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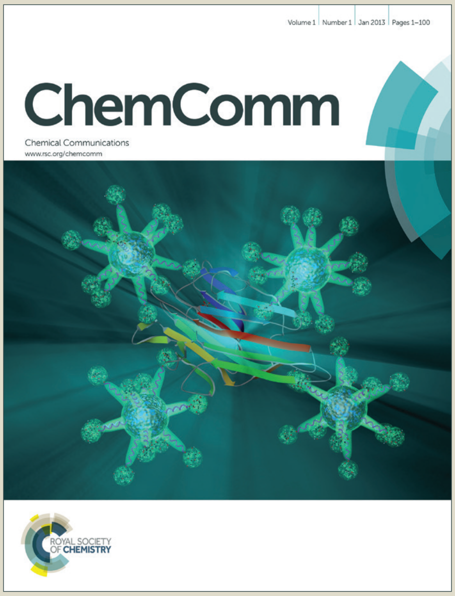

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ARTICLE TYPE

Mechanically Strong, Fluorescent Hydrogels from Zwitterionic, Fully π-Conjugated Polymers

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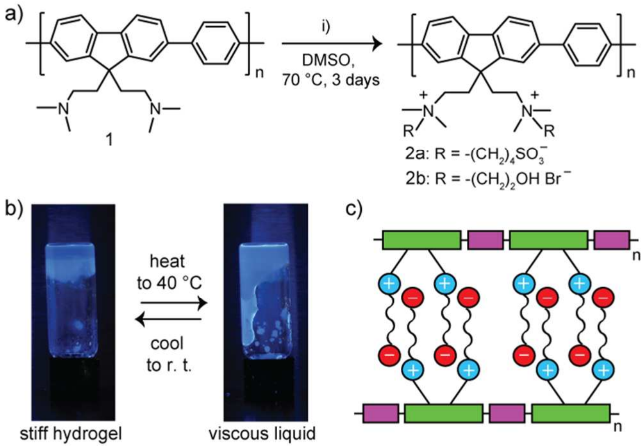
Mechanically strong supramolecular hydrogels (up to 98.9% water content) were obtained by the combination of a rigid, fully π-conjugated polymer backbone and zwitterionic side chains. The gels were characterized by SAXS, SEM and rheology measurements and are fluorescent, stimuli responsive (temperature, salts) and bind DNA.

Self-assembled, non-covalently cross-linked polymer hydrogels show dynamically reversible responses to external stimuli such as mechanical forces, temperature or ionic strength, and can have self-healing abilities.[1] Various reversible bonding interactions such as hydrogen-bonds, π-π-stacking and charge transfer interactions have been exploited for non-covalent polymer cross-linking,[1a-d,2] however, the mechanical performances of theses "honey-like" materials are often not ideal. Hydrogels with improved mechanical properties have been obtained through use of host-guest binding interactions,[1c,3] bundling into filaments of stiff polymers that mimick natural hydrogels from collagen or fibrin,[4] or Coulombic interactions.[5] For instance, mixing of poly-anions and poly-cations yields mechanically strong self-assembled materials,[5] although poly-cationic species are known to be toxic. Furthermore, multi-component gel preparation requires an accurately controlled mixing-ratio and can lead to undesired phase-separation.[1c,6] Single-component hydrogels from zwitterionic polymers are much more biocompatible, *e.g.* implanted zwitterionic gels did not cause a foreign-body reaction.[7]

Hydrogels that are assembled from conformational flexible components often show poor mechanical properties. This long-standing issue was recently addressed by the preparation of a composite material comprising of a "hard" inorganic component (an anionic clay) and "soft" organic component (a cationic dendrimer), which are held together by Coulombic interactions.[5a]



Scheme 1 a) Preparation of charged poly(fluorene phenylene) polymers from precursor **1**: For **2a**: (i) = 1,4-butane sultone, and for **2b**: (i) = 2-bromoethan-1-ol was used. b) "Inverted-vial-test" of the zwitterionic π-conjugated hydrogel **2a** at 10.0 wt% loading in water. The gel formation is thermally reversible. Photos were taken under UV illumination. c) Proposed inter-chain binding mechanism for zwitterionic polymers with a rigid polymer backbone and conformationally flexible side chains. See also Fig. SI9† for a 3D representation.

In this contribution, we present the formation of a mechanically strong hydrogel from a *single* polymeric component that combines a rigid, fully π-conjugated polymer backbone ("hard") and conformationally flexible zwitterion-carrying side chains ("soft"). Specifically, a fully π-conjugated fluorene-phenylene structure was selected as the rigid polymer spine and functionalized with flexible, R-Me$_2$N$^+$-butyl-SO$_3^-$ zwitterionic side-chains that were expected to act as solubilisers and non-covalent cross-linkers (Scheme 1).[8] In addition to zwitterionic polymer **2a**, a fully cationic polymer **2b** was also prepared from the common precursor **1** (Scheme 1a), ensuring comparable absolute MWs (~100 kDa by GPC, see Fig. SI1†) and polydispersities of both polymeric materials. The comparison of **2a** and **2b** allows for the direct analysis of the charge-influence on the materials properties, while both polymers are fully soluble in water. Visual inspection ("inverted-vial test") gave a first indication that zwitterionic polymer **2a** forms a hydrogel above 1.1 wt% polymer loading (Scheme 1b and Fig. SI2†), whereas fully cationic polymer **2b** does not, even at 10 wt%.

Small-angle x-ray scattering (SAXS) and scanning electron mi-

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croscopy (SEM) experiments were carried out in order to obtain structural information on the hydrogel material. SAXS analysis of a 1.1 wt% hydrogel of **2a** displayed a scattering pattern that exhibits a gradient very close to –2 (double-logarithmic representation) over most of the accessible q-range (Fig. SI3†). This behaviour suggests a sheet-like structure of the hydrogel in its hydrated state.[9] SEM measurements were carried out on freeze-dried samples, *i.e.* aerogels. The SEM images depicted in Fig. 1a revealed that the 100 kDa zwitterionic polymer **2a** forms a uniform, ordered and porous network with a 3D sheet-like structure. This observation is consistent with the SAXS pattern. A 60 kDa version of **2a** was also prepared from a lower MW precursor **1** in order to investigate the effect of the polymer length on the materials properties. Interestingly, SEM images of the 60 kDa zwitterionic polymer **2a** showed a very different morphology as compared to the 100 kDa variant (Fig. SI4†). The apparently "melted" structure indicates that the 3D network of the lower MW polymer is of much lower strength. These microscopic differences can be expected to cause a different macroscopic mechanical behaviour of the materials. This was probed by rheology measurements, as will be discussed in the next paragraphs.

A (hydro)gel can be regarded as a cross-linked polymer network for which the storage oscillatory shear modulus (G') dominates over the loss oscillatory shear modulus (G''), *i.e.*, $\tan(\delta) \leq G''/G'$.[3c,10] Indeed, frequency-dependent rheological experiments of hydrogel **2a** performed in the linear viscoelastic range displayed G' values that were larger than those of G'' across the whole frequency for all polymer loadings tested (Fig. 1b). These hydrogels are elastic ($\tan(\delta) \approx 0.30 - 0.60$), even at a very high water content (98.9 wt%). Increasing the polymer loading from 1.1 to 10.0 wt% raises G' and G'' by three orders of magnitude, *i.e.* control over the polymer loading provides a simple route for tuning of the mechanical properties of the hydrogel (Fig. SI5†). Noteworthy, the zwitterionic hydrogels show an impressive mechanical robustness, *e.g.* G' ≥ 2 kPa at ≥2.5 wt% polymer loading, which compares favourably to the strongest self-assembled hydrogels.[1c,3c] Moreover, the breakdown of the supramolecular structure occurs only with strain amplitudes of 2 - 4% (Fig. 1b), further demonstrating that zwitterionic polymer **2a** assembles into a stable non-covalently cross-linked network.

The lower MW zwitterionic polymer **2a** forms a less mechanically strong hydrogel material. The G' and G'' values, *i.e.*, the elasticity and the hardness, of the hydrogel **2a** were reduced by more than two orders of magnitude for the 60 kDa as compared to the 100 kDa polymer component (Fig. SI6†), corroborating the SEM results. Rheological experiments were also employed to assess the thermo-responsiveness of the hydrogel: A decrease by about four orders of magnitude in G', G'' and complex viscosity was observed upon increasing the temperature from 30 °C and 40 °C (Fig. SI7†), which was also consistent with the change in the visual appearance (Scheme 1b).

The emissive property provided by the π-conjugated polymer backbone of **2a** was utilized to characterize the sol-gel process. An approximately twofold increase in the emission intensity of zwitterionic polymer **2a** was observed within a narrow temperature range (30 °C to 40 °C), Fig. 1c, when cycling the temperature from 0 °C to 60 °C, which may be explained by the reduction of self-quenching effects upon spatial separation of the polymer chains

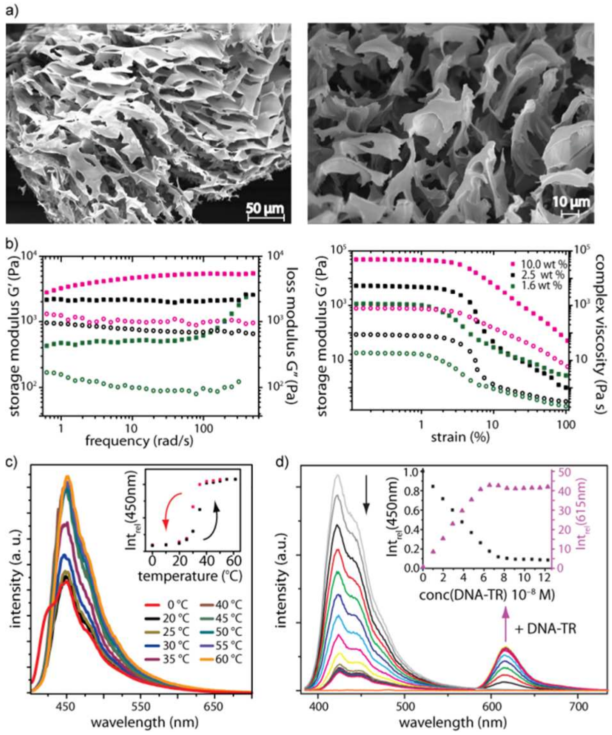

Fig. 1: a) SEM images of **2a** (100 kDa) aerogel, obtained by lyophilisation and cryo-drying of **2a** hydrogel at 2.5 wt% polymer loading. b) Oscillatory rheological analysis of **2a** zwitterionic hydrogels at different loadings (10.0 - 1.5 wt%) at 20 °C. Left: Frequency sweep preformed at 1% strain Right: Strain-amplitude sweep preformed at 10 rad/s. c) Emission spectra of hydrogel **2a** (2.5 wt%) at different temperatures. The insert plots the relative emission intensity during the heating (black squares) and cooling (red squares) cycle. d) Emission spectra (λ_{exc} = 380 nm) of **2a** in solution (1 µM) upon addition of ds-DNA tagged with Texas Red (DNA-TR, 0 to 130 nM). The insert shows the relative emission intensity at 420 nm (pF8 emission) and 615 nm (TR emission). The emission spectra of ds-DNA-TR alone (10 µM) is displayed as an orange line. DNA binding studies with **2a** as a hydrogel are show in the ESI.

The gel-sol conversion is reversible and the supramolecular hydrogel reforms upon cooling to room temperature because the inter-chain interactions are dynamic and non-covalent. In fact, a small hysteresis effect is noticeable; the gel-sol transition point is at 35 °C, the re-solidification temperature point is at 30 °C.

Additional photophysical characterizations of **2a** (100 kDa) in its hydrogel and solution state were carried out for probing the cross-linking mechanism, in particular ionic bonding and π-π-stacking interactions. According to literature studies, π-π-aggregated polyfluorene polymers typically display red-shifted and broadened absorption bands, additional high-wavelength emission bands and longer emission lifetimes.[26,27] We have not observed such effects for our materials - only a slight broadening of the absorption band around 388 nm could be noticed in the gel-state (Fig. SI8†). The absence of these effects, and the fact that only **2a** and not the structurally related cationic polymer **2b** forms a hydrogel, suggests that the non-covalent cross-linking is primarily mediated by the zwitterionic side chains and not by π-π-aggregation. A final piece of evidence for an ionic cross-linking mechanism is the destruction of hydrogel **2a** upon addition of salts such as sodium chloride or silver nitrate.

A simple model that takes the molecular features and the experimental observations into account, can rationalize the hydrogelation propensity of polymer **2a**. Firstly, as shown pictorially in Scheme 1c and Fig. SI9†, all cationic and anionic moieties on the side chains can take part in *inter*-chain ionic-bonding through a zipper-like arrangement. Conversely, on a single polymer chain *intra*-chain ionic bonding is less effective, because full back-folding of the *n*-butyl-SO_3^- moiety towards the ammonium group is entropically unfavourable, and the rigidity of the polymer backbone prevents single polymer-chain collapse, which in flexible polymers can provide a feasible pathway for charge stabilization. Secondly, the side-chains on a fluorene-type polymers are extending perpendicular to plane of the main chain, such that each single polymer can interact through the zwitterions-zipper with two neighbouring polymers simultaneously (Fig. SI9†). This can naturally lead to an extended sheet-like structure. Crude MMFF force field energy optimisation of aggregated oligomers confirmed the plausibility of this simplified binding model.

In addition to the crucial rigidity of the conjugated polymer backbone of **2a**, its emissive properties can also be of use for potential sensing applications. Conjugated polymers were frequently exploited in chemosensors, such as for the detection herbicide paraquat (viologen) and for DNA.[7c,11] While many DNA-chemosensors are highly positively charged, also zwitterionic polymer are effective.[11d] Indeed, **2a** interacts strongly with DNA, both in solution (Fig. 1d) and as a hydrogel (Fig. SI10†). The conclusive piece of evidence is the appearance of large Förster energy transfer (FRET) effects upon addition of a dye-labeled double stranded (ds)DNA oligonucleotide fragments (28 base pairs) to a solution, or to a hydrogel of zwitterionic polymer **2a**. The observation that a zwitterionic polymer, and the fact that zwitterionic polymer **2a** effectively self-aggregates and hydrogelates, may be of further use for the design of alternative DNA transfection systems.

"Smart" hydrogels that exhibit a function, for instance in electrostimulated drug release and biosensing are attracting considerable interest.[5d,12] For instance, (semi)conducting π-conjugated materials such as polyanilines and polythiophenes have been "gelated" as composites, blends or chemically cross-linked materials,[12b,13] but less laborious and better batch-to-batch reproducible supramolecular assembly strategies facilitate their fabrication.[5d,e] Our hydrogelation approach, *i.e.* the combination of a rigid π-conjugated polymer backbones and conformationally flexible zwitterionic side chains, appears directly applicable to these (semi)conducting polymers, which could provide a simple and reliable synthetic methodology for smart hydrogels.

Conclusions

In conclusion, combination of a rigid, fully π-conjugated polymer backbone and zwitterionic polymer side-chains provides a novel design strategy for the formation of mechanically strong, self-assembled hydrogels. The zwitterionic π-conjugated poly(fluorene phenylene) polymer could be readily synthesized in two high-yielding steps, followed by a facile processing step which results in a 3D-sheet-like hydrogel structure with a very high water content of up to 98.9 wt%. The zwitterionic hydrogel was found to be mechanically strong and elastic. The mechanical properties can be tuned *via* a variety of parameters such as the polymer loading, polymer chain length, temperature and the ionic strength. The supramolecular hydrogel formation is thermally reversible, displaying a sharp gel-sol transition at approximately 35 °C, which may be exploitable in biological systems. It was also demonstrated that this zwitterionic polymer, both in solution and as a hydrogel, binds DNA.

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