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

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

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A simple supramolecular hydrogel based on 1,3:2,4-di(4-acylhydrazide)benzylidenesorbitol (DBS-CONHNH$_2$), is able to extract acid-functionalised anti-inflammatory drugs via directed interactions with the self-assembled gel nanofibres. Two-component hydrogel-drug hybrid materials can be easily formed by mixing and exhibit pH-controlled drug release.

Gels are fascinating soft materials with potential applications in drug delivery.[1] In recent years, self-assembled low molecular weight gels (LMWGs)[2] have begun to emerge as an exciting potential technology for this application, with considerable advantages over more traditionally-used polymer gels. LMWGs are of interest as a result of the ability to program desired functionality into the gelator using basic organic synthesis prior to assembly,[3] with the added advantages of low gelator loading (<1%) and *in situ* gelation.

Active pharmaceutical ingredients (APIs) can be formulated into LMWGs using one of two strategies: (*a*) covalent conjugation and (*b*) physical encapsulation.[4] Covalent conjugation of APIs is less relevant here, but has included attachment of a wide range of drugs for potential enzyme-mediated or hydrolytic release.[5] However, these prodrug gelators have to be developed *a priori* for each new application. Physical encapsulation of an API effectively forms a multi-component gel[6] – the drug may form direct interactions with the gelator, or be passively adsorbed within the gel matrix. Organogels based on pharmaceutically relevant oils can be used for API formulation, [7] for example Naproxen salts form organogels capable of controlled API release.[8] However, hydrogels offer potentially greater biocompatibility. Many studies have focussed on peptide derived gels.[9] From the earliest example reported by Vegners and co-workers,[10] through an influential study from van Esch and co-workers, peptide-acid gels were used to formulate amine-functionalised APIs – exhibiting a degree of controlled release.[11] Many literature reports have focussed on dyes or model drugs, but peptide-derived gels have also been used to release genuine APIs as varied as doxorubicin and vancomycin.[12] Other LMWGs based on different building blocks, including G-quartets,[13] imidazolium amphiphiles[14] and fatty-acid-modified disaccharides,[15] have been tested for drug delivery. In attempts to control API release, Kim and co-workers demonstrated controlled release of AZT from a biotinylated amino acid, with streptavidin modifying release rate,[16] while Yang and co-workers used a fatty-acid-modified amino acid hydrogel with the pH of the receiving medium having a small impact on salicylic acid release.[17] Other reports have controlled gelation using (e.g.) pH,[18] photoirradiation,[19] or crosslinking reactions[20] to trigger or inhibit drug release.

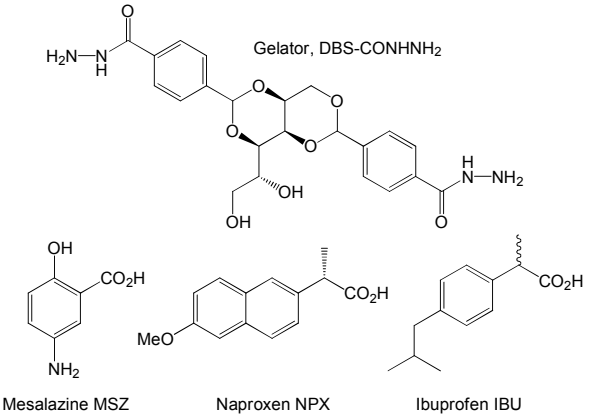

Figure 1. Structure of gelator (DBS-CONHNH$_2$) and APIs (MSZ, NPX and IBU).

Recently, we have worked with gelators based on the 1,3:2,4-dibenzylidene sorbitol (DBS) framework.[21] This family of gelators is of particular interest, as DBS is widely used in the cosmetics industry and considered to have an acceptable toxicity and environmental profile. A recent patent uses DBS to formulate pseudoephedrine, preventing easy extraction of the API for use in the synthesis of illicit drugs.[22] However, DBS generally only gels organic solvents. Importantly, in recent work, we modified the aromatic 'wings' of DBS with carboxylic acid[23] or acyl hydrazide[24] functional groups to generate hydrogels – significantly expanding

the scope of this family of gelators. Gels formed by 1,3:2,4-di(4-acylhydrazide)benzylidene sorbitol (DBS-CONHNH$_2$, Fig. 1) were tolerant to a wide pH range (2-11.5) and could adsorb large amounts of pollutant dyes.[24] As such, we became interested in using this gelator for API formulation. For this preliminary study, we selected anti-inflammatory drugs – 5-amino-salicylic acid (mesalazine, MSZ), ibuprofen (IBU), and naproxen (NPX) (Fig. 1). Enhanced formulation of such drugs is of great value. For example, NPX has recently been reported to cause significant problems such as ulceration in the stomach.[25] Furthermore, MSZ has been formulated into gels to enable intestinal release and better treat intestinal inflammation, avoiding adverse effects on the stomach.[26]

We synthesised DBS-CONHNH$_2$ using our previously disclosed method.[24] Initial API loading experiments were then carried out with MSZ. We exposed a pre-formed DBS-CONHNH$_2$ hydrogel to a supernatant solution of MSZ. Diffusion of the API allowed simple gel loading, which was followed using UV-Vis spectroscopy. MSZ was insufficiently soluble in pure water, but dissolved in acetate buffer (pH 4.65) – on standing the gel under this solution, MSZ was removed (Fig. 2). There was relatively rapid adsorption of MSZ into the gel, which was complete in under 24 hours. Interestingly, ca. 1 molar equivalent was adsorbed (an excellent API/gelator loading of 0.32 g/g), and indicating 1:1 DBS-CONHNH$_2$:MSZ interactions. Treatment of the adsorption data indicated pseudo-second order adsorption kinetics, as commonly observed for this type of process (see ESI).[27] After loading MSZ, the T_{gel} value of DBS-CONHNH$_2$ was depressed by ca 20ºC. We propose that interactions of gel nanofibres with MSZ cause this, possibly because the complex is slightly more soluble that DBS-CONHNH$_2$ alone (see below).

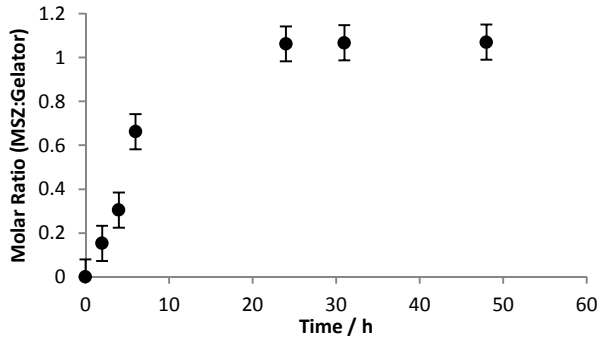

Figure 2. Uptake of MSZ from solution into DBS-CONHNH$_2$ hydrogel.

Although this study demonstrated the ability to load a simple API into our gel, this formulation method could not be extended to our other selected APIs, as their solubility in water was too poor – indeed the logP values of MSZ, NPX and IBU are 1.2,[28] 3.34[29] and 3.50[29] respectively, reflecting increased hydrophobicity as is true for many APIs. We therefore developed an alternative formulation approach by mixing API and gelator as solids, and forming gels from the two-component mixture. An equimolar mixture of API and gelator were sonicated in water for 15 min, and a heat-cool cycle led to gelation for each of MSZ, IBU and NPX. Interestingly, the APIs did not precipitate under these conditions, suggesting they are being incorporated into the nanostructure. However, if the gelator:API molar ratio was changed to 1:2, then the (excess) API did precipitate out of the hydrosol, supporting the view that there is a stoichiometric interaction between acid-functionalised APIs and this gelator. The loading possible with these systems is in the range of 0.32-0.49 g/g (API/gelator), depending on the molar mass of the API – significantly higher than many literature values. The presence of an equimolar amount of API enhanced thermal stability (T_{gel}) in the case of NPX and IBU (from 92ºC to >100ºC) but depressed thermal stability in the case of MSZ (from 92ºC to 79ºC). We propose that the higher logP values of the former drugs encourage network aggregation.

We then performed rheological studies with a parallel plate geometry on gels of DBS-CONHNH$_2$ (10 mM) formed in the absence of presence of APIs (also 10 mM) (Table 1 and ESI). The presence of API has some impact on the storage and loss moduli. Notably, these moduli increased in the presence of IBU and NPX, and decreased in the presence of MSZ. This is in general agreement with the observed T_{gel} values, and reflects the view that the more hydrophobic drugs (IBU and NPX) reinforce the 'solid like' nature of these materials, while the more hydrophilic drug (MSZ) decreases the ability of DBS-CONHNH$_2$ to form a solid-like network.

Table 1. Rheological Performance of DBS-CONHNH$_2$ (10 mM) hydrogels in the presence and absence of APIs (10 mM).

API	Max G' (kPa)	Max G'' (kPa)
None	14.5	2.6
MSZ	10.2	1.8
IBU	19.6	4.1
NPX	33.0	5.6

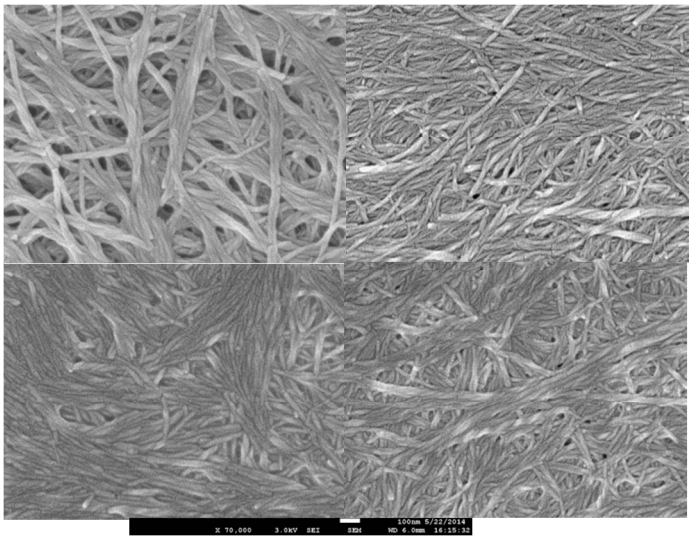

Figure 3. SEM images of dried xerogels formed by DBS-CONHNH$_2$ in the presence of: (top left) no additive, (top right) IBU, (bottom left) MSZ, (bottom right) NPX. [Scale bar; 100 nm]

We then studied these API-gel hybrids using scanning electron microscopy (SEM, Fig. 3). Although this technique images dried xerogels rather than solvated systems, it is effective for comparability studies in closely related systems. The xerogel of DBS-CONHNH$_2$ exhibited extended and intertwined bundles of fibres having a diameter of *ca.* 43 nm. In the presence of all APIs, nanoscale fibres were once again observed, demonstrating that the

API does not inhibit gel assembly. Furthermore, no microcrystals (which may have been associated with unincorporated API) were observed, supportive of specific interactions between APIs and gelator fibres. In the presence of APIs the diameters of the fibre bundles are *ca.* 36 nm, 29 nm and 29 nm respectively for the hydrogels with IBU, NPX and MSZ. It would therefore appear that the interaction of the API with the nanofibres somewhat limits the ability of the fibres to bundle.

Infra-red (IR) spectroscopy is a powerful way of monitoring interactions within such materials. Interestingly, the presence of the drug molecules shifted the N-H stretch of DBS-CONHNH$_2$ from 3194 cm^{-1} to lower wavenumbers by up to 16 cm^{-1}. This is consistent with the formation of interactions between this functional group and APIs. The O-H peak of sorbitol shifted by ca. 20 cm^{-1} to longer wavelength, consistent with possible involvement of this hydrogen bonding group. The C=O peak was less affected (max shift 6 cm^{-1}) and the aromatic C=C stretches and the C-O stretches were completely unchanged, which would appear to rule out π–π or solvophobic interactions between the gel fibres and these APIs. As such, IR supports specific interactions between the polar NH$_2$/OH units of the gelator and the API carboxylic acid.

For NPX-loaded gels, the unique chromophore of the naphthalene, distinct from DBS-CONHNH$_2$, meant we carried out some additional spectroscopic experiments. A CD band associated with chiral gelator DBS-CONHNH$_2$ at ca. 275 nm only emerges on self-assembly – this band can be attributed to the stacking of the aromatic 'wings' of the gelator into a chiral nanostructure (see supp. info.). This broad CD band was shifted slightly in the presence of NPX but maintained its key features. This would suggest the view that the API does not significantly disrupt chiral stacking of the DBS molecules and binds to the periphery of the nanofibres. Interestingly, a signal associated with NPX was also observed – a positive band at ca 310 nm and a negative band at ca 335 nm, demonstrating that this API is homogeneously incorporated into the gel in such a way that its chiral signature can be visually observed.

By following the evolution of the DBS-CONHNH$_2$ CD band over time, it is possible to gain insight into the kinetics of gel fibre growth. This can be fitted to the Avrami model which offers insights into the dimensionality of fibre growth (see ESI for details).[30] For the gelator alone (at 2.5 mM), the Avrami coefficient was 1.18, whereas in the presence of NPX, it was 1.08. In both cases the gelation was fast and was effectively complete in < 5 miin. This would suggest that in both cases fibre formation is primarily one-dimensional, as supported by SEM imaging.

Fluorescence spectroscopy using an excitation wavelength of 272 nm, and comparing emission spectra of DBS-CONHNH$_2$ in the absence and presence of NPX was informative (Fig. 4). For the gelator alone, there was an emission band at 340 nm. For NPX, an emission band at 360 nm was observed. For the combination of gelator and NPX, the gelator emission band at 340 nm disappeared, while the band at 360 nm associated with NPX increased in intensity. This effect was most marked at a gelator:API molar ratio of 1:1 (see ESI for full data). Although these experiments are performed at relatively high concentration, the results suggest energy transfer from gelator to NPX can take place within the gel, leading to loss of the gelator emission and enhancement of that of NPX. This can be mediated by close interactions between the chromophores of the individual components – consistent with a model in which NPX is bound in close proximity to the gelator aromatic rings.

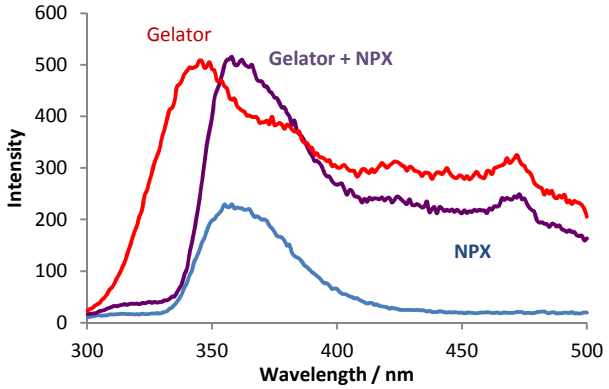

Figure 4. Emission spectra of a) DBS-CONHNH$_2$, b) NPX and c) equimolar mixture of DBS-CONHNH$_2$ and NPX.

The release of APIs was investigated, and NPX could easily be followed because of its longer wavelength UV-Vis absorption. At pH 7 and incubated at 37ºC, only 33% of the drug was released over 24 hours, whereas at pH 8 this rose to 100% (Fig. 5). We suggest that deprotonation of the API assists its release from the gel network under the conditions of higher pH. Using pH-adjusted water at pH 8, rather than buffered water slowed the release rate, explained by API release effectively lowering the pH of the receiving solution in this case. The release of MSZ and IBU could not be followed in this assay owing to inconsistent release profiles and difficulties with calibration/solubility. We suggest that the lower solubility of IBU limits release in this assay – although we anticipate release of this API in the presence of apolar biomembranes would still be possible.

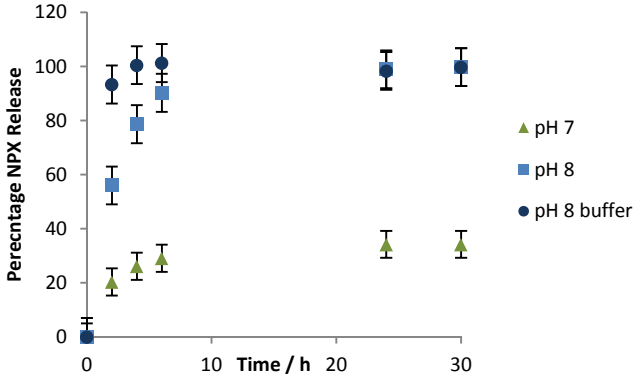

Figure 5. Release profiles of NPX from DBS-CONHNH$_2$ hydrogel into a supernatant aqueous solution under different conditions of pH control.

These DBS-CONHNH$_2$ gels are therefore ideal for release of NPX at slightly elevated (e.g. intestinal) pH values. Given the recently disclosed adverse effects of NPX in the stomach,[25] this is a significant observation. However, it should be noted that in very low pH conditions (<pH 2) the gels became less stable – which may limit direct oral application. However, problems such as this can be solved by encapsulation within an enteric coated capsule. We would also suggest that co-formulation with a more stable gelator to form a hybrid system may help solve this problem.[31] These gels may also be

useful for slow-release delivery, with the gel forming *in situ* after (e.g.) subcutaneous injection. Importantly, DBS is widely applied in cosmetic products,[21] and nanoscale acyl hydrazides have also been demonstrated to have good biological compatibility,[32] which bodes well for the potential relevance of this technology.

In summary, this preliminary communication reports a simple gelation system which encapsulates anti-inflammatory APIs as a consequence of direct 1:1 API-gelator interactions in a novel and interesting two-component gelation system. The adsorption and release of the APIs is therefore not simply passive. Furthermore, the hybrid co-assembled systems exhibit strongly pH-controlled, bio-relevant release of NPX, with significantly greater levels of release at pH 8 (intestinal pH) than 7. Self-assembled gels are small-molecule drug-like systems which, in principle, have well-established routes to clinical application, and we propose formulation systems such as these have considerable potential.

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Notes and references

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Electronic Supplementary Information (ESI) available: Details of all assay methods, Tables of IR data, Rheology data, CD spectra, Avrami kinetic plots, fluorescence at different molar ratios. See DOI: 10.1039/c000000x/

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

Graphical Abstract:

A simple supramolecular hydrogel is able to extract acid-functionalised anti-inflammatory drugs via directed interactions with the self-assembled gel nanofibres and exhibits pH-controlled drug release.