Converting drugs into gelators: supramolecular hydrogels from *N*-acetyl-L-cysteine and coinage-metal salts†

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Here we present the concept of *metallophilic hydrogels*, supramolecular systems in which the gelator species are metal-thiolates that self-assemble through metallophilic attractions. The principle is applied for a small drug, the mucolytic agent *N*-acetyl-L-cysteine (NAC), which readily forms hydrogels in the presence of Au(III), Ag(I) and Cu(II) salts. The resulting transparent hydrogels present pH induced sol/gel transition. Scanning electron microscopy (SEM) measurements reveal a microporous structure in form of flakes for the three of them. The low pH at which these hydrogels are formed (pH < 4) limits their direct use as drug-delivery systems, but still this system constitutes a novel method for easy and fast conversion of small drugs into potent hydrogelators. Future developments will help to fully develop the idea in order to create a new class of supramolecular drug-delivery systems.

Introduction

Supramolecular gels,[1,2] and particularly supramolecular hydrogels,[1,3] have recently become a hot topic in materials chemistry. This is mainly due to their great potential for the creation of stimuli responsive systems for diverse applications, ranging from optoelectronics and light harvesting to regenerative medicine and drug delivery.[4]

The discovery of small molecules capable of gelating water has been produced in great part by serendipity and, to a lesser extent, by design. In terms of design, and despite the wide variety of structures, most of the low molecular weight hydrogelators reported so far have the common feature that they are composed of both hydrophilic and hydrophobic units. In this context, a wide variety of hydrogelator structures based on natural products such as amino acids, sugars, nucleosides, nucleotides or bile acids has been described.[1]

It results obvious that supramolecular hydrogels formed by bioactive molecules such as drugs, attract a lot of interest due to their potential applications in tissue engineering, biosensing or drug delivery. Recently, Xu and co-workers have introduced a new kind of biomaterials through the transformation of several therapeutic agents into analogues that form hydrogels, without compromising their pharmacological efficacy.[5] In another work, Bhuniya *et al.* have also synthesised supramolecular hydrogelators based on an anti-inflammatory drug.[6] The idea is to transform a drug into a hydrogelator and use the resulting hydrogel to deliver the drug itself to the target site, for example. However, in most of the cases the therapeutic agent needs to be chemically modified with a suitable group or small fragment in order to undergo self-assembly in water, and thus, gelation. This means having to make quite an effort in structural design and synthesis. We wanted to go further and explore a straightforward modification that could transform a small drug into a hydrogelator. In this communication we show how a thiol-containing drug can be easily converted in an efficient hydrogelator by the simple addition of a coinage-metal (or group 11) salt.

We have shown in a previous work that glutathione, a naturally occurring tripeptide, can be converted into a hydrogelator by the addition of Au^{III}, Ag^{I} and Cu^{II} salts.[7,8] Following the same principle, a non-natural low molecular weight thiol has also been used for the formation of a similar hydrogel.[9] In these systems, the gelation process takes place *via* the self-assembly of the resulting metal(I) thiolate species, forming supramolecular polymers in which the monomers are stabilised by the so called *metallophilic attractions*.[10,11] The existence of such polymeric species is known since decades, although their structure is not still fully understood. Having this in mind, such systems can be envisaged as a special class of metallo-supramolecular gels[12] in which the gelator molecules are noble-metal thiolates hold together by metallophilic forces, and could therefore be designated as "metallophilic hydrogels".

The use of gold(I) salts in medicine, called "chrysotherapy", is known since decades and it has been applied for the treatment of rheumatoid arthritis, cancer, HIV, bronchial asthma or malaria.[13] This was the reason that took us to start investigating the use of gold-sulfur chemistry for the conversion of drugs into hydrogelators. Silver and copper thiolates were also studied, due to their analogous affinity towards sulfur and their similar tendency to form "metallophilic polymers". Here we apply the method for converting NAC, a well established and non-toxic drug, into a hydrogelator. NAC is frequently used as a mucolitic agent or as an antidote in paracetamol intoxication,[14] and recently it has shown good results for the treatment of hepatitis B[15] and chronic hepatitis C.[16]

Experimental

Materials

N-Acetyl-L-cysteine (99%), $AgNO_3$ (99.9%), $HAuCl_4 \cdot 3H_2O$ (99.9%) and $CuCl_2 \cdot 2H_2O$ (99.0%) were purchased from Sigma-Aldrich and used as received.

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† Electronic supplementary information (ESI) available: EDS, FT-IR, ^{1}H NMR, TGA and UV-vis spectra are available. See DOI: 10.1039/c0ob00311e

Measurements

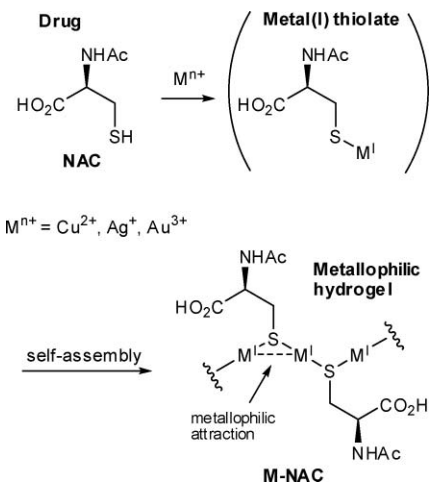
Proton nuclear magnetic resonance (^{1}H NMR) experiments were performed on a Bruker AVANCE III spectrometer at 500 MHz. All samples were measured at room temperature in D_2O. Fourier-transform infra-red (FT-IR) spectra were taken from a Nicolet Avatar 360 apparatus. All samples were measured in KBr disks. SEM micrographs were recorded on a JEOL JSM5610-LV apparatus. The specimen were prepared by placing a frozen sample of the hydrogel in the sputter coating chamber at high vacuum for 1 h, after which a thin layer of gold was applied. Energy dispersive X-ray spectroscopy (EDS) analyses were performed on an INCA-300 model from OXFORD, operated at an accelerating voltage of 20 kV. Ultraviolet–visible (UV-vis) spectroscopy experiments were performed on a Jasco V-570 spectrophotometer. Samples were measured at three different pH (3, 5 and 7). Thermo-gravimetric analyses (TGA) were performed on a Q500 apparatus from TA Instruments. The experiments were run under nitrogen, from room-temperature to 1000 °C at a rate of 10 °C min^{-1}.

Synthesis of hydrogels

For a typical preparation of a ~50 mM hydrogel, a solution of NAC (0.1 mmol) in water (1 mL) was added dropwise into a solution of the corresponding M^{n+} salt (0.1 mmol) in water (1 mL). This resulted in the formation of opaque and non-consistent hydrogels, which were converted into strong and transparent hydrogels by adding NaOH 1 N until complete dissolution, and then allowing the slow diffusion of acetic acid in a closed chamber.[17]

Results and discussion

The preparation of the hydrogels‡ is illustrated on Scheme 1. NAC reacts with $HAuCl_4$, $AgNO_3$ or $CuCl_2$ in water to give the corresponding metal(I) thiolate. Such thiolates spontaneously self-assemble to form polymeric species, this resulting in the formation of the corresponding hydrogel. The **Au-NAC**§ and



Scheme 1 Formation of NAC-based metallophilic hydrogels.

‡ The exact stoichiometry and reaction pathway for each metal is fully described in ref. 8 and references cited therein.

§ The preparation and some physical properties of this complex have been reported elsewhere.[22]

Ag-NAC hydrogels were stable an could be stored at room temperature for months. The **Cu-NAC** derivative, on the other hand, was very unstable and decomposed within hours acquiring a blue coloration, making difficult its characterisation and handling. Such instability can be attributed to the oxidation of Cu(I) to Cu(II) in air, as suggested by the blue color.

Hydrogels were purified by dialysis against pure water, and freeze-dried for characterization (see ESI†). The three xerogels gave very similar FT-IR spectra, with the absence of the S–H stretching band as the main characteristic feature. EDS semiquantitative elemental analyses of the dry compounds were consistent with a 1 : 1 metal : thiol ratio in all cases. TGA of the xerogels were also in good accordance with a M(I)–$SCH_2CH(CO_2H)NHAc$ molecular formula. The residues left after decomposition of all the organics (54.6% for **Au-NAC**, 38.5% for **Ag-NAC** and 31.0% for **Cu-NAC**) were comparable to the theoretical metal-content of the samples (51.7, 36.9 and 28.2%, respectively). The ^{1}H NMR spectra showed splitting and broadening of the NAC signals, what could be interpreted as an indication of the aggregation of thiolate species to form oligomeric species.

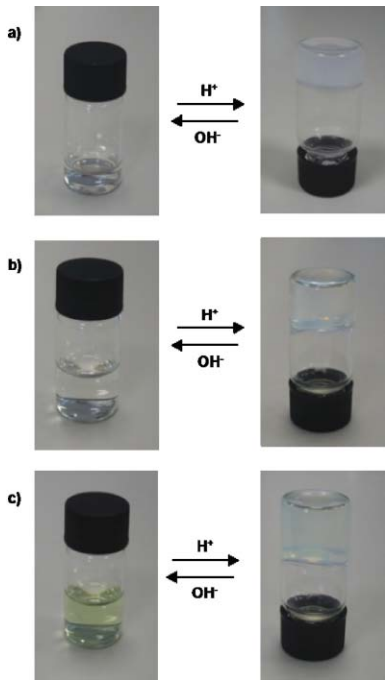
Good quality hydrogels were obtained by slowly diffusing acetic acid into a neutral aqueous solution of **M-NAC**, following a method described elsewhere.[17] The minimum gelator concentration for these compounds was ~20 mM, which is remarkably low for such a small molecule (this molecule represents, to the best of our knowledge, one of the smallest supramolecular hydrogelator reported so far). It is worth noticing that this compound does not possess a hydrophobic part, in contrast to the majority of low molecular weight hydrogelators, as the self-assembly occurs through the thiolate group.

These hydrogels presented pH responsiveness, what could be attributed to the presence of a free carboxylic group. Above pH 4 the hydrogels dissolved to give clear solutions, as the carboxylic groups are expected to be negatively charged. The gels were restored again upon the addition of an acid (Scheme 2).

The **Ag-NAC** and **Cu-NAC** hydrogels showed thermoresponsiveness, in line with the majority of supramolecular gels, although the latter one was quite unstable and its temperature-driven phase transition was difficult to reproduce. Thus, a typical 40 mM **Ag-NAC** hydrogel showed a phase-transition temperature of ~65 °C. Interestingly, the **Au-NAC** hydrogel presented a higher thermal stability, and could be heated up to the boiling point of water with no phase transition or apparent decomposition observed. A possible explanation could be given by the fact that aurophilic attractions (Au^I–Au^I) are energetically stronger compared to the argentophilic (Ag^I–Ag^I) or the cuprophilic (Cu^I–Cu^I) ones.[10]

The SEM micrographs of the xerogels are shown in Fig. 1. They all present a similar porous structure in the form of flakes,[18] very similar to that observed for M(I)–glutathione hydrogels.[7,8] It is interesting to notice that this structure differs considerably from that obtained for a non-peptidic argentophilic hydrogel, which showed a 1D microfibrilar network.[9]

Fig. 2 shows the UV/vis absorption spectra of **Au-NAC** at different pH values. An absorption band at ~320 nm (in form of shoulder) was observed at pH 3. This band could be assigned to the $d_{\sigma^*} \rightarrow p_\sigma$ transition observed by others for Au(I)-glutathione thiolates,[19] this being an indication of metal-metal interaction. The intensity of this band diminished at higher pH values, what



Scheme 2 Photographs of hydrogels **Au-NAC** (a), **Ag-NAC** (b) and **Cu-NAC** (c) showing pH-induced phase transition.

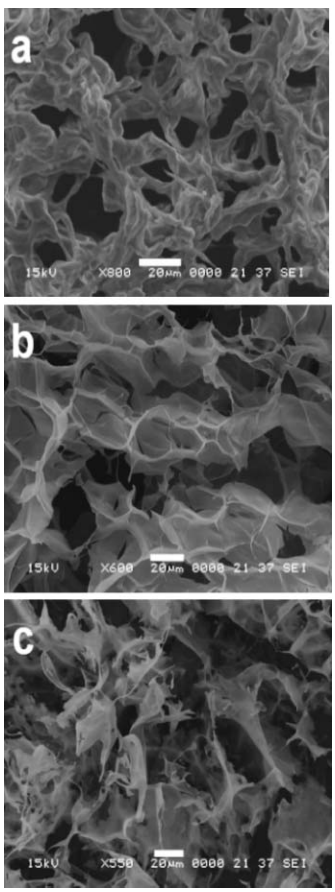


Fig. 1 SEM micrographs of xerogels **Au-NAC** (a), **Ag-NAC** (b) and **Cu-NAC** (c). Scale bar corresponds to 20 μm.

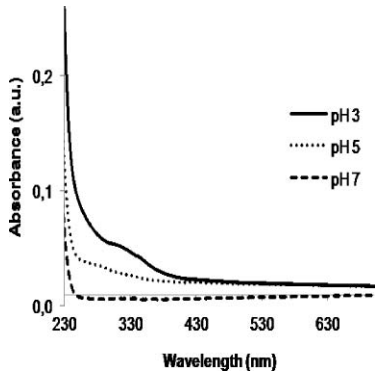


Fig. 2 UV/vis absorption spectra of **Au-NAC** at different pH values.

suggests that the Au(I)–Au(I) interactions become weaker. This is in agreement with a disaggregation of the gelator molecules and thus, the phase transition observed at higher pH. **Ag-NAC** and **Cu-NAC** spectra did not show any significant absorbance band (see ESI†). It is worth noticing that no absorbance related to surface plasmon resonance[20] was observed in any of the cases, meaning that monolayer-protected metal nanoparticles were not formed under these experimental conditions, as expected.

Conclusions

The idea of making a hydrogel with a drug itself acting as the gelator is now emerging as an alternative for the development of new generation drug-delivery systems. However, if that is going to occur we need to design practical methods to convert such drugs into gelators, avoiding their synthetic modifications to the maximum. Here we have shown a very straightforward method to achieve that, by taking advantage of the self-assembling tendency of copper(I), silver(I), and gold(I) thiolates. We have introduced the concept of "metallophilic hydrogel", and applied it for the fabrication of hydrogels from a small drug, *N*-acetyl-L-cysteine. Taking into account the extensive use of gold(I) thiolates as therapeutic agents for more than fifty years,[13] "aurophilic hydrogels" could be envisaged as potential candidates for the medical field.

Although *in vivo* experiments would be needed in order to study the drug-releasing ability of the system, it could be expected that the thiols that are present in the organism (glutathione and other cysteine-containing peptides) would displace the drug from the metal(I) ions by means of thiol-thiolate exchange,[21] resulting in a slow release of the drug.

The main practical limitation of the hydrogel described here is that the system gels at pH < 4, which can be undesirable for certain biological applications. Efforts are currently being focused towards the design of metallophilic hydrogels capable of forming gels at physiological pH.

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