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Coinage Metal–Glutathione Thiolates as a New Class of Supramolecular Hydrogelators

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Summary: Coinage metal salts (e.g. Au^{III}, Ag^{I} and Cu^{II} salts) readily react with thiols to give the corresponding metal(I) thiolates. These thiolate species are known to either self-assemble into insoluble polymeric species or to form soluble oligomers, depending on the nature of the thiol ligand. Here we demonstrate that this self-assembling ability can be applied to create supramolecular hydrogels. Glutathione (GSH), a naturally occurring tripeptide, has been used in combination with Au^{III}, Ag^{I} and Cu^{II} salts to obtain pH responsive hydrogels that are able to gelate 100 times their weight in water.

Keywords: coinage metals; glutathione; hydrogels; self-assembly; thiolates

Introduction

Supramolecular hydrogels[1] are a rapidly expanding area of research, in particular due to their possible practical applications in biomedicine such as tissue engineering or controlled drug release. Within this context, the most important advantages compared to conventional polymeric gels are a better biodegradability and the possibility of incorporating a drug or a bioactive molecule directly into the gelling component, without needing to capture it.[2]

We have previously reported a brief communication describing gold–glutathione (**GS–Au**) supramolecular hydrogels.[3] The method describes a new concept for binding together low molecular weight hydrogelator molecules, based on the self-assembling potential of gold(I) thiolates through *aurophilic attractions*.[4] Scheme 1 illustrates the proposed structure of such hydrogels, in which gold(I)–glutathione species self-assemble into polymeric 2D sheets.[5] The hydrophilic glutathione chains lye perpendicularly to both sides of the plane and water molecules are trapped in between the 2D layers, which results in the formation of a hydrogel.

Now we would like to present an extension of the method for the preparation of silver–glutathione (**GS–Ag**) and copper–glutathione (**GS–Cu**) supramolecular hydrogels. Silver(I) and copper(I) thiolates[5] have been reported to self-assemble in a similar manner to gold, forming polymeric structures stabilized by *metallophilic attractions*. Here we demonstrate that in addition to Au^{III} salts, glutathione can also be combined with Ag^{I} and Cu^{II} salts for the fabrication of pH responsive supramolecular hydrogels.

Experimental Part

Preparation of Hydrogels (1% w/w)

GS–Au: $HAuCl_4 \cdot 3H_2O$ (99 mg, 0.25 mmol) was dissolved in H_2O (6 mL) and added to a solution of glutathione (230 mg, 0.75 mmol) in H_2O (6 mL). After stirring for a few seconds a colorless solution was formed, and the pH dropped to 1.6. Then NaOH 1N (0.5 mL, 0.5 mmol) was added and a practically transparent gel formed immediately.

GS–Ag: $AgNO_3$ (42 mg, 0.25 mmol) was dissolved in H_2O (5 mL) and added to a

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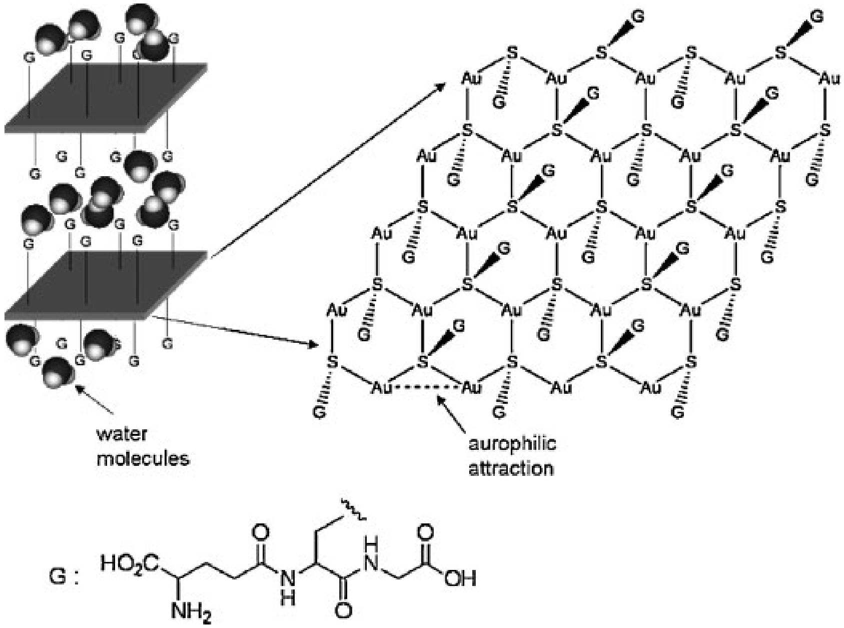


Scheme 1.
Schematic representation of the structure of **GS–Au** supramolecular hydrogels.

solution of glutathione (76 mg, 0.25 mmol) in H_2O (5 mL). A white suspension was formed immediately, as the pH dropped to 1.51. Then NaOH 1N (0.5 mL, 0.5 mmol) was added and a transparent solution was obtained. Finally, the addition of HNO_3 1N (0.13 mL, 0.13 mmol) gave a transparent hydrogel.

GS–Cu: $CuCl_2 \cdot 2H_2O$ (43 mg, 0.25 mmol) was dissolved in H_2O (5 mL) and added to a solution of glutathione (153 mg, 0.50 mmol) in H_2O (5 mL). After stirring for a few seconds a colorless solution was formed, and the pH dropped to 1.48. Then NaOH 1N (0.5 mL, 0.5 mmol) was added and a transparent gel formed immediately. The gel must be kept under an inert atmosphere in order to avoid oxidation and evolution of a blue color.

For **IR**, **SEM** and **EDS** analysis **GS–Au** and **GS–Cu** hydrogels were washed to eliminate the GS–SG released in the reaction. The gels were diluted in HCl 10^{-3} N (pH 3) and the resulting suspensions were then centrifuged and washed several times with HCl 10^{-3} N. The products were freeze-dried to give white solids (**GS–Ag** was freeze-dried without washing). The solids can be redissolved in water, and will form gels at the appropriate pH.

Results and Discussion

Au^{III}, Ag^{I} and Cu^{II} salts readily react with glutathione to give the corresponding metal(I) thiolate. The simplest reaction mechanism is for silver, as its salt is already in oxidation state +1 and does not need to be reduced. Thus, the reaction could be expressed as follows:

$$AgNO_3 + GSH \rightarrow GS-Ag + HNO_3 \quad (1)$$

In the case of gold(III) and copper(II) the metal needs first to be reduced to Au^{I} and Cu^{I}, respectively. The excess of GSH used acts as the reducing agent, and it is oxidized to GS–SG. Subsequently another equivalent of GSH reacts with the metal in oxidation state +1 to give the corresponding thiolate. Overall, $HAuCl_4$ reacts with 3 molecules of GSH to give 1 molecule of **GS–Au**, 1 molecule of GS–SG and 4 HCl

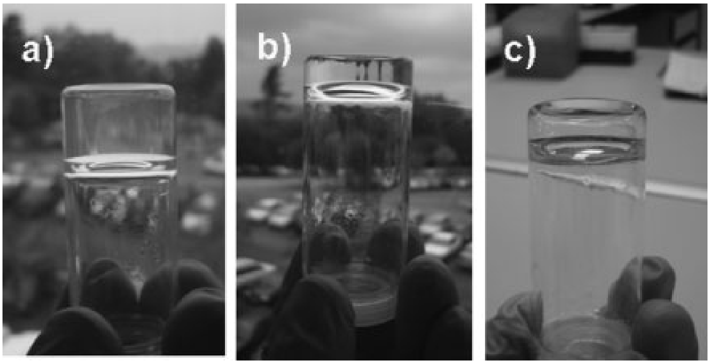


Figure 1.
Photographs of upside down turned **GS–Au** (a), **GS–Ag** (b) and **GS–Cu** (c) hydrogels (1% w/w).

molecules (Equation 2). Similarly, each $CuCl_2$ molecule needs to react with 2 molecules of GSH to give **GS–Cu**, half an equivalent of GS–SG and 2 molecules of HCl (Equation 3).

$$HAuCl_4 + 3\,GSH \rightarrow GS{-}Au + GS{-}SG + 4\,HCl \qquad (2)$$

$$CuCl_2 + 2\,GSH \rightarrow GS{-}Cu + 1/2\,GS{-}SG + 2\,HCl \qquad (3)$$

The proposed mechanism is supported by the following evidence: **GS–SG** is the mayor species present in the reaction supernatant,[6] and during the preparation of the hydrogels, the pH drops drastically indicating the release of acid.

These metal(I) thiolate species are able to gelate 100 times their weight in water. The resulting hydrogels are practically transparent and colorless, as shown in Figure 1. The gels are stable for months

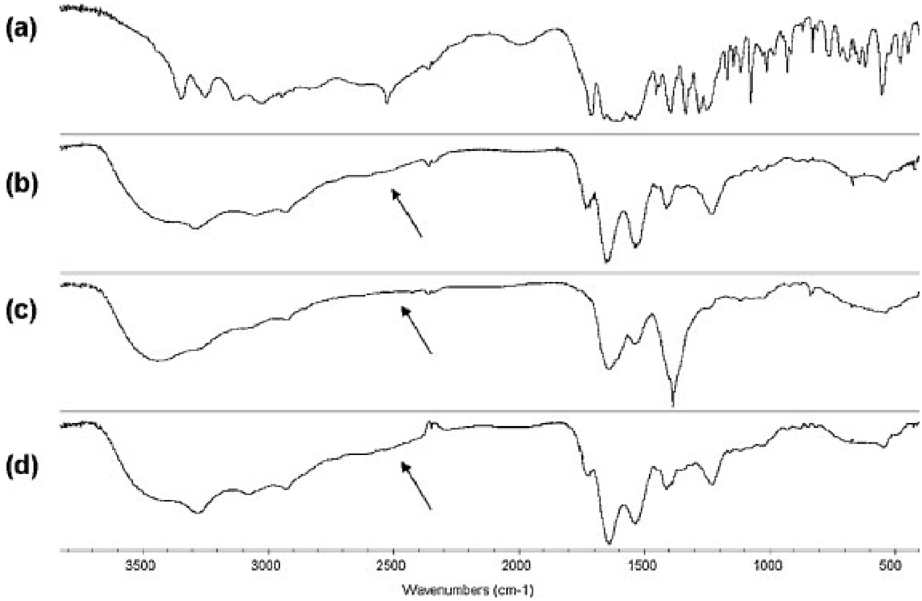


Figure 2.
IR spectra of free glutathione (a) and freeze-dried **GS–Au** (b), **GS–Ag** (c) and **GS–Cu** (d). The arrows indicate the absence of the S–H stretching band.

Table 1.
EDS elemental analyses of freeze-dried hydrogels. All results are given in atomic percent.

Sample	% C	% N	% O	% Na	% S	% Au	% Ag	% Cu	% Total
GS–Au 1	56.33	10.10	18.28	–	8.47	6.82	–	–	100.00
GS–Au 2	57.77	10.20	17.98	–	7.56	6.49	–	–	100.00
GS–Au 3	55.20	9.58	20.22	–	8.39	6.60	–	–	100.00
GS–Ag 1	8.04	14.85	53.94	19.95	1.55	–	1.68	–	100.00
GS–Ag 2	9.35	13.75	53.61	19.22	2.06	–	2.02	–	100.00
GS–Ag 3	4.18	18.03	55.26	20.63	1.01	–	0.89	–	100.00
GS–Cu 1	62.57	4.08	18.73	–	7.16	–	–	7.45	100.00
GS–Cu 2	63.06	4.84	17.75	–	6.83	–	–	7.51	100.00
GS–Cu 3	60.48	4.06	18.54	–	8.12	–	–	8.81	100.00

at room temperature, with the exception of **GS–Cu**, which decomposes after several days to give a blue solution (Cu^{II}). However this decomposition is considerably slowed down if the gel is kept under an inert atmosphere and protected from light.

The samples can be freeze-dried to give white porous solids. If water is added a gel is formed again. IR spectra of freeze-dried gold, silver and copper hydrogels are shown in Figure 2, together with the spectrum of free glutathione. The S–H stretching band at 2525 cm^{-1} is absent in the spectra of the metal thiolate derivatives. Interestingly, the supramolecular polymers show a much simpler spectrum than glutathione, which could be an indicative of a highly ordered structure.[7] The C=O and C–O stretching bands at 1724 and 1227 cm^{-1} respectively in the spectra of **GS–Au** and **GS–Cu**, which were freeze-dried at pH 3, indicate the presence of some protonated carboxylic acid. Those bands shift to 1630 and 1384 cm^{-1} in the spectrum of **GS–Ag**, freeze-dried at pH 5, indicating that at this pH all the carboxyl groups are deprotonated.

Table 1 shows the semiquantitative elemental analysis results obtained by energy dispersive x-ray spectroscopy (EDS) of the freeze-dried hydrogels. The results are given in atomic percent, and three different measurements are given for each of the hydrogels. In all cases the data is consistent with the proposed molecular formula, having a 1: 1 metal–thiol ratio. The presence of Na^+ in the case of **GS–Ag**

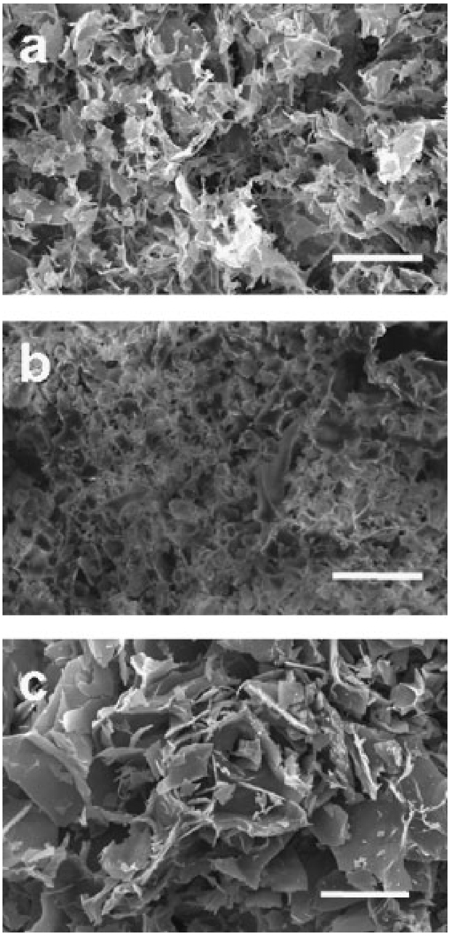
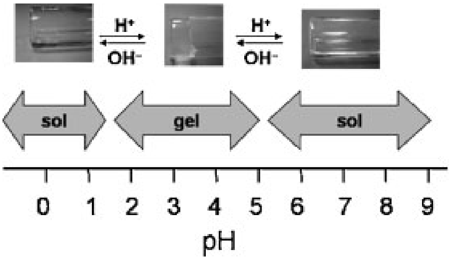


Figure 3.
SEM micrographs of freeze-dried **GS–Au** (a), **GS–Ag** (b) and **GS–Cu** (c) hydrogels. Scale bar corresponds to 50 μm.



Scheme 2.
pH-induced reversible sol-gel transition of coinage metal–glutathione supramolecular hydrogels.

arises from the $NaNO_3$ that results from the neutralization of NaOH with HNO_3; this hydrogel was not washed due to the fact that **GS–SG** is not formed in the process.

SEM images of the freeze-dried gels are shown in Figure 3. The porous microstructure appears to be similar for **GS–Au**, **GS–Ag** and **GS–Cu** derivatives. As a general rule, a porous network in the form of flakes is observed in all cases.

The gels do not present thermal reversibility, and can be heated up to $\sim$100 °C without observing phase transition. On the contrary, coinage metal-glutathione hydrogels demonstrate pH responsiveness (Scheme 2). The polymers behave as a gel in the pH 2–5 range. Above or below this pH range the systems behave as clear solutions, which can be reversibly converted into gels by the addition of acid or base, respectively. This sol-gel transition can be repeated many times with no loss of reversibility.

Conclusion

It has been shown that coinage metal salts and glutathione can be used for the construction of pH responsive supramolecular hydrogels. The novelty of the method relies on the use of metallophilic attractions as the driving key force to hold the hydrogelator molecules together, and the principle can be widely applied for the synthesis of designer hydrogels based on molecules other than glutathione. The principal requisite for a suitable hydrogelator molecule is the presence of at least one thiol group. The method offers the possibility of using thiol-containing drugs or bioactive molecules as the gelling agent, opening a new and interesting perspective for biomedical applications.

[1] M. de Loos, B. L. Feringa, J. H. van Esch, *Eur. J. Org. Chem.* **2005**, 3615–3631; L. A. Estroff, A. D. Hamilton, *Chem. Rev.* **2004**, *104*, 1201–1217; N. M. Sangeetha, U. Maitra, *Chem. Soc. Rev.* **2005**, *34*, 821–836.

[2] See for example Z. Yang, B. Xu, *J. Mater. Chem.* **2007**, *17*, 2385–2393.

[3] I. Odriozola, I. Loinaz, J. A. Pomposo, H. J. Grande, *J. Mater. Chem.* **2007**, DOI:10.1039/b713542d.

[4] The attraction between two or more nominally monovalent gold ions in compounds has been known in structural chemistry for a long time, and is called *aurophilic attraction*, or more generally, *metallophilic attraction*. See for example: P. Pyykko, *Angew. Chem. Int. Ed.* **2004**, *43*, 4412–4456.

[5] For a deeper understanding of these metal(I) thiolate structures see: F. Bensebaa, T. H. Ellis, E. Kruus, R. Voicu, Y. Zhou, *Can. J. Chem.* **1998**, *76*, 1654–1659; F. Bensebaa, T. H. Ellis, E. Kruus, R. Voicu, Y. Zhou, *Langmuir* **1998**, *14*, 6579–6587; I. G. Dance, K. J. Fisher, R. M. H. Banda, M. L. Scudder, *Inorg. Chem.* **1991**, *30*, 183–187; H. G. Fijolek, J. R. Grohal, J. L. Sample, M. J. Natan, *Inorg. Chem.* **1997**, *36*, 622–628; A. N. Parikh, S. D. Gillmor, J. D. Beers, K. M. Beardmore, R. W. Cutts, B. I. Swanson, *J. Phys. Chem. B* **1999**, *103*, 2850–2861; P. Espinet, M. C. Lequerica, J. M. Martin-Alvarez, *Chem. Eur. J.* **1999**, *5*, 1982–1986; L. M. Nguyen, M. E. Dellinger, J. T. Lee, R. A. Quinlan, A. L. Rheingold, R. D. Pike, *Inorg. Chim. Acta* **2005**, *358*, 1331–1336; N. Sandhyarani, T. Pradeep, *J. Mater. Chem.* **2001**, *11*, 1294–1299; W. Turbeville, N. Yap, *Catal. Today* **2006**, *116*, 519–525.

[6] The **GS-SG** can be removed by centrifuging and washing several times with HCl 10^{-3} N.

[7] In a way, these polymers can be envisaged as non-conventional polypeptides, for they are constituted of glutathione repeating units. The difference from conventional polypeptides is that here the short peptides are not linked by peptide-bonds, but they are linked by the side chain.