Chapter 5

Supramolecular Low Molecular Weight Hydrogelator Stabilization of SERS Active Aggregated Nanoparticles for Solution and Gas Sensing

Abstract
The potential of surface enhanced Raman scattering (SERS) spectroscopy in both laboratory and field analyses depends on the reliable formation of so called 'SERS hot spots', such as those formed during gold or silver nanoparticles aggregation. Unfortunately such aggregates are not stable in solution, since they typically grow until they precipitate. Here we describe the use of low molecular weight hydrogels formed through pH triggered self-assembly that occurs at a rate which matches well the rates of aggregation of Au or Ag colloids, allowing them to be trapped at the SERS-active point of the aggregation process. We show that the colloid-containing gels give SERS signals similar to the parent colloid but are stable over several months. Moreover, lyophilized gels can be stored as dry powders for subsequent use in the analyses of gases and dissolved analytes by contact with either solutions or vapours. The present system shows how the combination of pH switchable low molecular weight gelators and pH induced colloid aggregation can be combined to make a highly stable and low cost SERS platform for the detection of volatile organic compounds and microvolume analysis of solutions.

This chapter has been published as:
Introduction

Surface enhanced Raman scattering (SERS) is characterized by increased Raman scattering by molecules situated near to or on rough metal surfaces. Numerous materials have now been shown to give SERS enhancement, including sophisticated systems which, for example, attempt to create uniform plasmonic enhancements over large areas by controlling the nanostructure.\(^1\)\(^-\)\(^6\) However, for many practical applications aggregated metal nanoparticles continue to be of interest, both because of their low cost and simplicity and the large plasmonic enhancements they provide.\(^7\)\(^-\)\(^8\) Indeed the first single molecule SERS measurements used aggregated particles.\(^9\) It is now widely accepted that particle aggregation is necessary to create the so called “hot spots” of high local field intensities that are situated at the points where particles almost touch.\(^5\)\(^-\)\(^6\) Unfortunately, it is difficult to create stable aggregates of a given size and most experiments on aggregated colloids are therefore carried out in the time window where sufficient aggregation has occurred to give SERS enhancement but before the aggregates grow so large that they precipitate out of the suspension. This aspect has led to considerable work over the past decade aimed at controlling and stabilizing aggregates by trapping them within hydrogel hosts that act as particle scaffolds. The primary requirement is to trap the aggregates while still allowing access by the target molecules which must reach the surface to be enhanced. Many groups have focused on stabilizing aggregated particles in aqueous solutions by introducing (natural and synthetic) polymer based hydrogels.\(^10\)\(^-\)\(^16\) Stabilization of particles held within low molecular weight hydrogelators (LMWG) has been achieved through the use of gelators that interact strongly with aggregates, however, this approach resulted in enhancement of the Raman scattering of the gelator rather than the detection of added molecular targets.\(^17\)\(^-\)\(^18\)

In this contribution we demonstrate the use of a simple, biocompatible, pH switchable hydrogel, based on the self-assembly of a low molecular weight hydrogelator composed of a cyclohexane core decorated with three amino acid chains (Figure 1), as a new scaffold for colloidal SERS. Previous studies have shown that these gels are quite versatile, are tolerant to high concentrations of salts, are thermostable, non-toxic and environmentally benign.\(^19\)\(^-\)\(^21\) In principle, these LMWGs should be ideal scaffold materials since the gelation process can be switched, allowing the particle aggregation and trapping processes to be synchronized and controlled. Moreover, the gelators are expected to have relatively weak interactions with Ag or Au surfaces, so they should also allow analytes to access the particles. The model compounds used in this paper are thiophenol, chosen because it is a well-known SERS test material, which will allow ready comparison with other enhancing materials and aminothiophenol, because it is a solid with a low vapor pressure, which is useful for situations where evaporation/head space analysis is concerned. Aminothiophenol is used as a corresponding non-volatile analog.

![Figure 1. Structure of low molecular weight hydrogelators CH-Met and CH-Nle and a representation of trapped aggregated nanoparticles.](image-url)
Results and Discussion

In the present study thiophenol and aminothiophenol were selected as test analytes due to their chemical (both are aurophillic) and spectroscopic similarity and their volatile and non-volatile character, respectively. The Raman spectra of both compounds show a series of sharp, characteristic bands (Figure S1 and S2 at the end of the chapter), which are readily distinguishable from the Raman bands of other components, such as the LMWGs, citrate and inorganic anions employed in the present study.

Aggregation of Ag and Au colloid upon addition of inorganic acids resulted in a transient increase in SERS activity for aryl-thiols, which decreased concomitant with the subsequent precipitation of the colloid as expected. Under the present conditions, concentrations of ca. 10 µM gave strong signals for both thiophenol and aminothiophenol (Figure S3 and S4 at the end of the chapter) and hence this was used as the standard concentration throughout.

Addition of gold or silver colloid to solutions of either low molecular weight gelator (CH-Met or CH-Nle) at pH 10 did not result in significant changes to their colour (visible absorption) indicating that aggregation was not induced by the LMWGs. Addition of sufficient inorganic acid to the mixtures of colloid and LWMG to decrease the pH to 3 resulted in both the aggregation of the colloid (manifested in a change in colour and an increase in SERS scattering) concomitant with a dramatic increase in viscosity, indicating formation of a hydrogel. The Raman spectra of thiophenol and aminothiophenol obtained in the presence of the gelator are identical to those recorded with simple Ag and Au colloids aggregated by addition of acid to reduce the pH to 3 (Figure S5 and S6 at the end of the chapter). However, whereas the enhancement is lost as the particles settle in the absence of the LMWGs, the SERS spectrum obtained with the hydrogel present persists unchanged for at least several days.

Figure 2. UV-vis absorption spectra of (NaOH/HNO₃) aggregated colloid; (red) CH-Met (2 mg cm⁻³) hydrogel alone, (orange) gold colloid prior to precipitation, (light blue) colloid 10 s after addition of HNO₃ (to pH 3), and (dark blue) colloid held in hydrogel several minutes after addition of HNO₃. Spectra were recorded in 1 mm path length cuvettes positioned directly in front of the entrance to an integrating sphere to gather scattered as well as transmitted light. The NP concentration is estimated at 1.66*10¹² non-aggregated particles per 1 mL of gel.
Consistent with the SERS measurements, the absorption spectra of the Ag and Au colloids undergo a substantial red-shift and broadening upon a drop in pH to 3 (Figure 2 and Figure S7). The shifts are characteristic of the changes in surface plasmon resonance energy upon aggregation, and continue over time, ultimately leading to precipitation of the colloid. The opacity of the hydrogels due to scattering necessitated the use of an integrating sphere to record absorption spectra, however, similar initial changes to the visible spectrum upon aggregation (and gelation) were observed. Although the spectra of the silver colloid in the presence and absence of gelator are both broad, the spectra of the Au colloid shows a reduced extent of aggregation (less red-shift in surface plasmon resonance), which stops changing once the solution had undergone gelation, confirming that gelation inhibits further aggregation. The spectra of both Ag and Au colloids when trapped in the hydrogels did not undergo further change over several hours, whereas the spectra in the absence of the hydrogelators showed that the colloid underwent relatively rapid precipitation.

As reported earlier,19 the addition of salts to solutions containing the hydrogelators results in a substantial increase in the thermal stability of the hydrogels. The presence of the gold or silver colloids affected neither the melting temperature nor the rheological properties (G’ and G”) of the gels significantly (Figure S8), indicating a relatively weak interaction between the gel fibres and the colloidal particles. However, it should be noted that the concentration of gold nanoparticles is low (0.007 wt%), and hence any interaction between the gel fibres and gold nanoparticles is unlikely to impact substantially on the gels macroscopic properties. The thioether unit of the CH-Met gelator is unlikely to interact significantly with the gold nanoparticles, and indeed other related gelator structures such as CH-Nle gels show the same properties in terms of the SERS spectra obtained with silver and gold colloids and stability (Figure S9 and S10).

**Distribution of aggregated colloid in hydrogel matrices**

A key challenge in the application of SERS spectroscopy lies in quantitative analysis. In solution the time-averaged spectrum is essentially constant due to Brownian motion. In the gel state, the partially aggregated gold colloid is trapped spatially within the hydrogel fibre matrix and the strength of the SERS spectrum is dependent on the number of aggregated particles within the confocal volume. Hence, the spatial uniformity, which is dependent on the rate of gel fibre formation relative to the rate of colloid aggregation following the pH jump, will determine the reproducibility of SERS signal intensity. Mapping of Ag and Au colloid trapped within a hydrogel containing aminothiophenol (10 µM) in a 1 cm cuvette with 0.1 mm steps (over an area of 8 by 9 mm) was carried out and the absolute intensity of the band at 1550 cm⁻¹ used to generate heat plots (maps using other bands are essentially identical). The heat plot obtained with Ag colloid indicated that the spatial distribution was not uniform, especially in comparison to the heat plot obtained with the Au colloid, which indicates that aggregation of the Au colloid is slower and therefore suspended at an earlier stage than for Ag colloid (Figure 3). The average intensity is ca. 47 % (stdev 10%) and 69% (stdev 3.6%) of the maximum intensity for hydrogel stabilized Ag and Au colloids, respectively. These data are consistent with the absorption spectra of the colloids also (vide supra). The difference in uniformity of the hydrogel stabilized Ag and Au nanoparticles in the present case highlights a general challenge in using absolute intensity in quantitative work. The flexibility of the present system in terms of the acids used to the gel forming pH jump does offer the prospect of using the inorganic anions as internal reference signals which could correct for changes in focus or laser power, but of course for critical quantitative analysis a SERS-active internal standard is preferable since it could also correct for differences in the number average of Raman hotspots with the confocal volume.5 Furthermore, over long periods of laser excitation, local heating induces movement of the colloidal particles through the gel matrix and hence a minor drift in signal intensity over extended periods of irradiation (vide infra, Figure 4).
Detection of gases by hydrogel stabilized colloids through reversible gas uptake and release.

The open hydrogel scaffold provides for a sufficiently rigid matrix to prevent/limit convection and translation movement of the aggregated nanoparticles but simultaneously is a primarily aqueous state that allows diffusion of molecules partitioning from the head space. Their stability allows even relatively slow processes such as diffusion of gas into the matrix to be measured. The spatial distribution of the SERS spectrum obtained from a hydrogel stabilized gold colloid after saturation of the headspace above the gel with thiophenol gas was determined. The Raman bands of thiophenol increased in intensity steadily and eventually levelled off over a period of 5 h. Measurement of the spatial distribution of the Raman spectrum from 0 to 1 cm depth shows clearly the penetration depth of the thiophenol over this period. As expected for mass transfer by diffusion only, the signal is highest at the surface of the gel in contact with the gas and, after a certain depth, gradually decreases. Release of the gas from the cuvette when opened occurred slowly when uncapped but held overnight within a closed sampling compartment (ca. 35 L) and more quickly when the cuvette was placed in an air flow with eventual near complete loss of the signal of the analyte.

**Long term stability of SERS scaffolds**

The stability of gels containing colloids stored in sealed vials at ambient temperatures was apparent from the absence of changes in morphology (e.g., crystallization or the appearance of fluid) over at least a 3 month period. The SERS response to injection of thiophenol gas into the headspace above the gel was qualitatively similar in all cases (using the strong nitrate band as a pseudo-internal reference, Figure S11 and S12). However, for longer term storage lyophilization of the gel was explored as a means of preserving the stabilized aggregates in a dry form, which can be reconstituted before use, mixed directly with analyte solutions or used as dry powder for gas analysis.

Figure 3. Hydrogel containing aggregated (a) Ag and (b) Au colloid; the areas imaged by Raman spectroscopy are indicated by a red square. (c) SERS spectrum of aminothiophenol at 785 nm. Intensity maps (at 1550 cm⁻¹) for (d) Ag and (e) Au colloid containing hydrogels.
Figure 4. (a) Intensity of four Raman bands of thiophenol over time in a cuvette with 1 mL of CH-Met hydrogel containing aggregated gold colloid with a droplet of thiophenol placed in the headspace above the gel. After 5 h the cap and thiophenol droplet were removed, and after 21 and 22 h (†) the cuvette placed open in a fume hood for a few min and after 23 h left to stand in a fume hood for 1 h (‡). (b) Signal intensity as a function of depth into the hydrogel before removal of cap. (c) Raman intensity at 1550 cm⁻¹ within the hydrogel. *changes in intensity are due to repositioning cuvette.

SERS activity before and after reconstitution of lyophilized gels.
The hydrogels discussed in the present contribution have previously been shown¹⁹ to be stable upon lyophilization, so that subsequent reconstitution of the gel by addition of pure water followed by a heating/cooling cycle restores the gel’s original properties (e.g., rheology, melting point etc.). However, with the particle containing lyophilized gels, although the gel properties recovered fully, the blue colour of the colloid/gel mixture was lost upon heating, presumably due to increased aggregation when the gel structure was disrupted at high temperature. The SERS spectra obtained (after addition of thiophenol through exchange from the head space to the gel) from colloid containing
hydrogel reconstituted by heating and cooling, showed primarily bands due to SERS enhancement of the Raman scattering of citrate (Figure S13), present as a stabilizer of the gold colloid, in addition to that of the thiophenol. The pronounced surface enhancement, even after reconstitution by heating/cooling, albeit marginally weaker than for the original gel, together with the change in colour indicate that further aggregation of the colloid has occurred but not precipitation. However, the rapid heating cooling cycle is unlikely to be easily reproducible, from a quantitative perspective, which together with interference from enhancement of scattering from citrate, make this approach less useful. More significantly, however, addition of a drop of water containing the analyte (thiophenol) directly to the lyophilized (dried) gels resulted in the appearance of a strongly enhanced SERS signal (Figure 5).

Similarly, the spectra obtained from lyophilized hydrogels containing Ag and Au colloid upon addition of 100 µL of aqueous aminothiophenol (10 µM) are similar to those obtained with aggregated Au colloid alone (figure 6). Furthermore, the presence of water is not essential for SERS spectra to be obtained from the lyophilized gels, as demonstrated by the intense SERS spectrum obtained from a sample stored in a seal box in which the head space was saturated by thiophenol gas and subsequently removed to air for analysis (Figure 7). The ready uptake and retention of thiophenol by the lyophilized hydrogel via the head space resulting in a substantial SERS enhancement is unexpected but likely reflects the open porous structure of the hydrogel framework facilitating gas ingress. This property is important as it opens the opportunity to use this class of support for long term gas analysis by SERS since the colloid is locked in its partially aggregated state by the absence of solvent but is still accessible to gaseous as well as liquid analytes.

Figure 5. Raman spectrum of (ca. 1 µM) thiophenol in (black) CH-Met hydrogel with NaNO3 aggregated gold colloid (the glass background signal has been removed by scaled subtraction), (red) Raman spectrum obtained by addition of 10 ml of aqueous thiophenol (5 µM) placed on top of a lyophilized gold colloid containing hydrogel powder, and (blue) reconstituted hydrogel with thiophenol (ca. 1 µM) * SERS bands of citrate. (spectra are obtained at lexc 785 nm, with 4x 5, 10 and 10 s acquisitions, respectively).
Conclusion

The organic gelators shown here are excellent as scaffolds for nanoparticle aggregates since the pH switching that induces supramolecular aggregation and thereby gelation also induces particle aggregation concomitantly. The rate of particle aggregation is on a similar timescale as gel fibre formation and hence the colloid is trapped in the aggregated state but precipitation is prevented. The hydrogel scaffolds may interact with the colloid through its carboxylic acid groups in the same manner as citrate stabilizes gold colloids, however, the similar behaviour of the CH-Met and CH-Nle hydrogelators indicate that the sulphur unit in the former is not involved. Importantly the hydrogel gives a low SERS response and hence interference with the spectra of analytes is minimized. The stability of the hydrogel colloids to lyophilization and its open structure are important in the analysis of volatile

Figure 6. Raman spectra of a 100 µL droplet of water contain (10 µM) aminothiophenol placed on a lyophilized gel containing (a) Au colloid, (b) Ag colloid and (c) Raman spectrum of (10 µM) aminothiophenol obtained in aggregated gold colloid with HNO$_3$.

Figure 7. SERS spectrum at 632.8 nm of Au colloid stabilized in a lyophilized CH-Met hydrogel which has been exposed to thiophenol vapour.
target molecules. For the materials in the hydrogel state the ability of the target molecules to access the enhancing surface is not unexpected since the aggregates are held within an open hydrogel fibre network (with 0.1 wt% of the structure comprising of the gel fibres) and hence although convection is precluded, diffusion is unaffected. More importantly for practical purposes, the accessibility of the surface is retained even after the gels have been lyophilized and hence have a substantially long lifetime. Rehydration with analyte containing solution brings the analyte molecules directly in contact with the released particles, allowing SERS detection. Finally, the ability to take up analytes from the headspace, reversibly, in the hydrogel and the detection using a lyophilized powder opens up many opportunities for application in long term real-time air analysis.

Supporting information
The Supporting Information containing the preparation of gold and silver colloids, TEM images, SERS and Raman spectra, and UV−vis spectra, is available free of charge on the ACS Publications website at DOI: 10.1021/acs.langmuir.7b01445.

Acknowledgements
We would like to thank prof. Steven Bell and dr. Wendy Lee for their help with SERS spectra.

Bibliography


Supporting Figures

Figure S1. (a) Raman spectrum of a neat solution of thiophenol. SERS spectra of 10 µM thiophenol (b) in the absence of colloid, (c) in Ag colloid and (d) in Au colloid.

Figure S2. (a) Raman spectrum of solid aminothiophenol. SERS spectra of 10 µM aminothiophenol (b) in the absence of colloid, (c) in Ag colloid and (d) in Au colloid.
**Figure S3.** SERS spectra in Au colloid of thiophenol (concentration in µM). Spectra were normalized to the NO$_3^-$ band at 1075 cm$^{-1}$.

**Figure S4.** SERS spectra in Au colloid of aminothiophenol (concentration in mM), normalized to the NO$_3^-$ band at 1075 cm$^{-1}$. 
Figure S5. Raman spectra of 10 µM of thiophenol in CH-Met hydrogel with (a) silver and (b) gold colloid, (c) without colloid.

Figure S6. Raman spectra of 10 µM of aminothiophenol in CH-Met hydrogel with (a) silver and (b) gold colloid, (c) without colloid.
**Figure S7.** UV-vis absorption spectra of (NaOH/HNO₃) aggregated colloid; (black) CH-Met (2 mg/mL) hydrogel alone, (blue) silver colloid prior to precipitation, (cyan) colloid 10 s after addition of HNO₃ (to pH 3), and (red) colloid held in hydrogel several minutes after addition of HNO₃. Spectra were recorded in 1 mm path length cuvettes positioned directly in front of the entrance to an integrating sphere to gather scattered as well as transmitted light.

**Figure S8.** Rheology of CH-Met hydrogel formed by pH switching with (black) and without (red) Au colloid.
Figure S9. Raman spectra of CH-Nle (2 mg/mL, NaOH, HNO₃) hydrogel with gold colloid (a) 10 µM aminothiophenol, (b) 10 µM thiophenol, and (c) no additive.

Figure S10. Raman spectra of CH-Nle (2 mg/mL, NaOH, HNO₃) hydrogel with silver colloid (a) 10 µM aminothiophenol, (b) 10 µM thiophenol, and (c) no additive. Note that the Raman bands of citrate are more pronounced than in the case of gold colloid.
**Figure S11.** Raman spectra obtained from CH-Met gel (NaOH, HNO₃) hydrogel stabilized Au colloid (left to stand in a sealed vial for 4 months) (black) before and (red) after exposure to thiophenol vapour.

**Figure S12.** Raman spectra obtained from CH-Met gel (NaOH, HNO₃) hydrogel stabilized Ag colloid (left to stand in a sealed vial for 4 months) (black) before and (red) after exposure to thiophenol vapour.
Figure S13. Raman spectra (785 nm) of (black) citrate heated in water and added to Au colloid followed by aggregation with NaNO$_3$, (red) hydrogel stabilized Au colloid following reconstitution by heating and cooling and addition of thiophenol, and (blue) lyophilized hydrogel stabilized Au colloid after addition of 10 μL of aqueous thiophenol. In all cases the concentration of thiophenol is ca. 5 μM.