

Fluorescence Electrochemical Microscopy: Capping Agent Effects with Ethidium Bromide/DNA Capped Silver Nanoparticles

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Abstract

Fluorescence microscopy and electrochemistry were employed to examine capping agent dynamics in silver nanoparticles capped with DNA intercalated with ethidium bromide, a fluorescent molecule. The capped NPs were studied first electrochemically, demonstrating that the intercalation of the capping agent promotes oxidation of the silver core, occurring at 0.50 V (vs Ag, compared with 1.15 V for Ag NPs capped in DNA alone). Second, fluorescence electrochemical microscopy revealed that the electron transfer from the nanoparticles is gated by the capping agent, allowing dynamic insights unobservable using electrochemistry alone.

Main text

Metallic nanoparticles (NPs) are used in a variety of contexts, including in materials and energy conversion,[1] consumer products,[2, 3] and more recently, medical applications.[4] A capping agent is critical to stabilise or functionalise the nanoparticle,[5, 6] and can affect a range of properties, including reactivity,[7] biocompatibility and toxicity,[8–10] catalytic activity,[11, 12] and susceptibility to electron transfer.[13] However, investigation into the electronic effects of capping agents are still relatively unexplored, with initial studies suggesting the capping agent plays an important role in electron transfer.[13–16] Although electrochemistry is a powerful technique, combining it with non-electrochemical methods can provide previously inaccessible insights into the electron transfer processes to or from nanoparticles.

Herein we use a combination of electrochemistry and fluorescence microscopy to investigate silver nanoparticles capped with DNA and a fluorescent intercalator, ethidium bromide, to elucidate the ways in which the capping agent gates electron transfer from the silver particles to the electrode.

Silver nanoparticles (core radius 22.4 ± 3.9 nm), were modified with DNA intercalated with EtBr (see SI sections S1- S3 for further experimental details and characterisation). DLS measurements indicated that the DNA/EtBr capped silver nanoparticles had an average hydrodynamic radius of 63.0 ± 21 nm as compared to 30.1 ± 0.2 for the same silver nanoparticles capped with citrate. The significantly increased particle dimensions evidences the presence of a relatively thick organic layer surrounding the metallic core.

SI section 4.2 provides data for the oxidation of an ensemble of silver nanoparticles modified upon a macro glassy carbon substrate. The linear sweep voltammetric response exhibits a peak at +0.25 V vs Ag wire corresponding to the oxidation of the silver nanoparticles.[‡] However, this method of electrode modification is well-documented to produce incomplete stripping and to alter the effects of the capping agent,[19–21] and so the nanoparticles were next examined in solution with a carbon microfibre electrode (of radius 33 μ m). The electrode was submerged in a 10 mM solution of NaNO_3 [§] containing 2.4×10^{-8} mol m⁻³ nanoparticles. The electrode was held at 0.7 V vs a Ag wire pseudo reference/counter electrode for 200s and sharp spikes in current were observed (Figure 1a, solid line, whereby the dashed line is recorded in the absence of NPs). Integrating the area under the spikes provides a charge (using Faraday’s 1st law) which can be converted into a radius (assuming that the particles are spherical and of a known density).[23] A histogram showing the distribution of radii is shown in Figure 1 (taken from scans recorded at 0.5 – 0.8 V - *vide infra*). The EtBr/DNA Ag nanoparticles have a mean radii of 18.0 ± 4.9 nm

[‡]For recent reviews on the electrochemical analysis of nucleic acids please see Ferapontova[17] and Palacek *et al.*[18]

[§]This electrolyte was chosen to ensure the reaction of the particles progressed from Ag to Ag⁺. KCl, for example, can see the reaction progress to form AgCl at lower overpotentials.[22]

calculated by this method, whilst the citrate nanoparticles have previously been sized as 18.5 ± 4.9 nm,[14] suggesting that each spike corresponds to one NP undergoing full oxidation to Ag^+ .[¶]

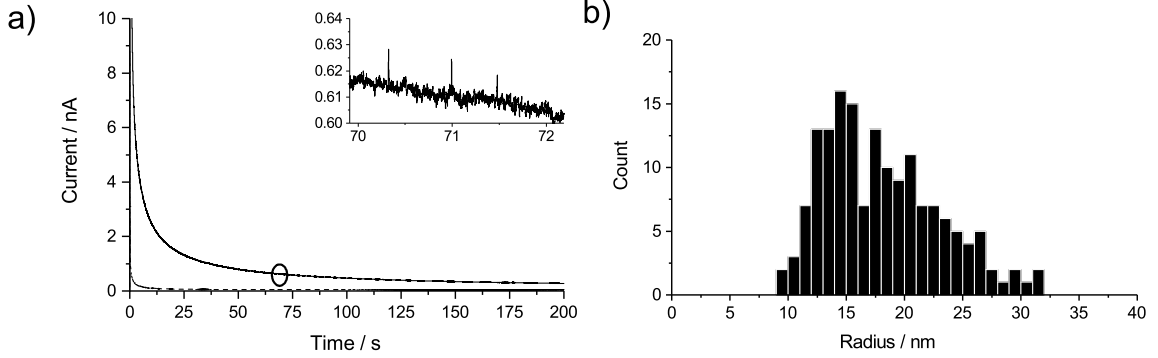


Figure 1: a) Representative chronoamperogram recorded at 0.7 V in the absence (dashed line) and presence (solid line) of EtBr/DNA Ag NPs. Inset is magnified circled region. b) Histogram of radii from integrated spikes in current.

To investigate the behaviour of the EtBr/DNA capped NPs, a potential dependency study was undertaken by holding the electrode at potentials from 0.0 to 0.8 V vs Ag for 200s and integrating the resulting spikes.

Figure 2a shows the potential dependency of the DNA and citrate capped nanoparticles as studied previously,[14] which show that the DNA capped nanoparticles do not undergo complete oxidation until 1.15 V, as determined by the potential at which the charge of the spikes reach *ca.* 3×10^{-13} C. In the case of the EtBr/DNA capped particles, full oxidation onsets at 0.5 V (Figure 2b, stars) some 0.65 V lower than the particles capped with DNA alone.

[¶]Note that recent reports suggest that only partial oxidation is observed under some conditions (namely by Unwin[24] and White[25]). However, White *et al.*[25] use rather large particles, whilst Unwin uses homebuilt equipment details of which are not reported so precluding others from repeating his work. It is possible that the potentiostat he used did not conserve charge accurately or, if it did, the many partial spikes ‘resolved’ are too close to the baseline to him to unambiguously resolve.

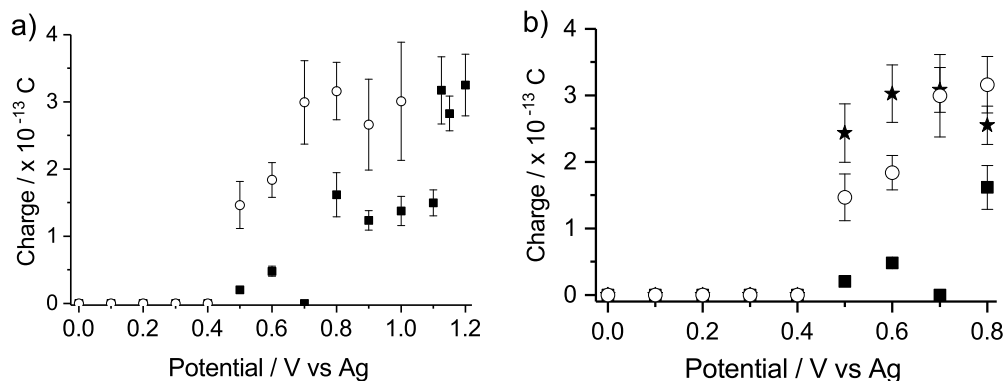


Figure 2: a) Average charge of spikes from DNA capped nanoparticles (black squares) and citrate capped (open circles) measured at each potential.[14] b) Average charge of spikes from DNA capped nanoparticles (black squares), EtBr/DNA capped nanoparticles (stars), and citrate capped (open circles) measured at each potential.

This suggests that the intercalation of the EtBr into the DNA is sufficiently disruptive such that the insulation provided to the nanoparticle by the DNA is lost upon intercalation. This may be generally significant.

To gain further insight into the effect of the capping agent on the electron transfer, the fluorescence intensity of individual particles were measured as a function of time and applied potential. A suspension containing the as prepared nanoparticles and 10 mM NaNO_3 was injected into a thin-layer opto-electrochemical cell (as described previously[26] and in full in the SI section S5) The cell was attached to a potentiostat built in house (as detailed in the SI section S4), and the interface was voltammetrically scanned between 0.0 and 0.8 V (at a scan rate of 10 mV s^{-1}). Four different general types of behaviour were exhibited by the fluorescent particles. Case 1 involves nanoparticles that are adhered to the wire physically but do not change their fluorescence intensity over the course of the electrochemical scan, suggesting that the particles are not in electrochemical contact with the fibre electrode. Case 2 describes nanoparticles that are in contact with the electrode from the beginning of the scan and undergo slow ($> 200 \text{ ms}$ duration) oxidation at low overpotentials. Case 3 and 4 both describe nanoparticles that come into contact with the electrode when it is being held at a potential above 0.4 V vs Ag (i.e. whilst the electrode is biased at a potential where the oxidation of silver is not kinetically limited). Case 3 involves nanoparticles that leave or become disconnected from the electrode shortly after undergoing oxidation. Case 4 describes nanoparticles that remain electrochemically connected throughout the scan.

Figures 3 a) and b) depicts the same carbon fibre wire before and after the voltammetric scan. The associated voltammetric response of the entire wire electrode is depicted in 3 c). Notably no distinct oxidative features are observable in the voltammetric profile and the behaviour shown in 3 c) predominantly arises due to the capacitive charging of the carbon fibre interface. Both case 1 (uncircled particles in Figure 3a) and b)) and 2 (circled in Figure 3a) and b)) nanoparticles are evidenced in Figure 3, and the change in intensity of the case 2 particles are discussed below.

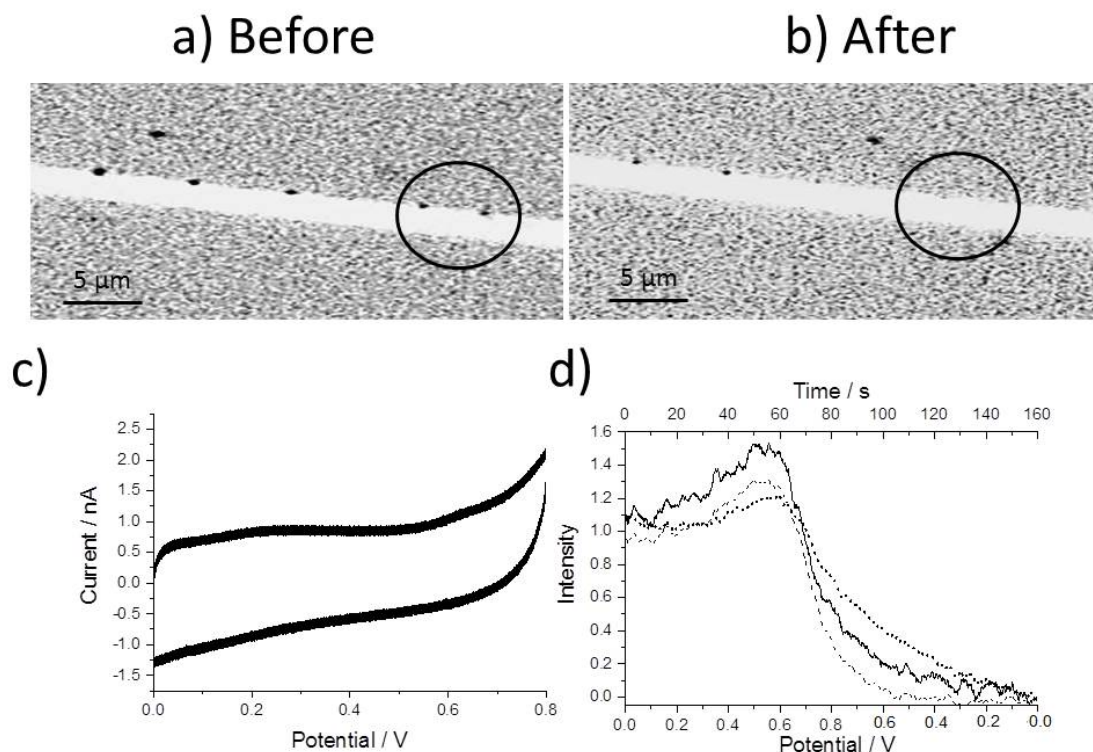


Figure 3: The carbon fibre wire a) before and b) after recording a CV (c)). The normalised fluorescence intensity of three nanoparticles that disappear during the scan is shown in d).

Figure 3 d) depicts three intensity profiles representative of case 2 particles, which decrease in intensity over the course of the electrochemical experiment as the potential is scanned at a rate of 0.01 V s^{-1} . Over the potential region in which the silver core would be expected to be oxidised i.e between 0.2 – 0.5 V, or 30 seconds, the fluorescence intensity of these individual particles increases (due to partial quenching of the fluorescence signal arising from the dye situated within the capping layer), and only at longer times do the intensity profiles decrease. The slow fluorescence intensity decrease (from 150 % relative intensity to 0 % over a 60 second window) likely represents the dissipation of the remaining capping agent after the removal of the core. Alternately, it might be that the decrease arises due to the oxidation of the EtBr, which occurs at potentials greater than 0.6 V (see SI section S2). However, it is noted that the slow fluorescence intensity decrease is still occurring at an experimental time of 120 s, where the potential of the electrode has been reduced to below 0.4 V.

An intensity profile of a case 3 nanoparticle is shown as a solid line in Figure 4d), whilst a case 4 nanoparticle is shown as a dot-dashed line in Figure 4d). A representative case 3 nanoparticle is shown before the intensity increase at frame 550 in Figure 4a) and after the increase in intensity at frame 900 in Figure 4b) is shown as a solid line in Figure 4d). These jumps in fluorescent intensity are observed in voltammetric scans containing sharp spikes in the current as shown in Figure 4c), which correspond to complete oxidation of individual nanoparticle cores. In the case

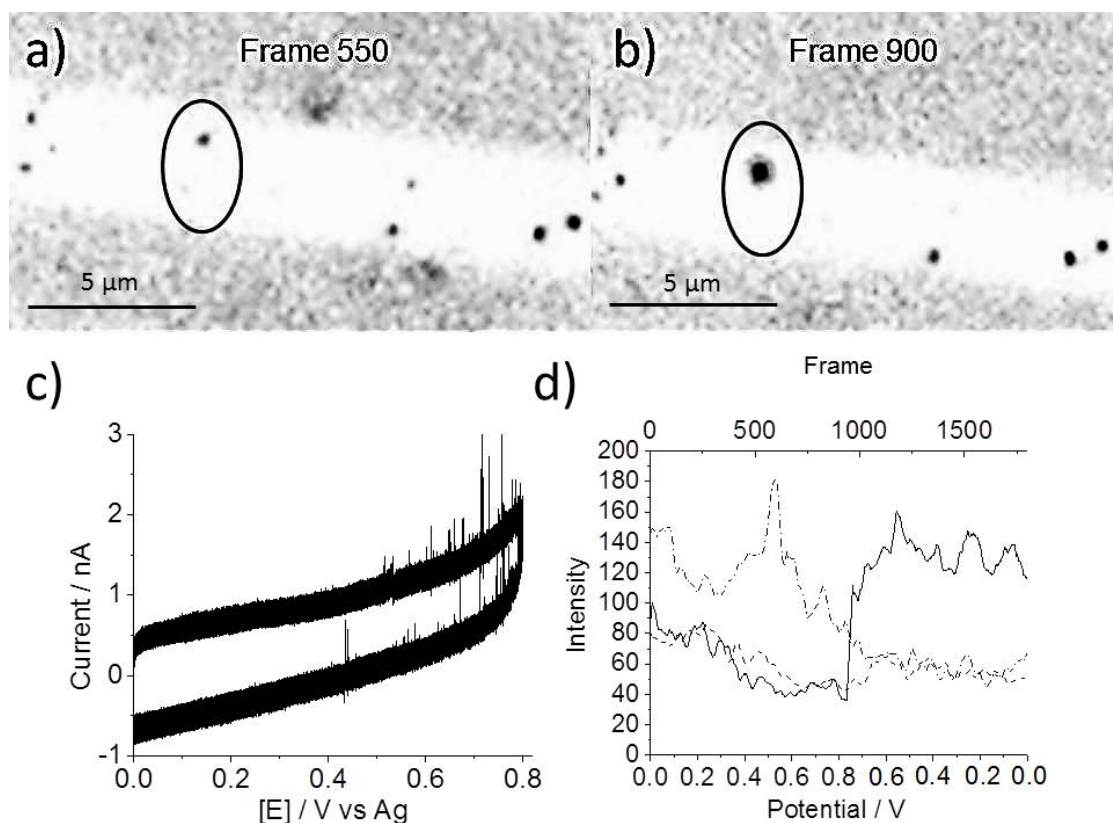


Figure 4: A nanoparticle increases in intensity from frame 550 ((a) to frame 900 ((b) as a CV is being recorded (c)). The change in intensity with frame and potential is shown in d), where the intensity of the nanoparticle circled is shown as a solid line, and the intensity of another nanoparticle whose intensity does not change is shown as a dashed line for comparison purposes.

of the nanoparticles that show a sharp increase in the fluorescence intensity (solid and dash-dot lines, Figure 4d)), it is likely that they come into electrical contact - whether through diffusing to the wire or the rearrangement of the DNA capping of an already adhered particle - at the point the spike is observed. Where the fluorescence shows a sharp decrease following the sharp increase (dash-dot line, case 3), it is likely that the capping agent is dissipating as seen in case 2.

In all the cases evidenced above, it is clear that the capping agent determines the outcome of the electron transfer from the nanoparticle. The use of Fluorescent Electrochemical Microscopy allows both fast and slow processes to be observed, revealing the gating of electron transfer the capping agent provides. Combining these results with electrochemical ones - whereby EtBr/DNA capped Ag NPs appear to undergo full oxidation of the nanoparticle at *ca.* 0.5 V (vs 1.15 V in the case of the native DNA)[14] - confirms that the capping agent is controlling the electrical access between the nanoparticle and the biased electrode and that intercalation promotes electrochemical access compared with native DNA.

Supporting Information

The Supporting Information includes thirteen additional figures and a detailed experimental section.

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