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Self-assembled sorbitol-derived supramolecular hydrogels for the controlled encapsulation and release of active pharmaceutical ingredients

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SUPPORTING INFORMATION

Contents

1. General Methods

Synthesis. DBS-$CONHNH_2$ was synthesised using our previously reported method, and all characterisation data were in agreement with those published.[1] All compounds required for synthesis were purchased from Sigma-Aldrich, as were all the carboxylic acid drug molecules and buffers.

Instrumentation. IR spectra were recorded on a PerkinElmer Spectrum Two FT-IR spectrometer. T_{gel} values were recorded using a high precision thermo-regulated oil bath. Scanning Electron Microscopy (SEM) was carried out on a LEO 1530 Gemini FEGSEM fitted with an Oxford instrument 80mm X-Max SDD detector. UV-Vis spectroscopy was performed on Shimadzu UV-2401 spectrometer. Fluorescence spectroscopy was carried out on a Hitachi F-4500. Both excitation and emission slit width was 10.0 nm, scan speed was set to 240 nm/min and PMT was set at 700 V. Circular Dichroism (CD) Spectroscopy was carried out using a Jasco J810 CD spectrophotometer – concentrations were below the gelation threshold.

Gel Formation. DBS-$CONHNH_2$ gels were made from a heat-cool cycle where by 0.5 ml of deionised water was added to dry DBS-$CONHNH_2$. This mixture was then sonicated for 10 minutes, heated until all the DBS-$CONHNH_2$ had dissolved, and then allowed to cool. Gel formation could be seen after 5 minutes but took slightly longer at lower concentrations of gelator.

T_{gel} Measurements. The T_{gel} values of DBS-$CONHNH_2$ gels were recorded using the standard reproducible table-top rheological method of tube inversion.[2] A range of gelator concentrations were investigated, all of which were formed in 0.5 ml of deionised water in standard sample tubes. The temperature was recorded when the gels fell under gravity.

Hybrid Gel-API Formation. The gelator and API were mixed as solids before adding water. In small vials DBS-$CONHNH_2$ (1.42 mg) was added to the API (MSZ (0.46 mg), NPX (0.69 mg), IBU (0.61 mg)). To this, deionised water (0.5 mL) was added, such that the concentration of

each of DBS-$CONHNH_2$ and API would be 6 mM. The mixture was then sonicated for 15 minutes, heated until dissolved, then allowed to cool and form a gel at room temperature.

Rheological Measurements. Dynamic rheological measurements were performed using a Kinexus pro^+ rheometer at 25^oC with parallel plate geometry (20 mm diameter) at a gap of 1 mm. All hydrogels (2 ml) comprising an equimolar amount of DBS-$CONHNH_2$ (10 mM) and the APIs (10 mM) were pre-formed in an in-house mould, de-moulded after being left to aged for at least 20 hours and carefully loaded onto the sample stage. Caution, which include gradual gap closing and careful sample trimming were ensured to minimize internal damage to the hydrogel networks. Oscillatory frequency sweeps were performed from 0.1 Hz (0.68 rad^{-1}) to 10 Hz (62.8 rad^{-1}) under a strain of 0.5 %. The shear moduli (G' and G'') were measured at a frequency of 1 Hz. Oscillatory strain sweeps measurements were performed within the linear viscoelastic region. Data were processed using rSpace software.

Scanning Electron Microscopy (SEM). SEM was performed on xerogels dried under ambient conditions from DBS-$CONHNH_2$ (6 mM) hydrogels in the absence or presence or API (6 mM). Samples were dried from water (0.5 mM) with gels being formed as described above.

2 IR Data

FTIR was performed on xerogels dried under ambient conditions from DBS-$CONHNH_2$ (6 mM) hydrogels in the absence or presence or API (6 mM). Samples were dried from water (0.5 mM) with gels being formed as described in Section 1.

Table S1 – IR absorption peaks corresponding to DBS-$CONHNH_2$ xerogel in the absence and presence of APIs.

	DBS-$CONHNH_2$	**DBS-$CONHNH_2$ +MSZ**	**DBS-$CONHNH_2$ +NPX**	**DBS-$CONHNH_2$ +IBU**
O-H	3262	3283 (+21)	3287 (+25)	3288 (+26)
N-H	3194	3180 (-14)	3186 (-8)	3185 (-9)
C=O	1638	1640 (+2)	1644 (+6)	1641 (+3)
C=C (Aromatic)	1571	1570 (-1)	1570 (-1)	1572 (+1)
C-N	1331	1339 (+8)	1340 (+9)	1332 (+1)
C-O	1091	1092 (+1)	1092 (-)	1093 (+2)

3 Rheological Data

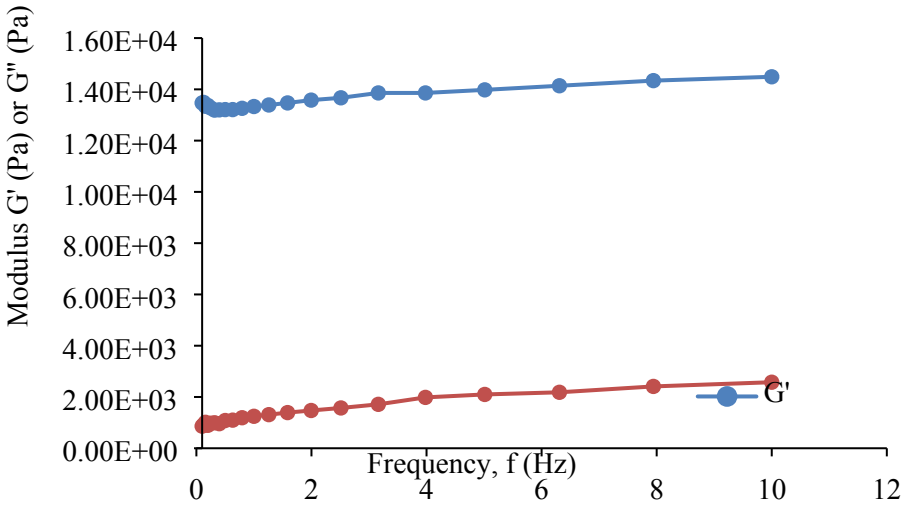

Figure S1. Rheology of DBS-CONHNH$_2$ in the linear viscoelastic region.

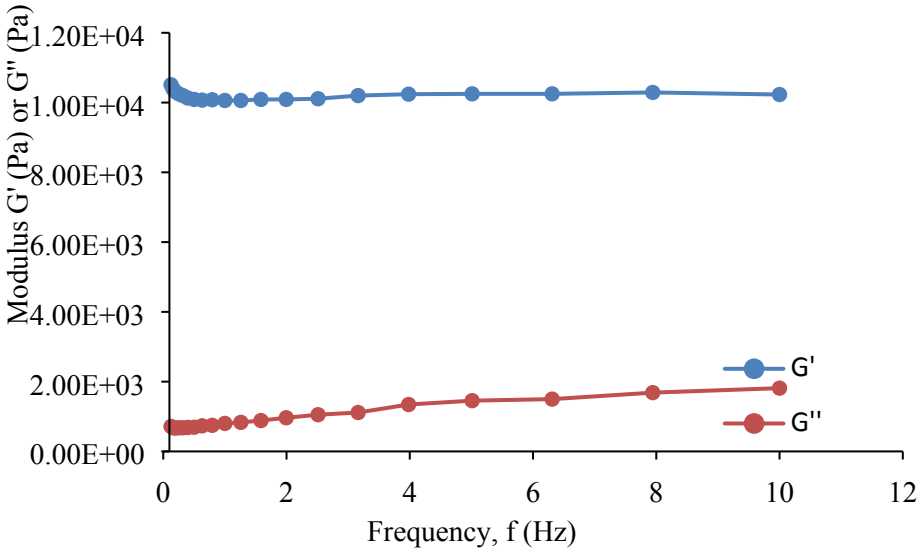

Figure S2. Rheology of DBS-CONHNH$_2$ in the linear viscoelastic region formed in the presence of MSZ.

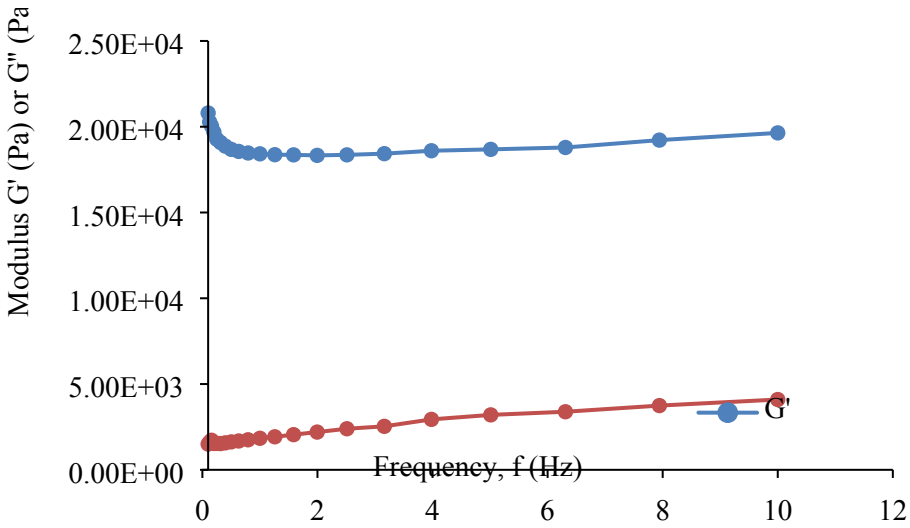

Figure S3. Rheology of DBS-CONHNH$_2$ in the linear viscoelastic region formed in the presence of IBU.

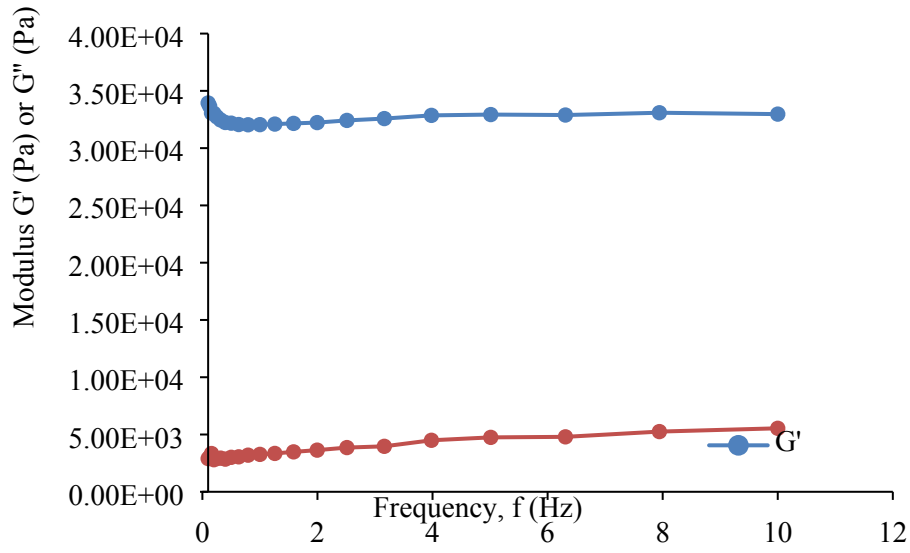

Figure S4. Rheology of DBS-CONHNH$_2$ in the linear viscoelastic region formed in the presence of NPX.

4 CD Spectra and Avrami Kinetic Plots

A sample of DBS-$CONHNH_2$ (2.5 mM) in the absence and presence of NPX (2.5 mM) was studied by CD spectroscopy (Fig. S5). Similarities in the CD band at 270 nm associated with DBS-$CONHNH_2$ were observed, as were bands associated with NPX at 310 and 335 nm.

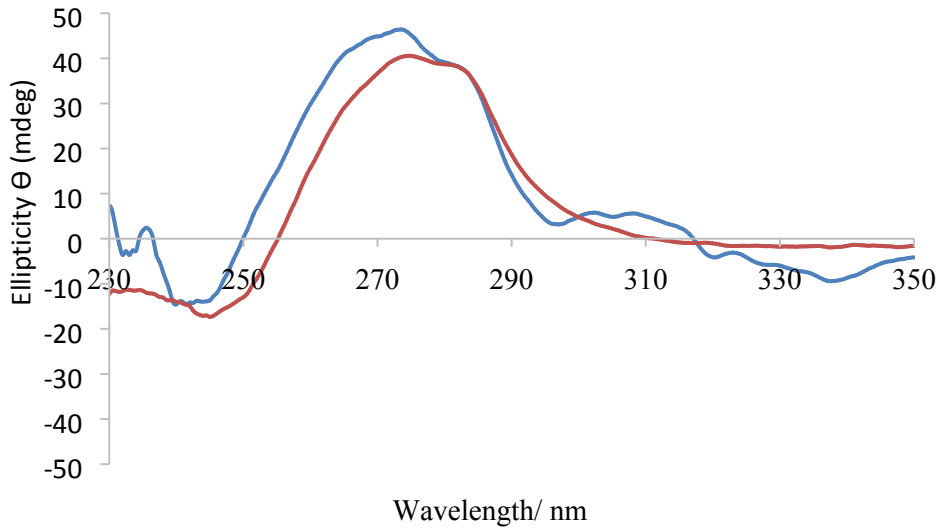

Figure S5. CD Spectra of DBS-$CONHNH_2$ (2.5 mM, red) and DBS-$CONHNH_2$ + NPX (both 2.5 mM, blue) after evolution of the gel.

The evolution of the CD spectrum over time at intervals of 30 s, until a maximum stable band was observed (Figs. S6 and S7).

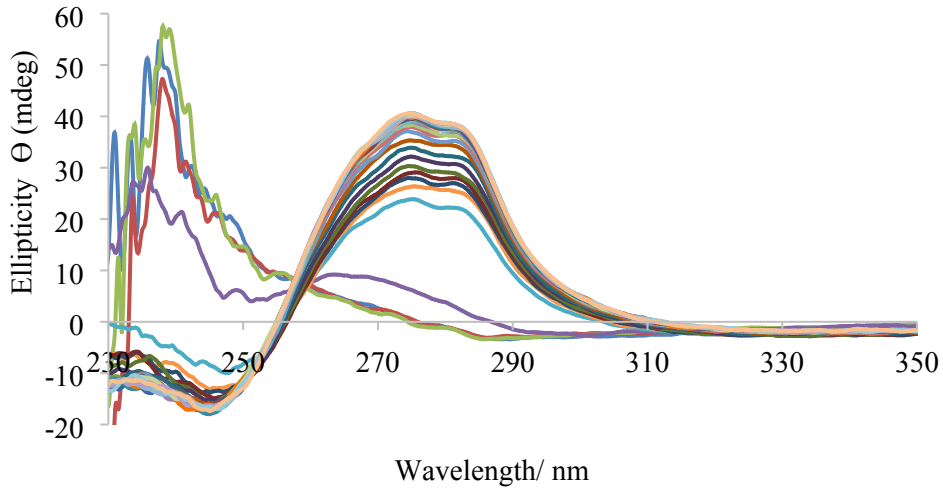

Figure S6. CD Spectra of DBS-CONHNH$_2$ (2.5 mM) evolving over time with CD spectra being measured every 30 s.

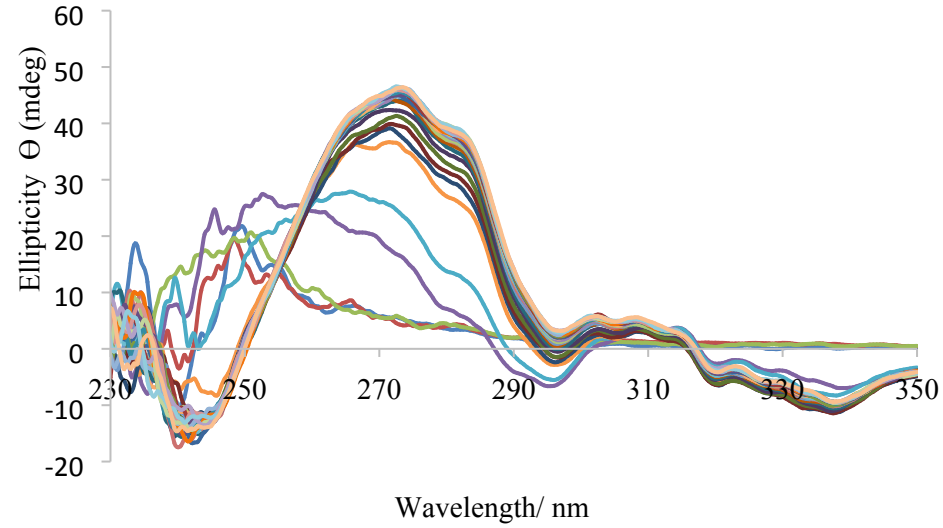

Figure S7. CD Spectra of DBS-CONHNH$_2$ and NPX (both 2.5 mM) evolving over time with CD spectra being measured every 30 s.

The time resolved CD spectrometry following the assembly process can be fitted using the Avrami treatment (equations 1 and 2).[1]

$$1 - X = exp(-Kt^{n}) \quad (1)$$

$$\ln\left(\ln\frac{1}{1-X}\right) = \ln K + n\ln t \quad (2)$$

As applied to gels, *X* is the volume fraction, *K* is a temperature dependent parameter similar to a rate constant, *n* is the Avrami exponent that reflects the growth leading to phase separation and *t* is the time. To interpret the CD data to Avrami theory, we used the peak maximum at 275 nm for DBS-CONHNH$_2$, and at 272 nm in the presence of NPX to collect kinetic data.

The fraction of the gel phase at time $= t, X(t),$ can be expressed as $X(t) = (I(t) - I(0))/(I(\infty) - I(0))$ where I is the intensity at time t, 0 and ∞. The Avrami exponent n is then obtained by plotting $ln[-\ln\frac{I(\infty) - I(t)}{I(\infty) - I(0)}]$ versus $\ln t$ (Figures S8 and S9). As reported elsewhere,[1d] it is challenging to assign time zero, as the gels form after a variable induction time, and under this concentration regime, gelation is very rapid. This can have a considerable impact, especially on the reported *K* values which are extrapolated out to an axis, which should therefore be treated with caution. For example, for DBS-CONHNH$_2$, gelation was effectively almost all complete within a 30 s window (differene between purple and blue lines in Fig. S6), but this could not really be captured within the data handling, which therefore focussed only on the latter stages of gelation, which occur more slowly.

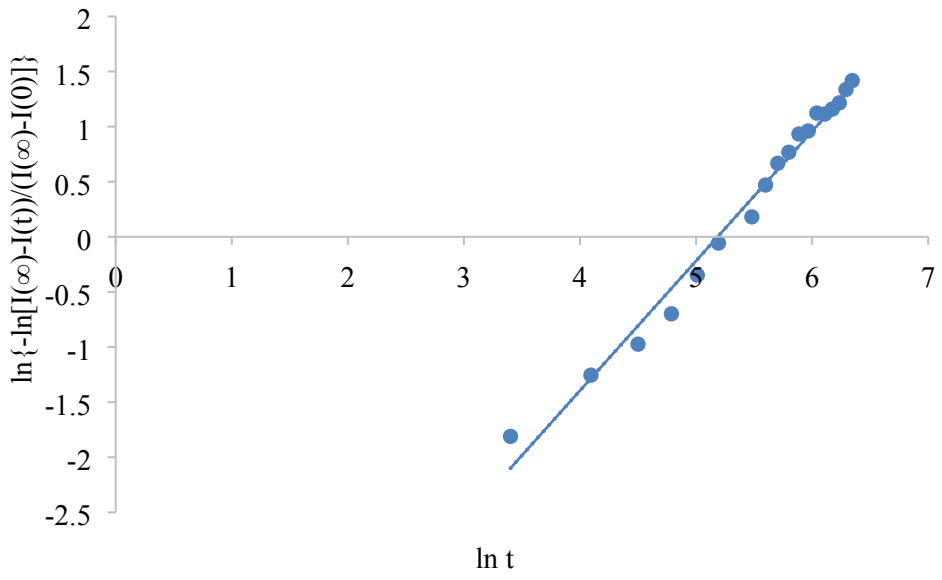

Figure S8. Avrami plot for the evolution of DBS-CONHNH$_2$ (2.5 mM) as monitored by CD spectroscopy (n = 1.18, K = 2.2x10^{-3} s^{-1}).

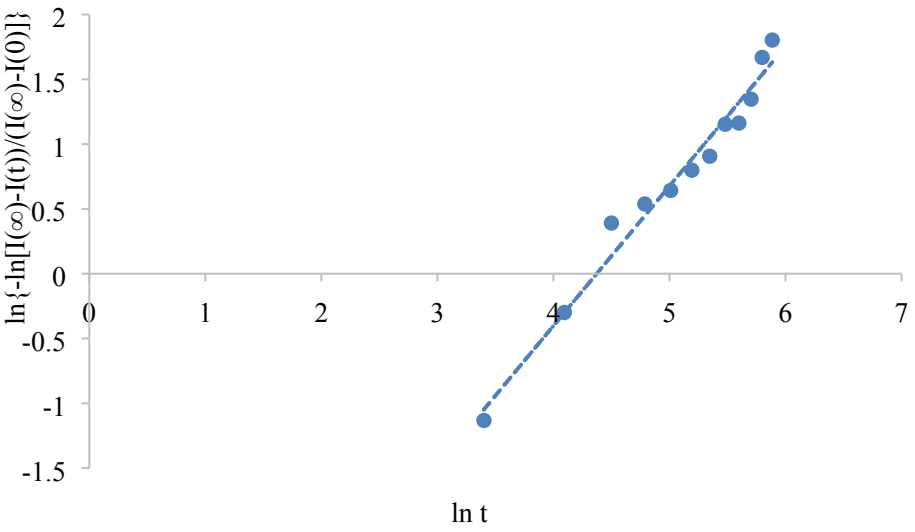

Figure S9. Avrami plot for the evolution of DBS-CONHNH$_2$ and NPX (both 2.5 mM) as monitored by CD spectroscopy (n = 1.08, K = 9.0x10^{-3} s^{-1}).

5 Fluorescence Spectra

Fluorescence studies of the Naproxen gels were carried out using 2.5 mM DBS-hydrazide (0.59 mg) and 2.5, 2.0, 1.5 and 1.0 mM of Naproxen (0.29, 0.23, 0.17 and 0.12 mg respectively). The gel and drug were mixed and sonicated for 15 minutes in sample vials with 0.5 ml of deionised water. They were then heated until everything was dissolved then transferred into a cuvette to cool and form a gel. The excitation wavelength used for all spectra was 272 nm and the measured range was from 300 to 500 nm.

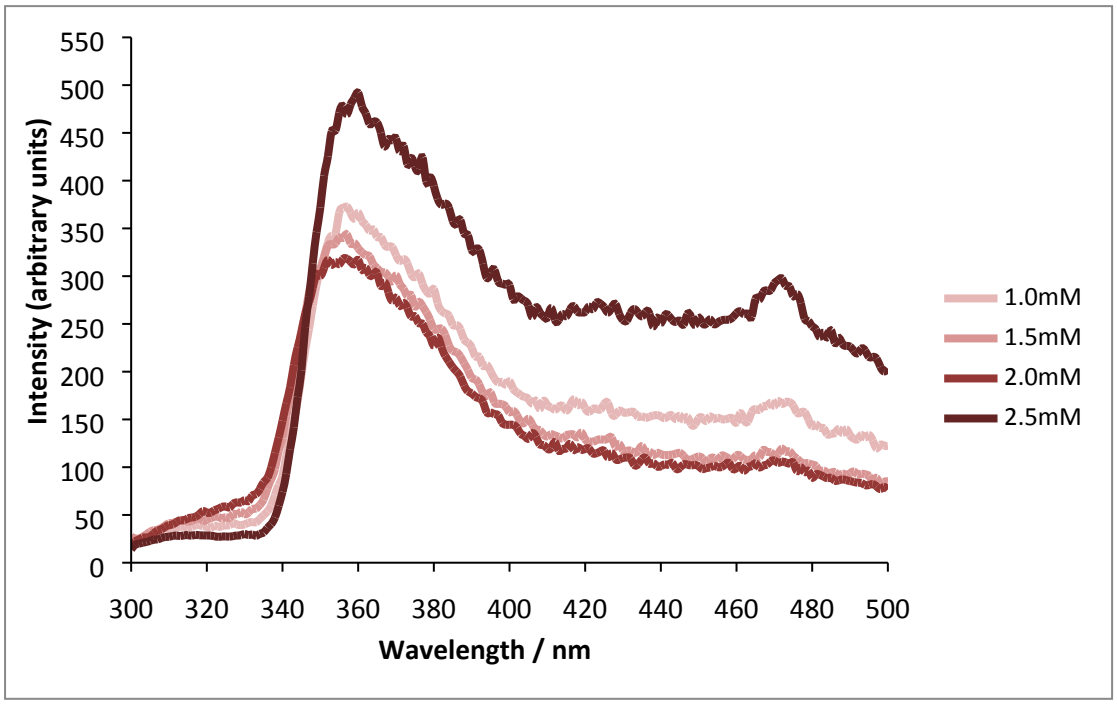

Figure S10. Fluorescence spectra of DBS-CONHNH$_2$ (2.5 mM) and increasing aliquots of NPX.

6 API Adsorption from Solution

MSZ was adsorbed from solution into the gels. A stock solution of 2.87 mM MSZ (22 mg) in acetate buffer (50 mL, pH 4.65) was prepared for absorption experiments. A calibration plot was performed by serial dilution in acetate buffer in order to determine UV-Vis absorption of MSZ at λ = 297 nm.

2 ml of MSZ stock solution was then added carefully on top of a pre-formed 10 mM DBS-hydrazide gel (2.37 mg in 0.5 mL of water). An initial UV-Vis absorption reading was taken and subsequent readings were taken every two hours. For each reading, 5 µL of the supernatant solution on top of the gel was taken and diluted to 2 mL in acetate buffer. After 24 hours, as MSZ levels dropped, 2 ml of the solution was taken from on top of the gel, a spectrum was recorded. The 2 ml was then put back on top of the gel and the experiment continued – this was repeated for each measurement. Data from this experiment are presented as Figure 2 in the main paper.

Two kinetic models were used to establish the mechanism for adsorption based on the experimental data. The pseudo-first-order rate equation given by Lagergren[2] as shown in equation 3 was used to study the adsorption kinetics.

$$\ln\left(q_e - q_t\right) = \ln q_e - k_1 t \qquad (3)$$

Where q_e and q_t are the amount of 5ASA adsorbed (mg/g) at equilibrium and time t (h), respectively, and $k_1$is the rate constant of adsorption (h^{-1}). The value of k_1 was calculated from the plots of $\ln\left(q_e - q_t\right)$ versus t as shown in Figure S11.

The pseudo-second-order model employed by Ho and McKay based on equilibrium adsorption is expressed in equation 3.[3]

$$\frac{t}{q_t} = \frac{1}{k_2 {q_e}^2} + \frac{1}{q_e} t \qquad (4)$$

Where k_2 is the second-order rate constant$(g/(mg\ hour))$ calculated from the slope and intercept of the plot $\frac{t}{q_t}$ versus t as shown in Figure S12.

The fitting shown in Figures S11 and S12 clearly demonstrates that pseudo-second-order kinetics fit much better than first order with an R^2 value of 0.986 compared to 0.890.

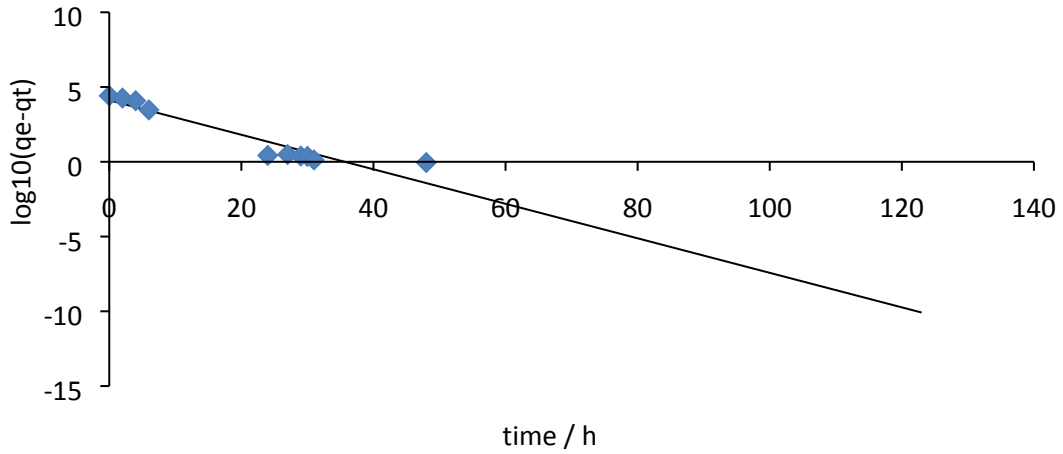

Figure S11. Pseudo-first-order kinetics for MSZ adsorption from solution onto DBS-hydrazide gel.

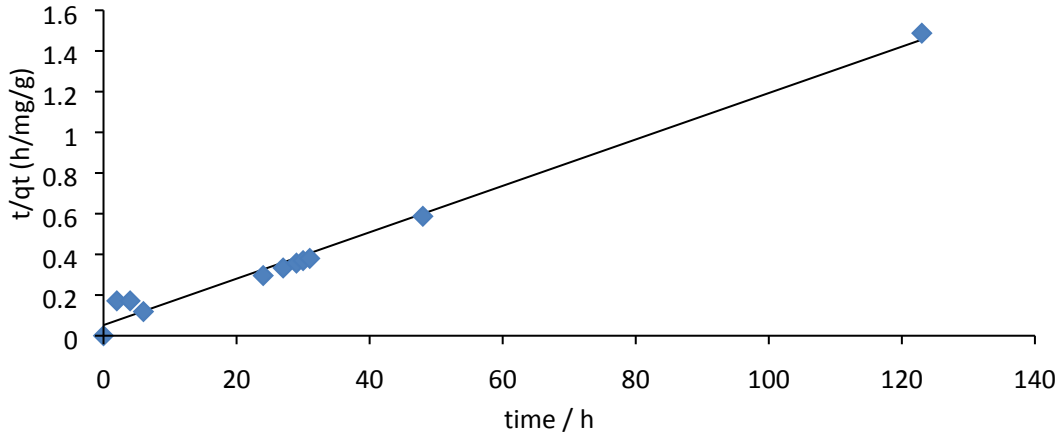

Figure S12. Pseudo-second-order kinetics for MSZ adsorption from solution onto DBS-hydrazide gel.

7 API Release Profiles

The gels used for API release were 6 mM DBS-$CONHNH_2$ (2.84 mg) and 6 mM Naproxen (1.38 mg) dissolved in water (1 ml). To these gels, 6 ml one of either (i) clean water at pH 7, (ii) water adjusted to pH 8 or (iii) phosphate buffer (2 mM) at pH 8 (2 mM), was added to the top of the gel. The gel was placed in an incubator at 37^{o}C for the duration of the experiment. The UV-vis absorbance was measured for each of the supernatant samples over time. The concentration of Naproxen released was determined through the construction of calibration curves.

8 References

1 (*a*) M. Avrami, *J. Chem. Phys.,* 1939, **7**, 1103-1112; (*b*) M. Avrami, *J. Chem. Phys.,* 1940, **8**, 212. (*c*) M. Avrami, *J. Chem. Phys.,* 1941, **9**, 177-184. (*d*) X. Huang, P. Terech, S. R. Raghavan and R. G. Weiss, *J. Am. Chem. Soc.,* 2005, **127**, 4336-4344.

2 S. Lagergren, *Kung. Sven. Veten. Handl.* 1898, **24**, 1-39.

3 Y. S. Ho and G. McKay, *Can. J. Chem. Eng.*, 1998, **76**, 822-827.