Towards In-Field Determination of Nitrate Concentrations via Diffusive Gradients in Thin-Films – Incorporation of Reductants and Colour Reagents.

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Supporting Information

AuNP-rGO Synthesis

The synthesis and functionalisation of graphene oxide (GO) commonly involves the treatment of pristine graphite with strong oxidants, producing functionally similar but structurally dissimilar graphite oxide sheets. Exfoliation by sonication in the presence of water formed a colloidal suspension of graphene oxide – which was further functionalised with chitosan and Au to produce the reduced GO supported Au nanoparticles (AuNP-rGO).

Graphite oxide was prepared using a modified Hummers method, as in Amanulla et al. (2017). A mixture of 1 g of NaNO₃ and 50 mL H₂SO₄ (AR grade), to which 2 g of natural graphite powder (≤44 µm) was added and stirred at 0 °C. 12 g of KMnO₄ was slowly added and the solution was continuously stirred for a further 2 hours, and then heated to 35 ± 5 °C for 30 minutes. Water (150 mL) was added slowly, and the solution heated to 90 °C under vigorous stirring for 15 minutes. 120 mL of 30 % H₂O₂ was added to the suspension until the colour became yellow. The prepared solid sample was allowed to settle and the decanted, before being washed with 5% HCl, and at least three times with MQ water, following sample settling. The graphene oxide suspension (50 mL, 1 mg mL⁻¹) was formed via the sonication (Soltec Sonica Ultrasonic Cleaner) of 50 mg graphite oxide in 50 mL 18.2 MΩ water for minutes. 30 minute sonication times have previously been reported to almost completely (97-99 %) form GO sheets of smaller than 1 µm.

Chitosan stabilised gold nanoparticles decorated reduced graphene oxide (AuNP-rGO) were also prepared as in Amanulla et al. Briefly, 0.5 g of low molecular weight chitosan (150 kDa) was added to 30 mL of deionised water and stirred, HCl (0.6 mol L⁻¹) was added dropwise until the chitosan was dissolved. Graphene oxide suspension (20 mL of 2.5 mg mL⁻¹) was added and the mixture stirred for 30 minutes, to which 0.1 mol L⁻¹ succinic acid and 50 mL of 1 mmol L⁻¹ HAuCl₄ were added simultaneously, and then heated at 60 °C whilst stirring under reflux until the colour turned wine red. NaOH (0.1 mol L⁻¹) was added 1 mL at a time to aid in the formation of the AuNP-chitosan. The gold nanoparticle/graphene oxide composite was oven dried at 60 °C.

The AuNP-rGO hydrogels were prepared using the hydrogel formation in particle suspension method. Briefly, 0.5g of the synthesised AuNP-rGO was added to the agarose/acrylamide gel solution (10 mL), and vigorously stirred. 70 µL of 10 % ammonium persulfate and 25 µL of tetramethylethylene diamine were added to the solution whilst stirring. The solution was quickly pipetted between glass plates separated by 0.4 mm inert spacers, carefully to ensure there were no air bubbles. Polymerisation occurred at 40 °C in an oven for approximately 100 minutes, or until polymerisation was complete.

The prepared AuNP-rGO hydrogels were washed 4 times in 18.2 MΩ water over 24 hours, to remove unreacted hydrogel reagents. The hydrogels were stored in 18.2 MΩ water at 4 °C.
**AuNP-rGO**

![Image](image_url)

Figure S1: (A) 5 g L\(^{-1}\) AuNP-rGO reacted with a range of nitrite masses (0 – 145 µg), (B) UV-vis adsorption of 5 g L\(^{-1}\) AuNP-rGO reacted with nitrite (peak at 586 nm), and (C) the red, green and blue (RGB) intensities versus the nitrite mass.

**Stability of AuNPs**

![Image](image_url)

Figure S2: Stability of 3 g L\(^{-1}\) AuNP-chitosan suspensions determined by UV-vis at 523 and 683 nm, and the 683:523 nm ratio compared to the initial 3 g L\(^{-1}\) AuNP-chitosan suspensions. Determined as the ratio of these components for one-week after initial analysis.

**Analysis of AuNPs**

The formation of the AuNP-rGO and AuNP-chitosan composites were confirmed via FTIR and XRD (Supplementary Figure 1). The broad and intense -OH stretch at 3380 cm\(^{-1}\), strong C=O stretching at 1734 cm\(^{-1}\), C=C conjugated ketone stretching at 1628 cm\(^{-1}\), C-O phenol stretch at 1228 cm\(^{-1}\), and C-O primary alcohol stretch at 1058 cm\(^{-1}\), present in sample 2 and not 1 (Supplementary Figure 1), are indicative of the formation graphite oxide from pure graphite \(^5\).

The rGO spectra is a combination of the chitosan and graphite oxide spectra \(^5\). The loss of the carboxylic acid C=O stretch at 1734 cm\(^{-1}\) of the graphite oxide upon reaction with chitosan, and the
presence of HN-CO stretching vibrations at 1648 cm\(^{-1}\), support the formation of amide linkages between the NH and COOH groups of the chitosan and graphene oxide (Figure 4.2, and Supplementary Figure 1). The intensity of the interaction of -OH groups of the graphene oxide and chitosan resulted in a more intense C-O primary alcohol stretch at 1093 cm\(^{-1}\) for the rGO, compared to the stretch at 1058 cm\(^{-1}\) for the graphite oxide. The chitosan strong C-H stretch at 2880 cm\(^{-1}\) was also present in the rGO, and persisted in the AuNP-chitosan and AuNP-rGO. The AuNP-chitosan and AuNP-rGO spectra were largely the same, and the intensity was diminished in comparison to the parent materials.

XRD analysis further confirmed the formation of the AuNP composites (Supplementary Figure 1C) and precursors (Supplementary Figure 1). A very strong peak at 9.8° and weak peak at 20° in the graphite oxide replaced the peaks present in the pure graphite. Chitosan provided a weak peak at 10.6° and a strong peak at 19.9°. The reduced graphene oxide peaks were a combination of the graphite oxide and chitosan peaks (9.9°, and 19.8° with shoulder at 21.8°).

AuNP-rGO peaks at 38.0°, 44.3°, 64.5° and 77.5°, and AuNP-chitosan peaks at 38.2°, 44.4°, 64.7° and 77.4° (Supplementary Figure 1C) confirm the reduction of Au(III) to Au(0). They are indicative of the (111), (200), (220) and (311) planes of face centred cubic Au. The peaks at around 27°, 32°, 54°, 57°, 66°, 73° and 75° correspond to NaCl\(^+\). The rGO peaks in the AuNP-rGO composite were significantly reduced in intensity. The chitosan peaks in the AuNP-chitosan composite largely disappeared (10.6°), and the peak at 19.9° broadened and flattened from 17° to 25.6°.

Figure S3: (A) Fourier-transform infrared chromatography, and (B) and (C) XRD: graphite (1), graphite oxide (2), rGO (3), chitosan (4), AuNP-chitosan (5) and AuNP-rGO (6). The spectra in (A) and (C) were artificially separated so the components were easily discernible, the relative transmittances and intensities were unchanged.
SEM imaging and elemental analysis (Supplementary Figure 3) further confirmed the formation of the AuNP-chitosan and AuNP-rGO composites. The two-dimensional graphene oxide and irregular spherical chitosan were present in the AuNP-rGO (Supplementary Figure 3A and C). The irregular spherical/globular chitosan structure was clearly visible in the AuNP-chitosan (Supplementary Figure 3B). Elemental analysis (Supplementary Figure 3E-F) of the bright areas in the back scattering images confirmed the presence of gold. It was considerably more difficult to find and analyse gold in the AuNP-chitosan composite, requiring much greater magnification, because the gold particles were much smaller than in the AuNP-rGO. This size difference resulted in different optical properties, due to the different surface plasmon resonance.

Figure S4: SEM images of AuNP-rGO (A and C), and AuNP-chitosan (B and D). Elemental analysis of the marked spots of the AuNP-rGO (E) and AuNP-chitosan (F). Example AuNPs circled in (D).
Fe p(AMA/AMPS) Hydrogel

Iron impregnated hydrogels were successfully synthesised based on the method developed by Sahiner et al. (2010). The light areas in Supplementary Figure 4 are the Fe particles suspended in and on the p(AMPS/AMA) hydrogel. Iron particles on the surface of the hydrogel are significantly larger than those within the polymeric network. The Fe particles are smaller in the hydrogels after reduction to Fe(0) and oxidation to Fe(III) by reaction with nitrate. The wrinkles in Supplementary Figures 4B and D are likely due to the rapid drying and shrinking of the hydrogels.

Figure S5: SEM images of iron impregnated p(AMA/AMPS) hydrogels. (A) and (C): hydrogels before iron reduction with NaBH₄. (B) and (D): hydrogels after reduction of nitrate to nitrite. (E) Elemental analysis of the site marked by the arrow in D.
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