Electronic Supplementary Material (ESI) for Soft Matter.

Probing the Self-assembled Structures and pK_a of Hydrogels Using Electrochemical Methods

Emily R. Cross[a] and Dave J. Adams[a,*]

School of Chemistry, University of Glasgow, Glasgow, G12 8QQ, U.K.

Supporting information

1 Materials

All chemicals were purchased from Sigma-Aldrich. Milli-Q water was used throughout. The gelator molecules were synthesised using previously established methods.[1-4] All gelators contain an aromatic group, with either one or two amino acids on the periphery. These gelators were chosen based on their ability to either form gels or not.[5] A range of amino acid and aromatic groups were used to show the method could be applicable to a variety of hydrogels.

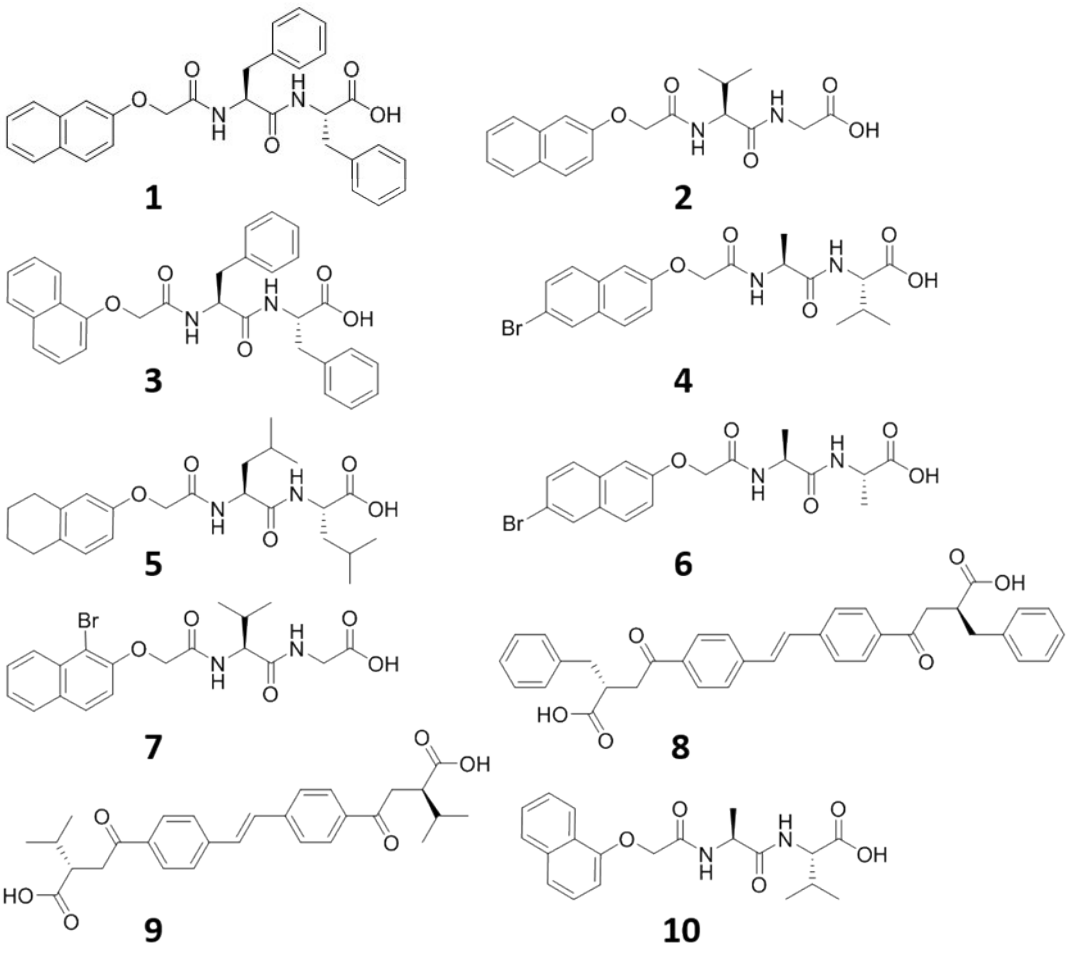

Figure S1. Gelators **1**-**10**

Hydrochloric acid (HCl) and glucono-δ-lactone (GdL) are used to lower the pH of the system. All gelator solutions are prepared using H_2O and NaOH (0.1 M aq.). The redox-active transition metal complex used was $[Ru(NH_3)_6]Cl_3$ (TM). This TM was used due to it forming a cation that is electrochemically reversible in aqueous solution.[6] The metal complex is also widely used in electrochemistry and there are many publications citing the correct diffusion coefficient values.[7, 8]

2 Methods

2.1 Preparation of dipeptide stock solutions

Each single component solution was prepared by weighing out 50 mg of gelator into 14 mL vials then adding deionised H_2O and NaOH (aq. 0.1 M, one molar equivalent for **1**-**6** and **8**-**10** and 2 molar equivalents for **7**) to a volume of 10 mL. The solution was stirred overnight to ensure all gelator had dissolved to provide solutions at a final concentration of each gelator of 5 mg/mL. For the multicomponent solution, single component solutions were prepared as above at a concentration of 10 mg/mL. The two single component solutions were then mixed in a ratio of 1:1 to provide a solution in which the concentration of each component were 5 mg/mL (so total gelator concentration of 10 mg/mL). All solutions were stored at room temperature.

2.2 Preparation of dipeptide solutions for electrochemical analysis

For each single component systems, 2 mL of the gelator solution were transferred using a pipette to a Sterilin vial containing 3.1 mg of $[Ru(NH_3)_6]Cl_3$ (5 mmol). The gelator solution was then transferred, by pouring, into a Sterilin vial containing 10 mg of glucono-δ-lactone (5 mg/mL). Immediately after, a modified Sterilin vial lid containing three electrodes (glassy carbon working electrode, Ag/AgCl (3 M) reference, Pt wire counter) was added as shown in Figure S2. Then, the electrochemical experiment was run as described in Section 2.4 & 2.5. For the multicomponent systems, 1 mL of each gelator solution at a concentration of 10 mg/mL were mixed together (so the total gelator concentration of each component were 5mg/mL). 2 mL of this gelator solution were transferred to a Sterilin vial containing 6.2 mg of $[Ru(NH_3)_6]Cl_3$ (10 mM). The solution was poured into 20 mg of GdL (10 mg/mL) and the electrochemical experiments carried out.

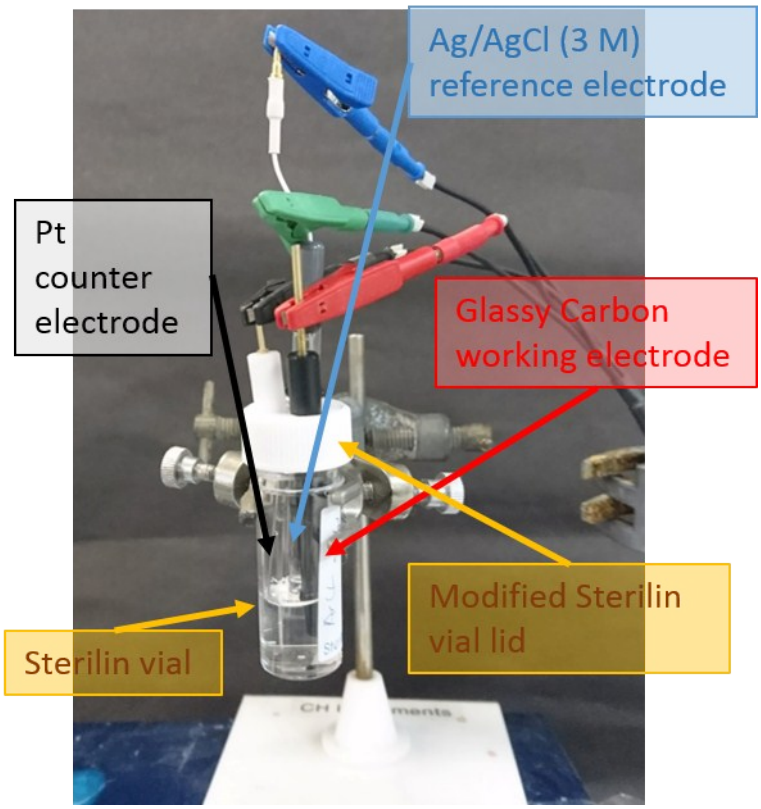

Figure S2. Image showing the electrochemical set up.

2.3 pH measurement

pH measurements were recorded using a Hannah PC turtle FC500 pH probe with a given error of +/- 0.1. For measuring the pH of gelation over time, 2 mL of gelator solution at pH 10 was added to GdL (5 mg/mL for single component and 10 mg/mL for multicomponent) in a 7 mL Sterilin vial. The pH measurements were recorded with an interval of 0.5 minutes over a period of 16 hours.

2.4 Cyclic voltammetry

The electrochemical set up as described in Section 2.2 was used to carry out cyclic voltammetry. Cyclic voltammetry measurements were carried out within a potential range of -0.5 to 0.2 V vs. an Ag/AgCl (3 M) ref. at a scan rate of 20, 40, 60, 80, 100, 200, 400, 600, 800, 1000 mV/s. Each CV measurement consisted of one scan. The diffusion coefficient was determined using the reduction current peak and the Randles-Sevcik equation (Equation S1).

$$i_p = 0.4463nFAC\left(\frac{nFvD}{RT}\right)^{\frac{1}{2}}$$

Equation S1. Randles-Sevcik equation where i_p is the peak oxidation or reduction current, n is the number of electrons transferred, F is Faraday's constant, A is the area of electrode, C is the concentration of TM, v is the scan rate, R is Rybergs constant, T is the temperature and finally D is the diffusion coefficient.

2.4.1 Cyclic Voltammetry over time.

Cyclic voltammograms were measured as described above consecutively for 16 hours (Figure S3). The peak reduction current was then converted to Diffusion coefficient using the Randles-Sevcik equation.

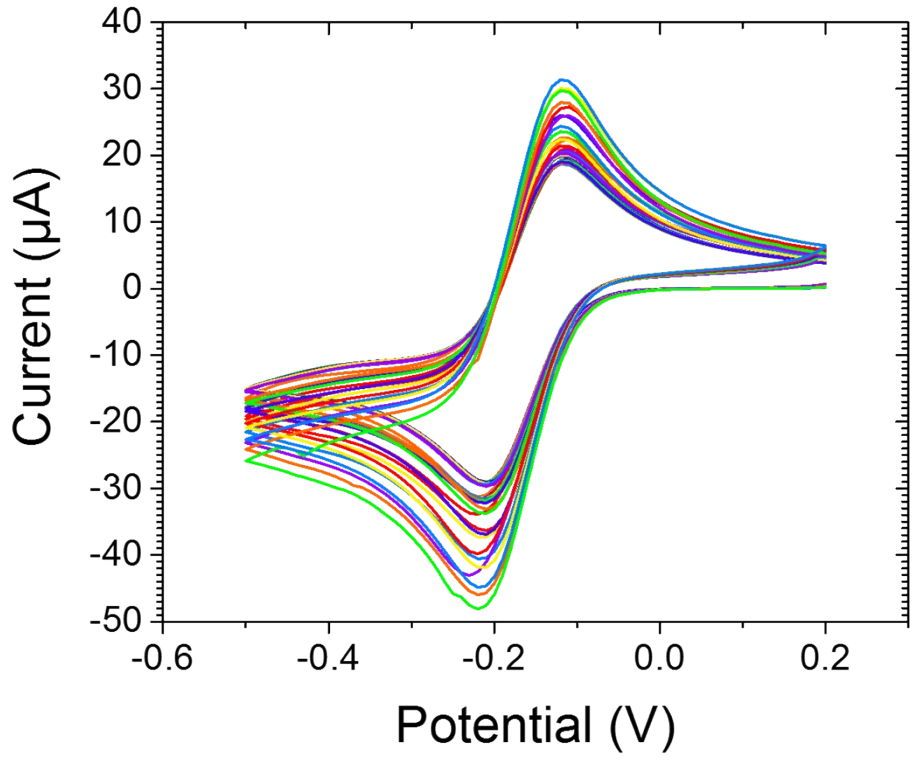

Figure S3. Example data for **1** showing 20 continuous CVs. From these graphs the peak reduction current is measured as a function of time.

2.4.2 Gel or crystal determination

Cyclic voltammetry as described above was used to measure the peak current of the TM at pH 9.5 and pH 4.5 (Figure S4). The difference between the reduction current peak of 9.5 and 4.5 was calculated. This value indicated whether a crystal or gel formed as discussed in the main text.

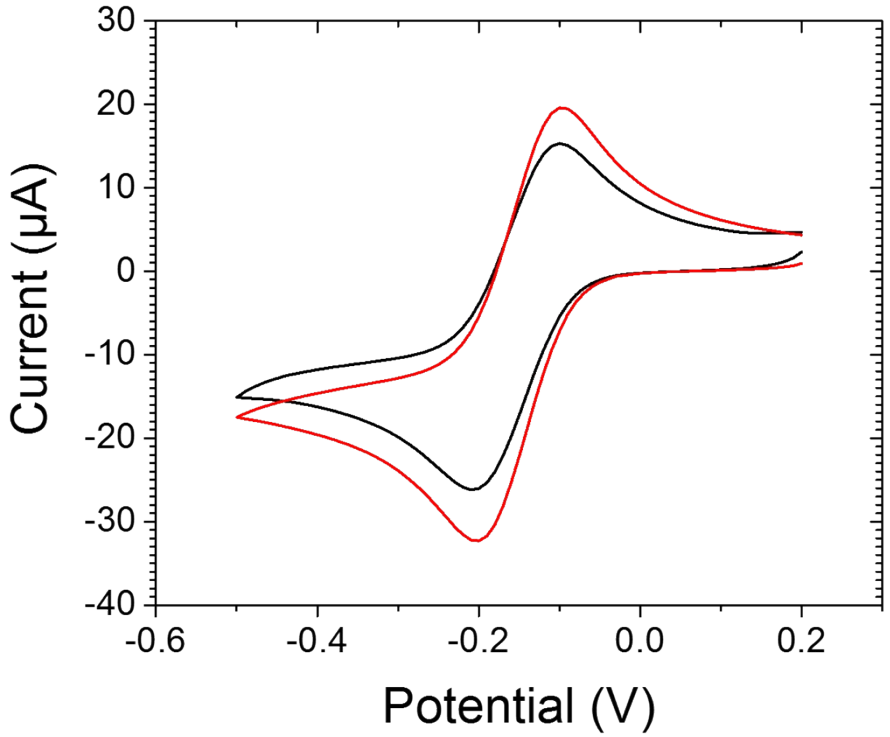

Figure S4. Cyclic voltammograms of **1** at pH 9.5 (black) and at pH 4.5 (red).

2.5 Multiple Pulse Amperometry (MPA)

The same electrochemical set up was used for MPA as described in Section 2.2. MPA measurement were carried out at potentials of -0.12 and -0.20 V for 1 s each. This was continuously repeated for 16 hours.

2.6 Rheological measurements

Rheological measurements were carried out using an Anton Paar Physical MCR301 rheometer. Time sweeps were performed using 50 mm diameter parallel plates with an angular frequency of 10 rad/s with a strain of 0.5 %. For measuring the time sweep

2 mL of gelator and TM solution as described in Section 2.22.1 was poured onto the bottom parallel plate. Mineral oil was added to the edges of the parallel plate to prevent drying of the sample. A time delay of 15 seconds was maintained from addition of GdL to sample acquisition. The time sweeps were recorded over 16 hours.

3 Summary of pK_a values.

Gelator	Literature	pH titration p*Ka* value	Cyclic voltammetry method p*Ka* value	Multiple pulse amperometry method p*Ka* value	Gel or No Gel
1	6.0[1]	6.0	~6.0	6.0	Gel
2	5.0[1]	5.0	-	5.0	Gel
3	-	6.8	-	-	Gel
4	5.8[1]	5.9	~5.8	-	Gel
5	-	5.8	-	5.7	No gel
6	4.9[1]	-	-	6.0	Gel
10	-	4.1	-	-	No gel
2 & **8** (in a multicomponent system)	5.8*[9] (**8**) & 5.0[1] (**2**)	-	-	6.6 (**8**) & 5.0 (**2**)	Gel

*Literature value states the gelator solution at 10 mg/mL.

Table S1. Summary of pK_a values for gelator molecules.

References

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