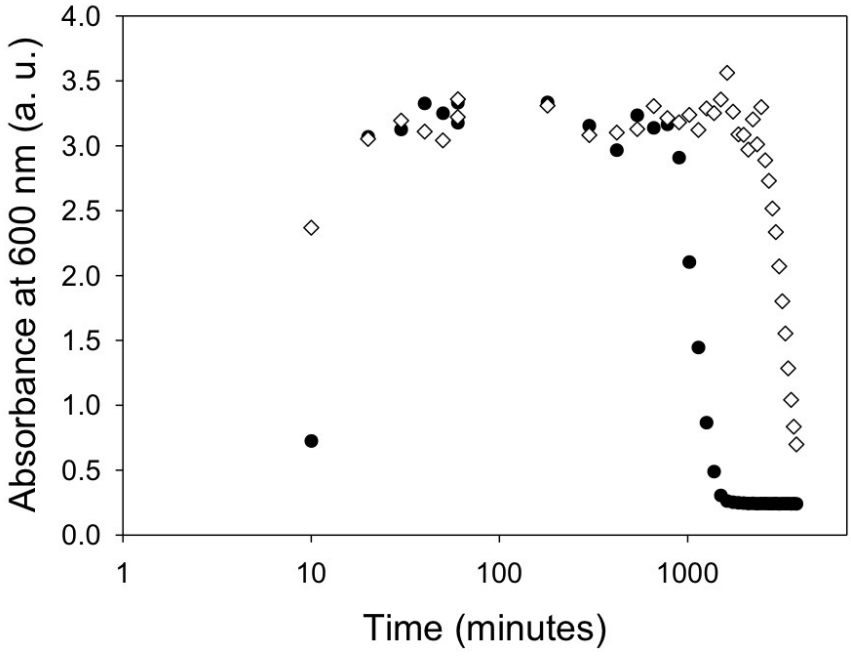

Figure S6. Graph showing the change in turbidity over time for gelation in air (represented by circles) and gelation under nitrogen (represented by diamonds).

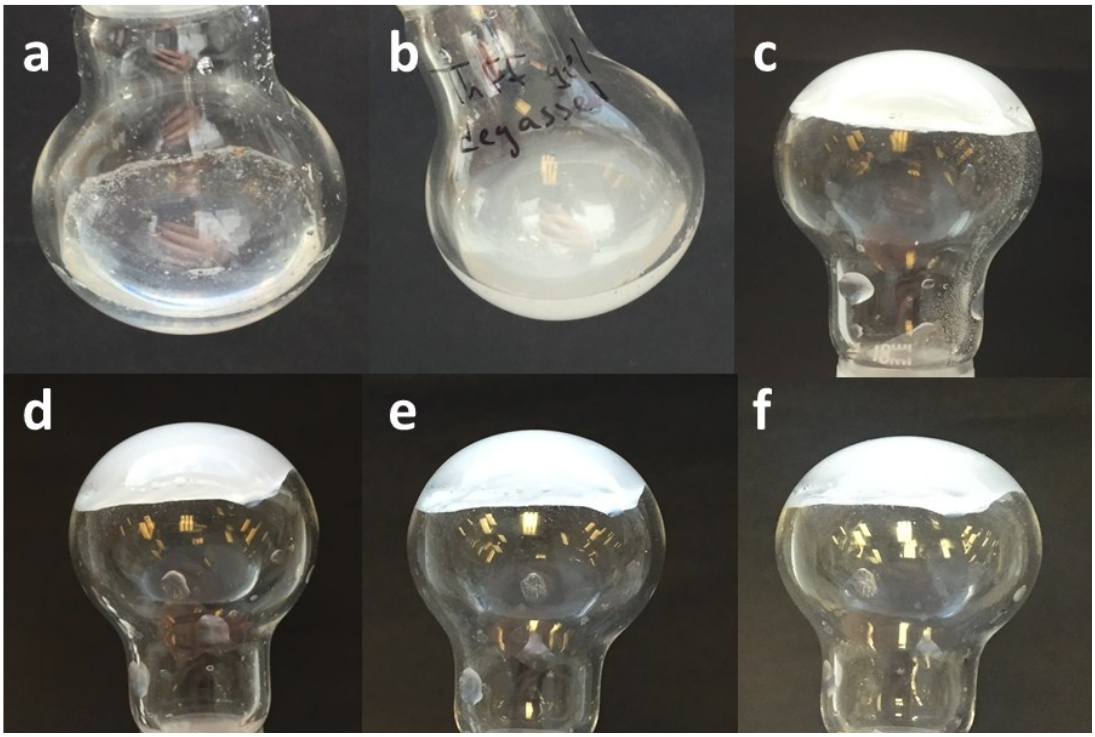

Figure S7. Photographs of a degassed solution sealed under nitrogen (a) immediately after the addition of GdL (b) after one hour (c) after 1 day (d) 2 days (e) 3 days and (f) 4 days.

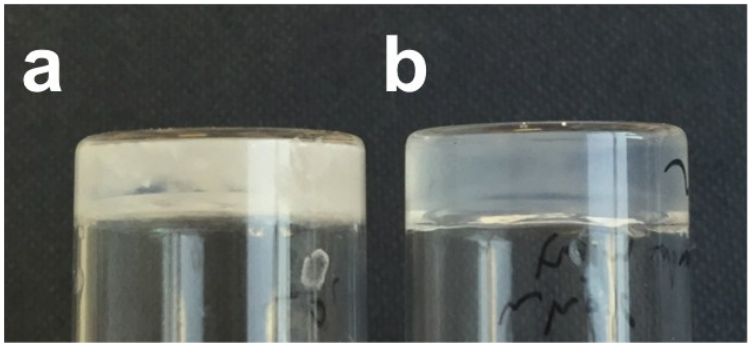

Figure S8. Photograph of gel after 24 hours (a) under air (b) under carbon dioxide.

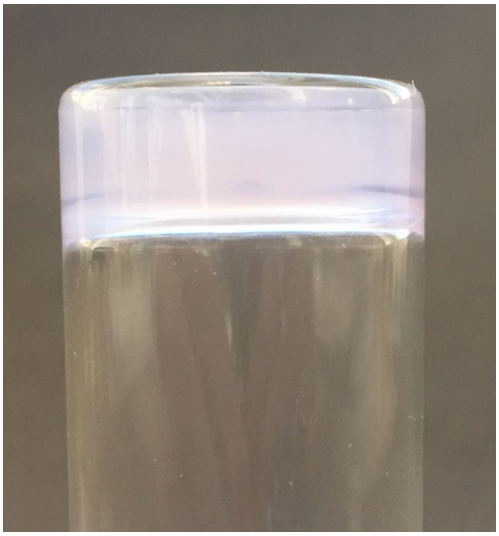

Figure S9. Photograph of an aged gel with bromophenol blue, showing no difference in colour between the transparent and turbid phase.

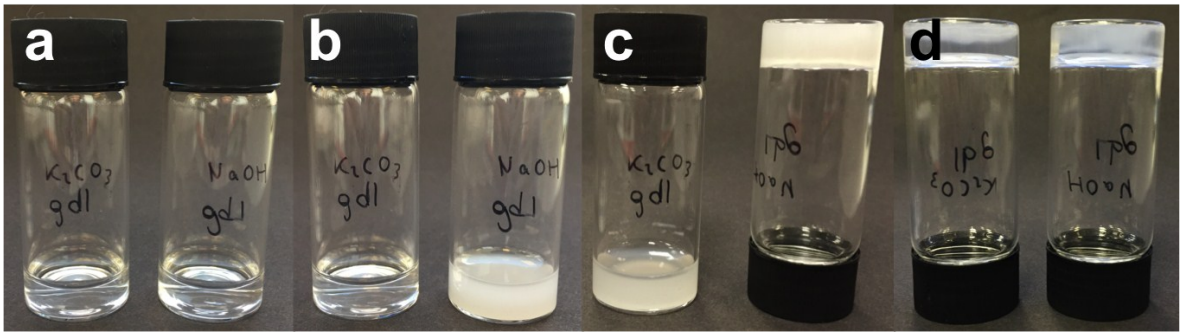

Figure S10. Photograph gelation process of gels with potassium carbonate and sodium hydroxide: (a) immediately after GdL addition (b) after one hour (c) after 6 hours (d) after one day. In all cases, the samples prepared with K_2CO_3 is on the left and the samples prepared with NaOH is on the right.

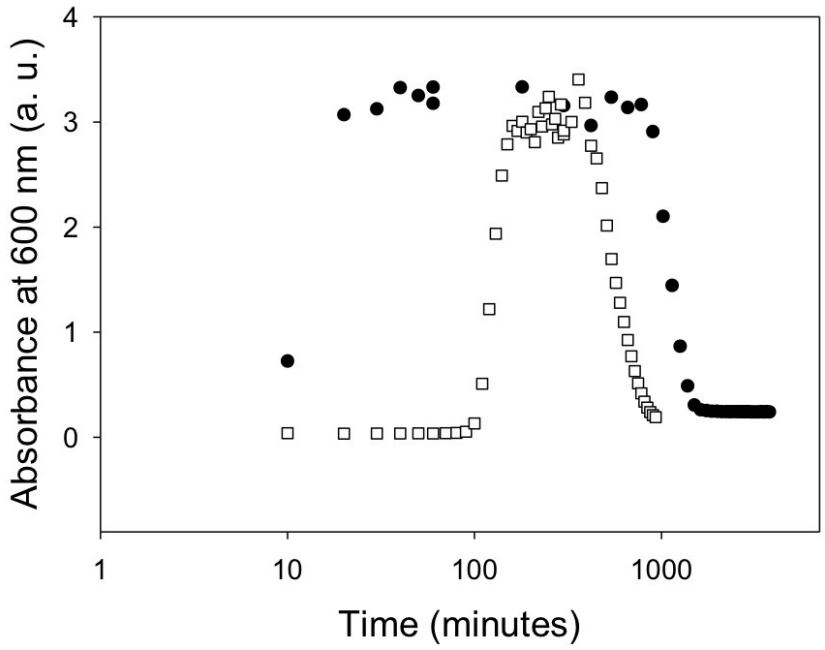

Figure S11. Graph showing change in turbidity over time for gels prepared with sodium hydroxide (represented by circles) and with potassium carbonate (represented by squares).

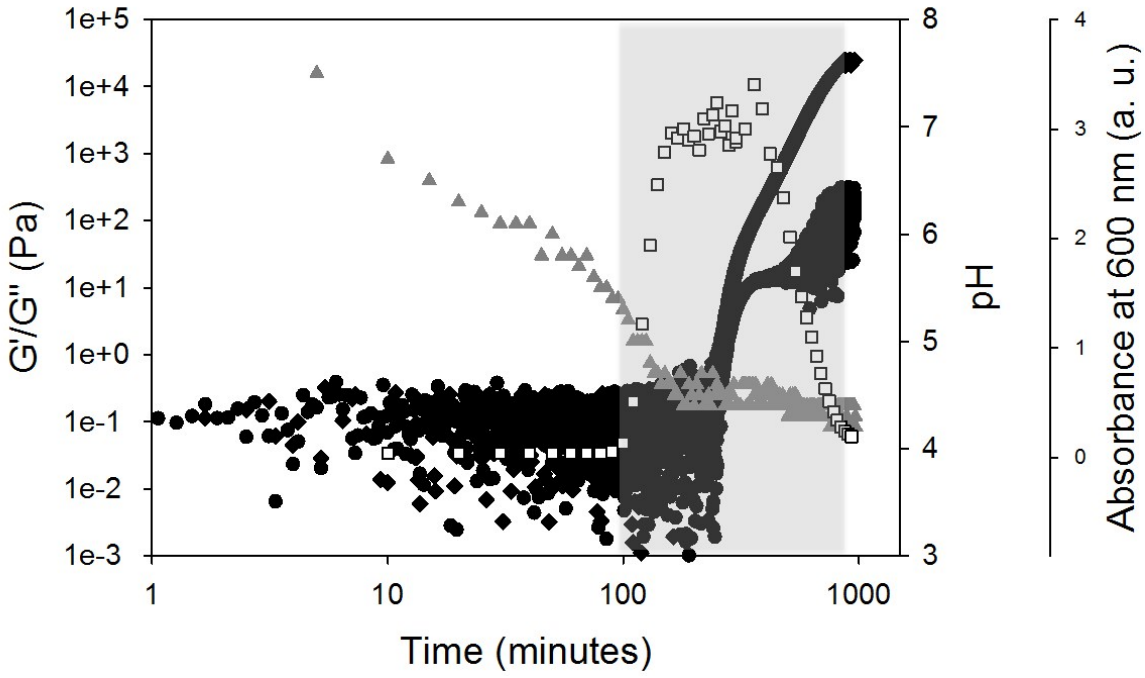

Figure S12. Graph showing the evolution of the gel network with solution made with potassium carbonate. The circles represent the storage modulus (G'), diamonds represent the loss modulus (G"). pH is represented by grey triangles. The change in absorbance at 600 nm (turbidity) is represented by open squares. The grey area indicates that the solution or gel is visually turbid.

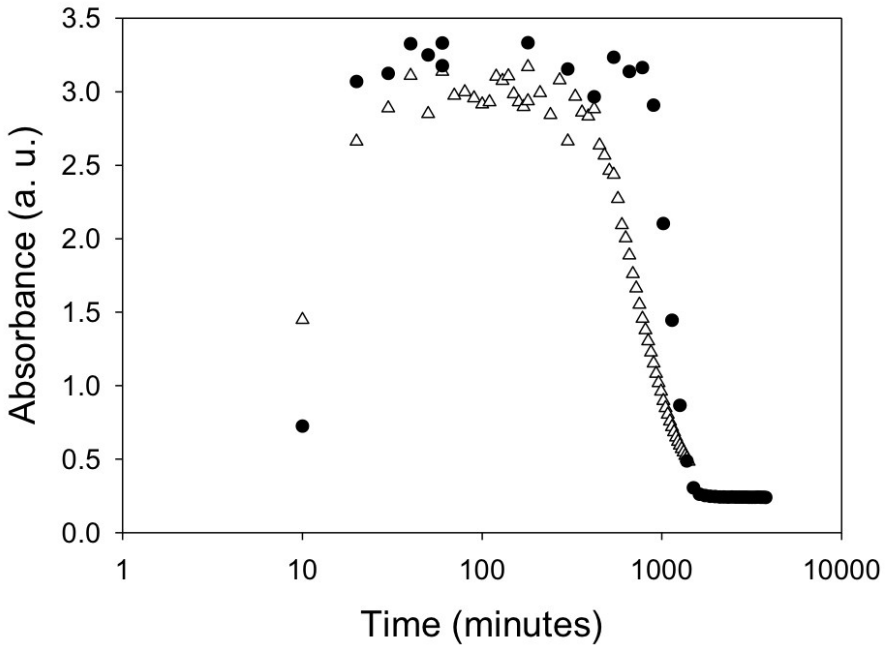

Figure S13. Graph showing change in turbidity over time for gels prepared using sodium hydroxide (represented by circles) and using potassium hydroxide (represented by triangles).

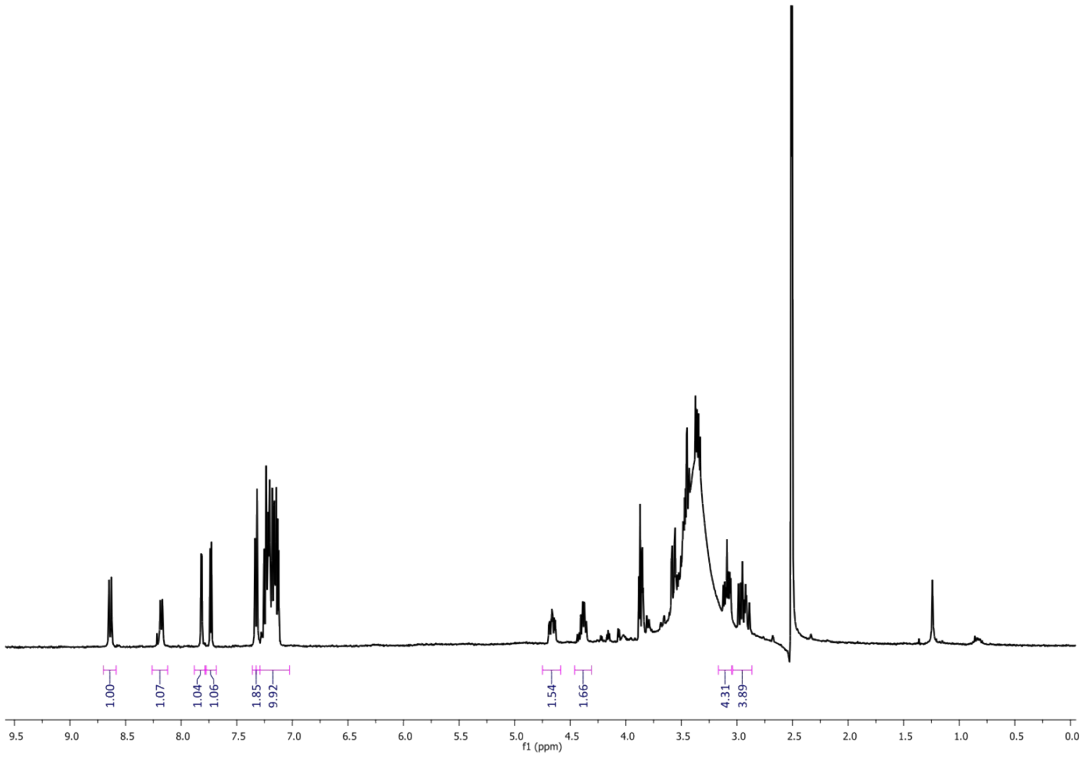

Figure S14. NMR spectrum of freeze-dried turbid gel in d_6-DMSO. The peaks between 2.70 and 4.00 ppm are mainly from the hydrolysed products of GdL.

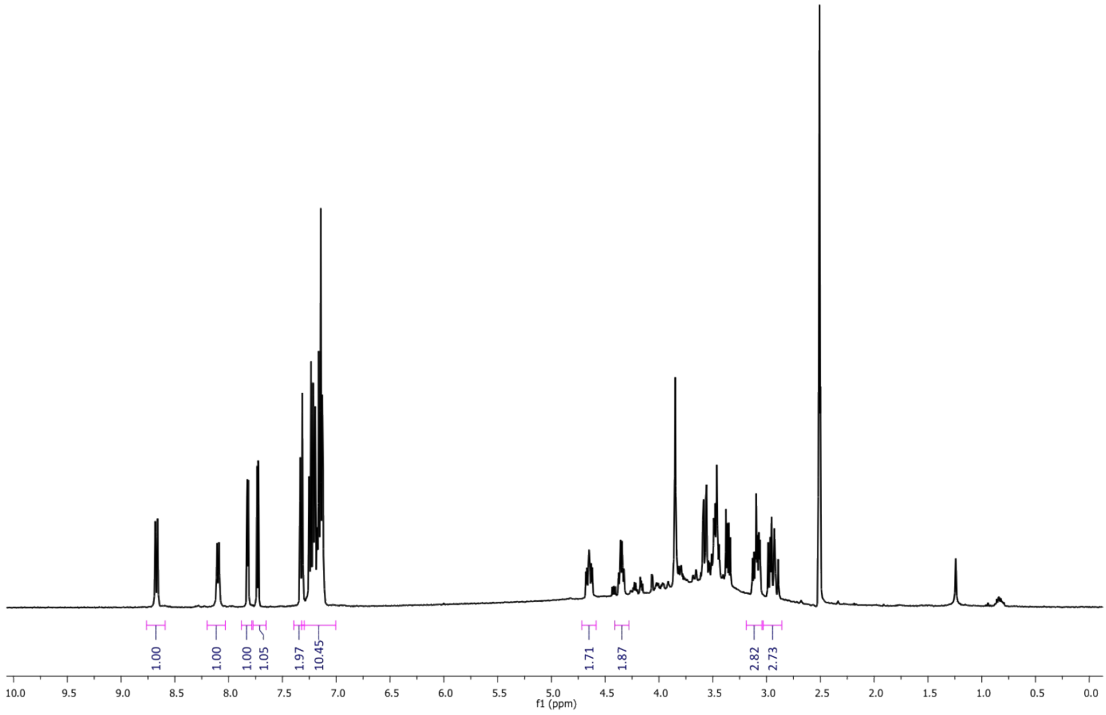

Figure S15. NMR spectrum of freeze-dried transparent gel in d_6-DMSO. The peaks between 2.70 and 4.00 ppm are mainly from the hydrolysed products of GdL.

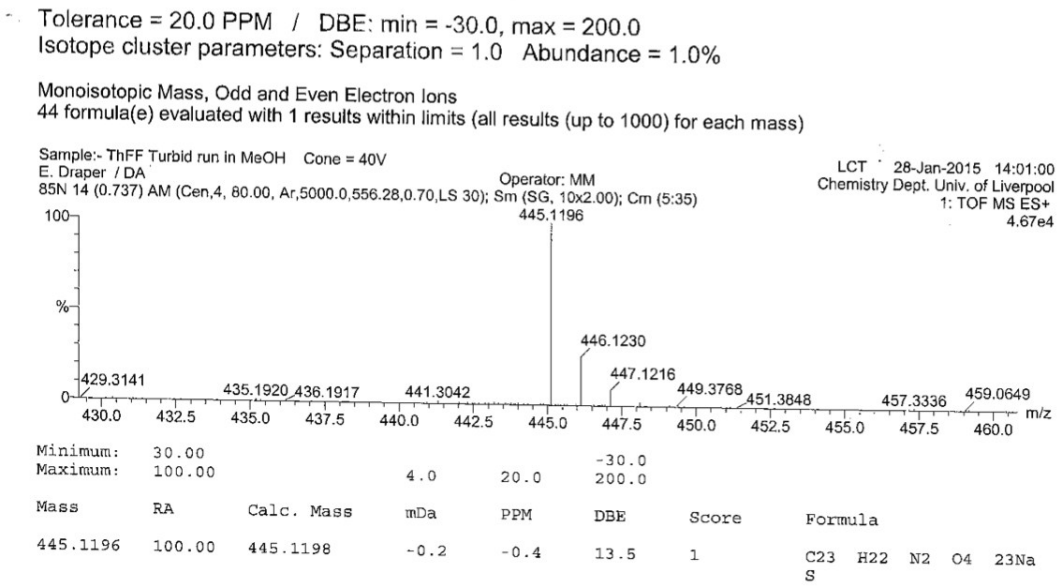

Figure S16. Mass spectrum of freeze-dried turbid gel

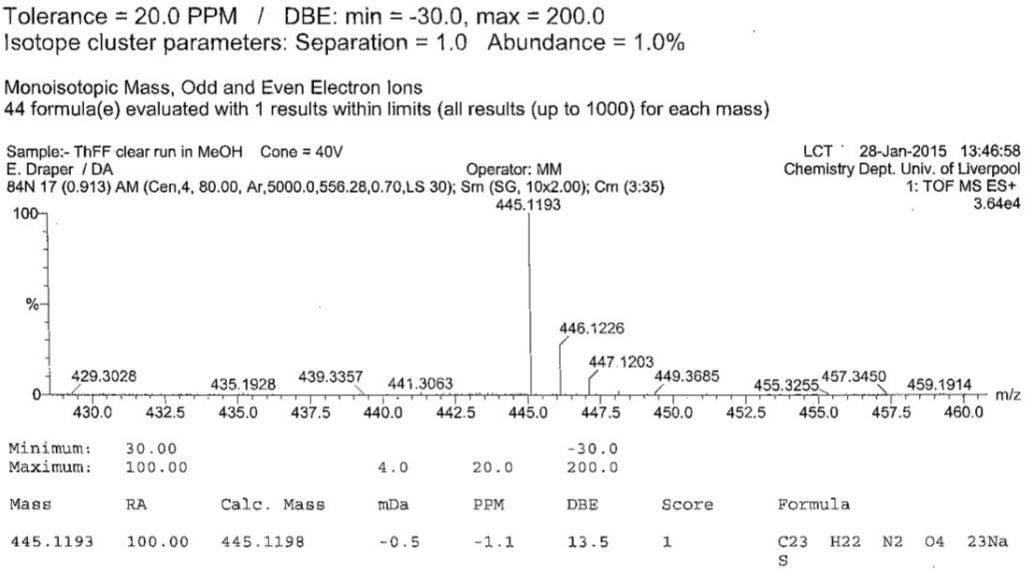

Figure S17. Mass spectrum of transparent freeze-dried gel.

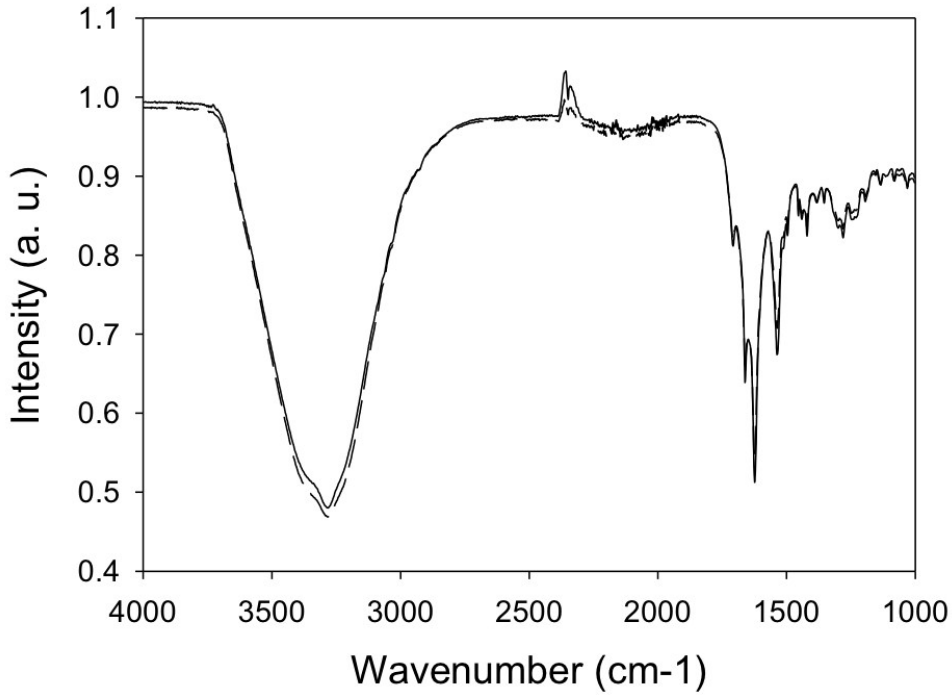

Figure S18. Infrared spectrum of the turbid gel (solid line) and transparent gel (dashed line).

References

1. C. Colquhoun, E. R. Draper, E. G. B. Eden, B. N. Cattoz, K. L. Morris, L. Chen, T. O. McDonald, A. E. Terry, P. C. Griffiths, L. C. Serpell and D. J. Adams, *Nanoscale*, 2014, **6**, 13719-13725.